Etiology and Pathogenesis of Periodontal Disease

Alexandrina L. Dumitrescu

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Dedication

The important thing is not to stop questioning.
Curiosity has its own reason for existing.
Albert Einstein, 1879–1955

Foreword

In his 1968 book, *General Systems Theory: Foundations, Development, Applications*, Ludwig von Bertalanffy observed that "… science is split into innumerable disciplines continually generating new subdisciplines. In consequence, the physicist, the biologist, the psychologist and the social scientist are, so to speak, encapsulated in their private universes, and it is difficult to get word from one cocoon to the other…."

The same might have been true about subdisciplines *within* the fields of medicine and dentistry as well. But times are changing. The concept that oral diseases and disorders reflect and affect overall health has been gaining wide acceptance, especially over the past decade. As an illustration, a quick search of PubMed's electronic database of biomedical journals yields approximately 225 research and review articles published any time before 1980 that mention both periodontal diseases and cardiovascular diseases; in just the last 10 years, that number has nearly quadrupled.

We are seeing an ever-increasing amount of research that links periodontal disease to an astonishingly large and diverse set of systemic health outcomes other than cardiovascular diseases. These include low birth weight, osteoporosis, diabetes, cognitive decline, obesity, and others. We are gaining a better understanding of the roles that pathogenic bacteria, the intra-oral media in which they thrive, and the local and systemic immunologic responses they elicit play in the etiologies of periodontal and systemic diseases. We are also gaining insights into the nature of interactions between periodontal disease and other intra-oral conditions and treatments. Since many of these associations are bidirectional, uncovering the true cause and effect relationships presents methodological challenges, and so it is not surprising to find conflicting reports and opinions.

This volume represents a truly comprehensive update and critical review of the complex interrelationships of periodontal diseases with our total health and well-being. Individual chapter topics cover disease microbiology and etiology; genetic, chronic systemic disease, and psychological factors; effects of periodontal disease and treatments on restorative and endodontic outcomes, and the impacts of malocclusion and orthodontic intervention. As these chapters illustrate, we are talking to one another, and this collection of papers will serve as an important resource for researchers and providers interested in the causes, prevention, and treatment of periodontal disease.

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Preface

The past decade has witnessed a remarkable growth of knowledge concerning the etiology and pathogenesis of periodontal disease. Biologic processes, including the characteristics of the biofilm and of the host inflammatory and immune responses, tend to vary among individuals, despite producing a similar clinical picture or diagnostic category. Studies on the microbiota associated with periodontal disease have revealed a wide variety in the composition of the subgingival microflora. Other factors that may influence the biologic phenotype and clinical expression of disease include unique environmental exposures, psychological (behavioral) factors, as well as differences in genetic and possibly epigenetic composition.

A strong relationship between periodontal health or disease and systemic health or disease was also revealed. This means a two-way relationship in which periodontal disease in an individual may be a powerful influence on an individual's systemic health or disease as well as the most customary understood role that systemic disease may have in influencing an individual's periodontal health or disease. There is increasing evidence that individuals with periodontal disease may be at increased risk for adverse medical outcomes: mortality, cardiovascular disease, metabolic syndrome, diabetes mellitus, adverse pregnancy outcomes, respiratory disease, rheumatoid arthritis, renal disease, cancer, inflammatory bowel disease, Alzheimer disease, and osteoporosis.

In this book we propose an holistic view, by delineating the multiple systemic and local factors that contribute to the clinical presentation of periodontal disease in a specific individual: dental plaque, calculus, microbial composition, immune response, systemic diseases, behavioral determinants, genetic variants, and local factors that should allow a more accurate diagnosis of periodontal disease, prognosis, provide insight into the customized treatment for the periodontal patient, as well as the identification of individuals of high risk.

As Socransky et al. stated in 1987, the task of defining the etiological agents of periodontal disease is a cyclical process with continual re-evaluation and refinement.

This book, dedicated to the science and practice of periodontology as a contribution to understand, treat, and prevent this disease, would be of interest to periodontists, undergraduate and postgraduate dental students, dental educators, and researchers.

Tromsø, Norway — Alexandrina L. Dumitrescu

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Etiology of Periodontal Disease: Dental Plaque and Calculus

1

Alexandrina L. Dumitrescu and Makoto Kawamura

Dental plaque is a unique ecosystem. It is a microbial biofilm, a diverse microbial community found on the tooth surface embedded in a matrix of polymers of bacterial and salivary origin. The biofilm is a thin basal layer on the substratum, in contact with, and occasionally penetrating, the acquired enamel pellicle, and with columnar, mushroom-shaped multibacterial extensions into the lumen of the solution, separated by regions ("channels"), seemingly empty or filled with extracellular polysaccharide. The bacteria in a biofilm communicate with each other by sending out chemical signals. These chemical signals trigger the bacteria to produce potentially harmful proteins and enzymes. Several plaque models, as well as dental calculus localization, composition, morphology, formation, assessment, association with periodontal disease pathology, and anticalculus agents, are presented.

1.1 Dental Plaque

Dental plaque is a unique ecosystem. Several hundred bacterial species inhabit the human oral cavity (Tanner et al. 1998), and these multiple bacterial species form a community of dental plaque (Kolenbrander 2000; Okuda et al. 2004). Bacteria in periodontal pockets use gingival crevicular fluid as the nutrient source of carbon and nitrogen, as well as essential growth factors, such as minerals and vitamins. These bacteria then proliferate and communicate by signals to each other (Carlsson 2000; Palmer et al. 2001a; Okuda et al. 2004). In order to maintain the ecosystem, various anaerobes anchor to each other by forming aggregated bacterial masses (Kigure et al. 1995; Okuda et al. 2004). The regulation of bacterial gene expression in response to changes in cell density is known as quorum sensing. Quorum-sensing bacteria synthesize and secrete extracellular signaling molecules called autoinducers, which accumulate in the environment as the population increases (Okuda et al. 2004). Gram-positive bacteria generally communicate via small diffusible peptides, while many gram-negative bacteria secrete acyl homoserine lactones (AHLs) (Whitehead et al. 2001), which vary in structure depending on the species of bacteria that produce them. AHLs are involved in quorum sensing whereby cells are able to modulate gene expression in response to increases in cell density. Another system involves the synthesis of autoinducer- 2 (AI-2); its structure is unknown, but a gene product, LuxS, is required (Federle and Bassler 2003; Winzer et al. 2003). This system may be involved in cross-communication among both gram-positive and gram-negative bacteria, as homologues of LuxS are widespread within the microbial world (Marsh 2004). Several strains of *Prevotella intermedia, Fusobacterium nucleatum*, and *Porphyromonas gingivalis* (formerly *Bacteroides gingivalis*) were found to produce such activities (Frias et al. 2001; Wu et al. 2009). It was also revealed that the signals produced by subgingival bacteria induce both intra- and inter-species responses in the mixed-species microbial communities that exist in the oral cavity (Okuda et al. 2004).

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1.1.1 Dental Plaque as a Microbial Biofilm

Dental plaque can be defined as "*matrix-enclosed bacterial populations adherent to each other and/or to surfaces or interfaces*" (Costerton 1995).

Dental plaque is a *microbial biofilm*, a diverse microbial community found on the tooth surface embedded in a matrix of polymers of bacterial and salivary origin (Bradshaw and Marsh 1999). Bradshaw and Marsh (1999) showed the biofilm as a thin basal layer on the substratum, in contact with, and occasionally penetrating, the acquired enamel pellicle, and with columnar, mushroom-shaped multibacterial extensions into the lumen of the solution, separated by regions ("channels") seemingly empty or filled with extracellular polysaccharide (TenCate 2006; Costerton and Lewandowski 1997; Costerton et al. 1994, 1995). The bacteria in a biofilm communicate with each other by sending out chemical signals. These chemical signals trigger the bacteria to produce potentially harmful proteins and enzymes (Overman 2000) (Fig. 1.1).

The biofilms are considered as etiological communities that evolved to permit the survival of the community as a whole and having a molecular organization, physiochemical properties and growth characteristics. Organization of micro-organisms within biofilms confers, on the component species, properties that are not evident with the individual species grown independently or as planktonic populations in liquid media (Gilbert et al. 1997). The basic biofilm properties are (Overman 2000):

- Cooperating community of various types of microorganisms: Microorganisms are arranged in microcolonies.
- Microcolonies are surrounded by a protective matrix.
- Within the microcolonies are differing environments.
- Microorganisms have a primitive communication system.
- Microorganisms in the biofilm are resistant to antibiotics, antimicrobials, and host response.

1.1.1.1 Inter-species communication: explaining the biological effects of bacterial biofilms

Communication among the different species within biofilms appears to be the key to understanding how plaque can act as a single unit, and how specific bacteria emerge and impair the balance with the host. *Physical* (coaggregation and coadhesion), metabolic and physiological (gene expression and cell-cell signaling) interactions yield a positive cooperation among different species within the biofilm: the metabolic products of some organisms may promote the further growth of other bacteria or prevent the survival of others. A key role in the cooperative processes is

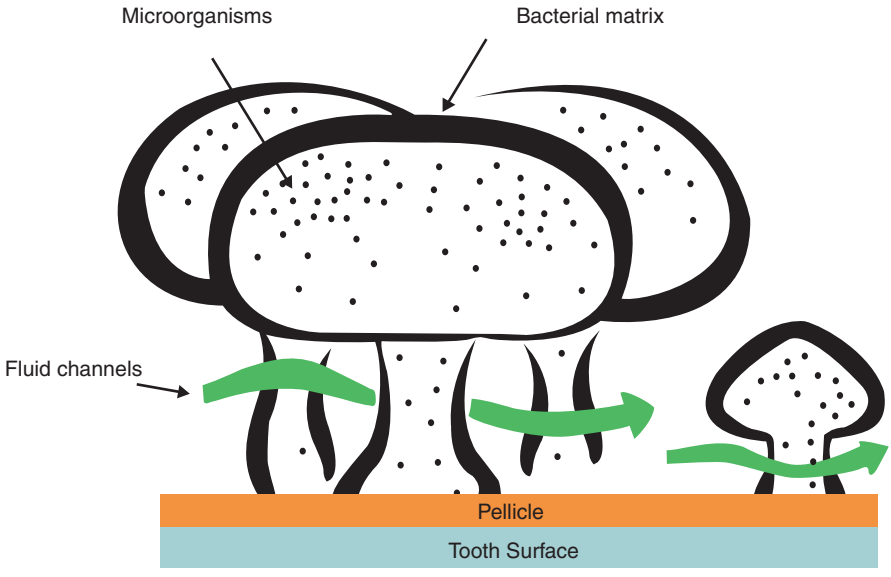

Fig. 1.1 *Diagrammatic representation of the structure of the plaque biofilm*. Note the relatively open water channels between discrete microcolonies in which bacterial cells are enclosed in a dense expolysaccharide matrix. The *arrows* indicate convective flow within the water channels (modified from Costerton et al. 1994) (with permission from Annual Reviews Publishing)

played by *Fusobacterium nucleatum,* which is able to form the needed "bridge" between early, i.e., *Streptococci* spp., and late colonizers, especially obligate anaerobes. In the absence of *Fusobacterium nucleatum, Porphyromonas gingivalis* cannot aggregate with the microbiota already present, such as the facultative aerobes *Actinomyces naeslundii, Neisseria subflava, Streptococcus mutans, Streptococcus oralis* and *Streptococcus sanguinis* (formerly *Streptococcus sanguis*). The presence of *Fusobacterium nucleatum*, on the other hand, enables anaerobes to grow, even in the aerated environment of the oral cavity. Other microorganisms are also able to link otherwise noncommunicating bacteria (i.e., *Streptococcus sanguinis* forms a "corn cob" complex together with *Corynebacterium matruchotii* (formerly *Bacterionema matruchotii*) and *Fusobacterium nucleatum*), and this may represent the basic event leading to biofilm initiation and development (Sbordone and Bortolaia 2003).

The pattern of colonization and coaggregation is often unidirectional,which is proof that some bacteria need to have the environment prepared by other microbiota in order to colonize. *Porphyromonas gingivalis* can adhere to oral *streptococcus* spp. and *Actinomyces naeslundii*, forming small coaggregates resistant to removal, if the substratum has been previously exposed to *Streptococcus gordonii*. Lacking *Streptococcus gordonii*, only few *Porphyromonas gingivalis* cells manage to attach and are easily removed (Sbordone and Bortolaia 2003).

Using DNA probes and checkerboard DNA-DNA hybridization analysis, Socransky et al. (1998) have been able to provide a clear explanation of this colonization pattern and the positive cooperation among *subgingival microbiota*. They describe how bacteria tend to be grouped in clusters according to nutritional and atmospheric requirements, with the exception of *Actinomyces viscosus, Selenomonas noxia* and *Aggregatibacter actinomycetemcomitans* (formerly *Actinobacillus actinomycetemcomitans*) serotype b which do not belong to any group (Sbordone and Bortolaia 2003). The red cluster consisted of *Porphyromonas gingivalis Tannerella forsythia* (formerly *Bacteroides forsythus*) and *Treponema denticola*. The orange cluster consisted of *Fusobacterium nucleatum* subsp., *Prevotella intermedia* and *Prevotella nigrescens, Peptostreptococcus micros,* and *Campylobacter rectus, Campylobacter showae, Campylobacter gracilis, Eubacterium nodatum,* and *Streptococcus constellatus*. The three *Capnocytophaga* sp., *Campylobacter concisus, Eikenella corrodens* and *Aggregatibacter actinomycetemcomitans* serotype *a* formed the green cluster, while a group of streptococci made up the yellow cluster. *Streptococcus mitis, Streptococcus sanguinis* and *Streptococcus oralis* were the most closely related within this group. *Actinomvces odontolyticus* and *Veillonella parvula* formed the purple cluster. Two *Actinomyces naeslundii* genospecies (formerly *Actinomyces viscosus*), *Selenomonas noxia* and *Aggregatibacter actinomycetemcomitans* serotype b, did not cluster with other species (Socransky et al. 1998) (Fig. 1.2).

The authors describe how each cluster appeared to influence the others. The species within complexes are closely associated to one another: Most periodontal sites harbor either all or none of the species belonging to the same complex, while individual species or pairs of species are detected less frequently than expected, reinforcing the hypothesis of the community theory rather than the germ theory. Precise interrelations are established between complexes as well. Microbiota belonging to the red cluster are seldom detected in the absence of the orange complex, and the higher the detected amounts of orange complex bacteria, the greater is the colonization by red complex members. Yellow and green clusters show a similar preference for each other and a weaker relation with the orange and red complexes, while the purple complex shows loose relations with all the other clusters. Such relations can be explained by mechanisms of antagonism, synergism and environmental selection (Socransky et al. 1998; Sbordone and Bortolaia 2003).

Clinically, yellow and green complexes are associated with shallow pockets (probing depth <3 mm), while the orange and red ones are related to increasing periodontal indices and more advanced lesions. P.gingivalis, B. forsythus and T.denticola are detected in deeper pockets (probing depth >4 mm) and bleeding on probing-positive sites. Given the consecutive colonization of orange and red clusters, altering the former might prevent the emergence of the latter, though it is quite difficult to interfere with the colonization mechanisms and relations among the species as they are yet to be completely understood (Sbordone and Bortolaia 2003).

Specific microbial complexes in *supragingival plaque,* which were similar to those found in subgingival plaque samples with a few minor differences, were recently described by Haffajee et al. (2008a–c) (Table 1.1). Red complex community was formed containing the three species, previously identified as the red

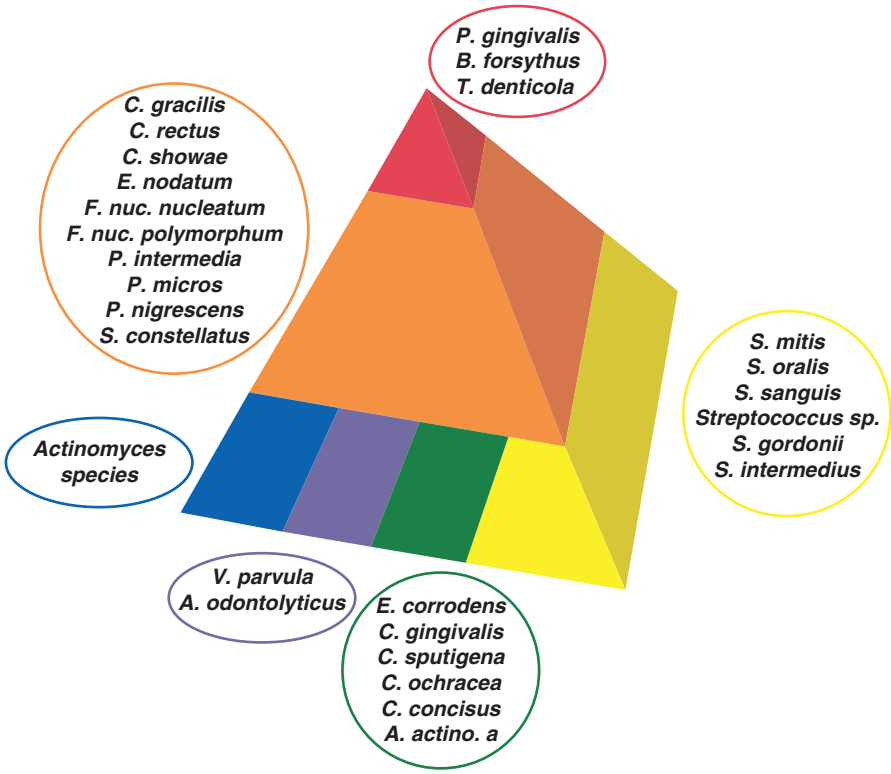

Fig. 1.2 *Diagram of the association among subgingival species*. The base of the pyramid is comprised of species thought to colonize the tooth surface and proliferate at an early stage. The *orange complex* becomes numerically more dominant later, and is thought to bridge the early colonizers and the *red complex* species which become numerically more dominant at late stages in plaque development (Socransky and Haffajee 2002; with permission from Wiley-Blackwell Publishing)

complex in subgingival plaque namely, *Tannerella forsythia, Porphyromonas gingivalis*, and *Treponema denticola. Eubacterium nodatum* was also part of this complex and *Treponema socranskii* was loosely associated with these four species. A number of species previously identified in subgingival plaque as orange complex species were also detected as part of an orange complex in supragingival plaque. These included *Campylobacter showae, Campylobacter rectus, Fusobacterium nucleatum* subsp. *nucleatum*, *Fusobacterium nucleatum* subsp. *vincentii, Fusobacterium periodonticum, Fusobacterium nucleatum* subsp. *polymorphum, Campylobacter gracilis, Prevotella intermedia*, and *Prevotella nigrescens*. These taxa were joined by *Gemella morbillorum, Capnocytophaga ochracea, Selenomonas noxia*, and *Prevotella melaninogenica*. Yellow complex was primarily formed of the *streptococcus* sp. Streptococcus *mitis, Streptococcus oralis, Streptococcus gordonii, Streptococcus sanguinis* and, somewhat separately, of *Streptococcus anginosus, Streptococcus intermedius*, and *Streptococcus constellatus*. These species were joined by *Leptotrichia buccalis, Propionibacterium acnes, Eubacterium saburreum, Peptostreptococcus micros*, and *Aggregatibacter actinomycetemcomitans*. A tight cluster of *actinomyces* sp, including *Actinomyces israelii, Actinomyces naeslundii* one, *Actinomyces odontolyticus, Actinomyces gerencseriae*, and *Actinomyces naeslundii* two was formed. A green complex consisting of *Capnocytophaga sputigena, Eikenella corrodens*, and *Capnocytophaga gingivalis,* as well as a loose purple complex consisting of *Neisseria mucosa* and *Veillonella parvula* was formed (Haffajee et al. 2008a) (Fig. 1.3). While plaque mass was associated with differences in proportions of many species in supragingival biofilms, tooth location also was strongly associated with species proportions in both univariate and multivariate analyses (Haffajee et al. 2008b, c).

The relationship between the microbial composition of supragingival plaque samples and the clinical measures of inflammation was quite strong, and on probing, many species were found to be significantly elevated in mean counts at sites that exhibited gingival redness or bleeding. The species that were most in number, adjacent to the inflamed sites, were members of the orange and red complexes. This relationship of orange and red complex species with inflammation was in accordance with the findings related to subgingival biofilms. There was a strong relationship of supragingival counts to

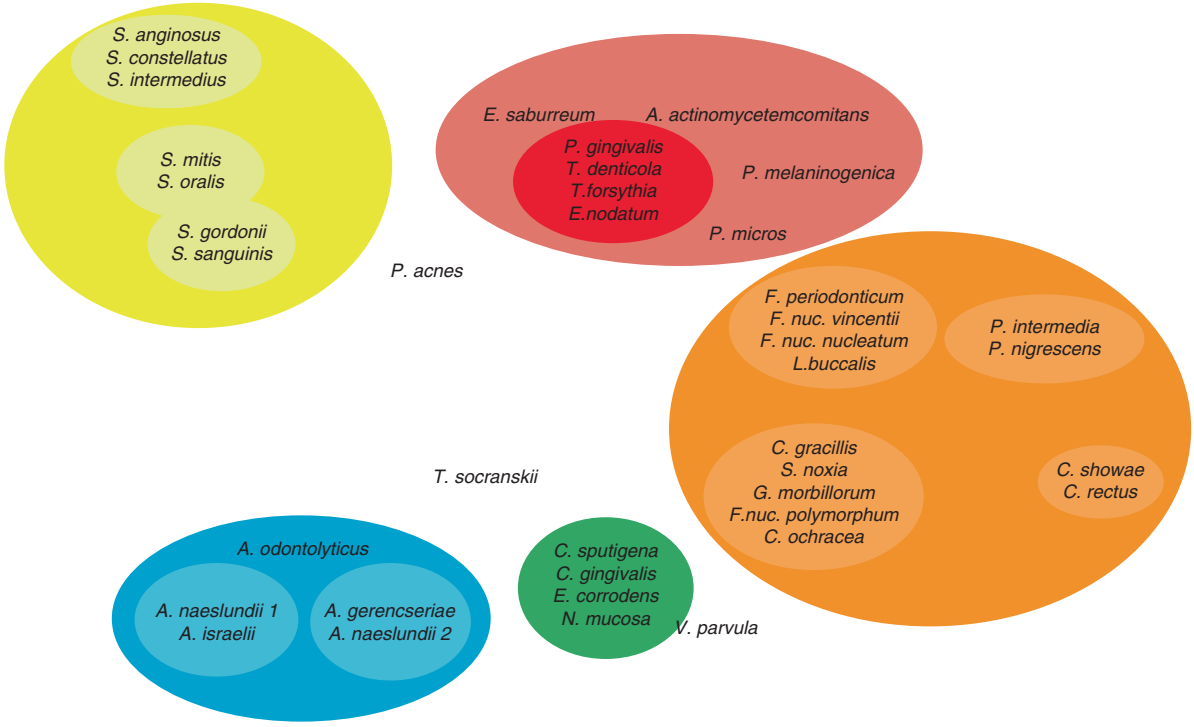

Fig. 1.3 *Diagrammatic representation of the relationships of species within microbial complexes and between the microbial complexes in supragingival biofilm samples*. (Haffajee et al. 2008a) (with permission from Wiley-Blackwell Publishing)

measures of pocket depth and attachment level. When counts of species in the different pocket depth categories were examined for sites that did or did not exhibit inflammation, the increased levels of orange and red complex species were still observed at the sites with deep pockets irrespective of the level of inflammation (Haffajee et al. 2008a). (Table 1.1).

A consequence of biofilm growth that has profound implications for their control in the environment and in medicine is a markedly enhanced resistance to chemical antimicrobial agents and antibiotics (Marsh 2004). Mechanisms associated with such resistance in biofilms are thought to be related to the following: (1) modified nutrient environments and suppressed growth rates within the biofilm; (2) direct interactions between the exopolymer matrices and their constituents, and antimicrobials, affecting diffusion and availability; and (3) the development of biofilm/attachment-specific phenotypes that can result in reduced sensitivity to inhibitors. Growth on a surface may also result in the drug target being modified or not expressed in a biofilm, or the organism may use alternative metabolic strategies. Bacteria grow only slowly under nutrient depleted conditions in an established biofilm and, as a consequence, are much less susceptible to change than faster-dividing cells.The structure of a biofilm may restrict the penetration of the antimicrobial agent; charged inhibitors can bind to oppositely charged polymers that make up the biofilm matrix (diffusion–reaction theory). The agent may also adsorb to and inhibit the organisms at the surface of the biofilm, leaving the cells in the depths of the biofilm relatively unaffected. The matrix in biofilms can also bind and retain neutralizing enzymes (*e.g.*, β-lactamase) at concentrations that could inactivate an antibiotic or inhibitor. In addition, it has also been proposed that the environment in the depths of a biofilm may be unfavourable for the optimal action of some drugs (Gilbert et al. 1997; Marsh 2004 Stewart and Costerton 2001) .

Although it has been shown that bacterial species residing in biofilms are much more resistant to antibiotics than the same species in a planktonic state, antibiotics that have been used frequently in the treatment of periodontal infections (Teles et al. 2006). van Winkelhoff et al. (1996) and Slots and Ting (2002) have revealed that systemically administered antibiotics provided a clear clinical benefit in terms of mean periodontal attachment

Table 1.1 Microbial complexes in subgingival and supragingival plaque (according to Socransky et al. 1998 and to Haffajee et al. 2008a)

Cluster	Bacterial species	
	Subgingival plaque	Supragingival plaque
Purple cluster	*Veillonella parvula, Actinomyces odontolyticus*	*Veillonella parvula, Neisseria mucosa*
Yellow cluster	*Streptococci* sp.: *S.mitis, S.oralis, S.sanguis, S.gordonii, S.intermedius*	*Streptococcus* sp.: *S. mitis, S. oralis, S. gordonii, S. sanguinis, S. anginosus, S. intermedius, S. constellatus, Leptotrichia buccalis, Propionibacterium acnes, Eubacterium saburreum, Peptostreptococcus micros, Aggregatibacter actinomycetemcomitans*
Green cluster	*Eikenella corrodens, Capnocytophaga gingivalis, Capnocytophaga sputigena, Capnocytophaga ochracea, Capnocytophaga concisus Aggregatibacter actinomycetemcomitans serotype a*	*Capnocytophaga sputigena Eikenella corrodens Capnocytophaga gingivalis*
Orange cluster	*Prevotella intermedia, Prevotella nigrescens, Peptostreptococcus micros, Campylobacter gracilis, Campylobacter rectus, Fusobacterium periodonticum Fusobacterium nucleatum* subsp. *nucleatum, Fusobacterium nucleatum* subsp.*vincentii, S.constellatus, Eubacterium nodatum, Campylobacte showae, Fusobacterium nucleatum* subsp. *polymorphum*	*Campylobacter showae, Campylobacter rectus Fusobacterium nucleatum* subsp. *nucleatum, F. n.* subsp. *vincentii, Fusobacterium periodon ticum, F. n.* subsp. *polymorphum, Campy lobacter gracilis, Prevotella inter- media, Prevotella nigrescens, Gemella morbillorum, Capnocytophaga ochracea, Selenomonas noxia, Prevotella melaninogenica*
Red cluster	*Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Actinomyce viscosus, Selenomonas noxia, Aggregatibacter actinomy- cetemcomitans serotype b*	*Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Eubacterium nodatum, Treponema socranskii*

level "gain" post-therapy, when compared with groups not receiving these agents. Meta-analyses performed by Herrera et al. (2002) and Haffajee et al. (2003) indicated that adjunctive systemically administered antibiotics can provide a clinical benefit in the treatment of periodontal infections. However, it must be pointed out that not every study found that adjunctive systemically administered antibiotics provided a benefit to the subject in terms of clinical or microbial outcomes beyond control mechanical debridement therapies (Teles et al. 2006).

The supra- and subgingival habitats present distinct opportunities for colonization by bacterial species. The supra- and subgingival biofilms form a continuum, at least on the tooth surface. *Thus, major changes in the supragingival environment are likely to bring about shifts in the subgingival microbiota*. It is thought that supragingival plaque control decreases inflammation and gingival crevicular fluid flow, resulting in less nutrition for the subgingival organisms. *Removal of supragingival biofilm* may also directly affect the contiguous subgingival plaque because the supragingival bacteria may provide nutrients for the subgingival plaque. Several microbial changes in the subgingival microbiota have been reported as a result of supragingival instrumentation, including a reduction in the total number of subgingival microorganisms, a reduction in the levels of *spirochetes*, an increase in the proportion of gram-positive organisms, a reduction in the frequency of detection of *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans* and *Fusobacterium nucleatum*, and a decrease in the levels of subgingival species, such as *Prevotella intermedia, Fusobacterium nucleatum* and *Porphyromonas gingivalis*. The cumulative evidence indicates that persistent, meticulous supragingival plaque control affects both the amount of subgingival biofilm and its composition. These effects seem to be more dramatic in shallow to intermediate pockets, but also impact the microbiota of deep sites. However, the magnitude of the impact of the alterations in the supragingival biofilm on the subgingival biofilm does not seem to be enough to arrest disease progression in severe cases with deep periodontal pockets (Teles et al. 2006).

Subgingival debridement is by far, the most commonly used antiinfective therapy in the treatment of periodontal diseases. Its primary goal is to remove soft and hardened microbial deposits from the pathologically

exposed root surfaces. The immediate effect of scaling and root planing is an enormous disruption of the subgingival biofilm. Curettes, when used as a sampling method, can remove up to 90% of the subgingival plaque. It was suggested that the impact of scaling and root planing on the subgingival microbiota can last beyond the first 3 months posttherapy, in spite of a lack of subgingival reinstrumentation (Teles et al. 2006).

The proposed benefits of *periodontal surgery* over scaling and root planing include better access for cleaning of the root surfaces, pocket reduction (or elimination) and exposure of root surfaces for proper cleaning by the patients. Of all periodontal therapies, surgery is the procedure that most drastically alters the periodontal pocket environment. Hence, it is logical to anticipate that these techniques, particularly those aiming at total pocket elimination, will result in dramatic changes in the subgingival microbiota. Additional beneficial changes in the subgingival microbiota after surgery, when compared with scaling and root planing alone, were reported (Teles et al. 2006).

Despite the best efforts by clinicians and patients, certain subgingival sites will become *recolonized by periodontal pathogens after active therapy*. There are several potential sources for the reinfection of the gingival crevice, including: regrowth of residual cells present in the depths of the pocket; neighboring supra- and subgingival biofilms still colonized by the species in question; other intra-oral sites; and exogenous sources, through vertical or horizontal transmission. A key role of supragingival plaque control in retarding the resurgence of pathogens within the pockets after mechanical therapy was clearly established, suggesting a clear involvement of supragingival plaque as a major source of reinfecting organisms. In fact, the observation that periodontal pathogens, including members of the red complex (*Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*), can colonize supragingival biofilms of both, subjects with periodontitis and healthy individuals (although at lower levels and at a reduced prevalence), highlights the importance of this habitat as a source of reinfecting microorganisms. The alternative explanation consists of the fact that the pathogenic species could have remained in the depths of the healed pockets, and been provided with an enhanced source of nutrients from the gingival crevice fluid, resulting from the inflammation triggered by early colonizers of the supragingival microbiota. It has long been hypothesized that the sulci of neighboring teeth, or even teeth from distant quadrants, can foster the recolonization of treated sites (Teles et al. 2006).

Traditionally, the oral cavity of a periodontal subject is treated in several sessions during which antiinfective therapy is applied to different areas of the mouth, divided into quadrants or sextants. Based on the idea that the microbiota of nontreated sites could compromise the healing of treated quadrants, a new therapeutic approach was devised, based on the principle of full-mouth disinfection. In this approach, the full dentition receives scaling and root planing within 24 h, in order to minimize reinfection of treated sites by pathogens present on untreated teeth. As other oral surfaces (saliva, tonsils, oral mucosa, tongue) also harbor periodontal pathogens, the therapy, additionally involved the disinfection of these surfaces using chlorhexidine. This technique resulted in superior pocket depth reduction and clinical attachment level gain when compared with the typical weekly/biweekly quadrant treatment regimen for scaling and root planing. Reported microbiological improvements included a decreased percentage of *spirochetes* and motile rods, greater reduction in the levels of pathogenic species such as *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Peptostreptococcus micros* and *Campylobacter rectus*, and diminished levels of "black-pigmented *bacteroides*" (Teles et al. 2006).

Oral implants provide a unique opportunity for the observation of initial subgingival colonization patterns, since it is started with a "pristine" bacteria-free surface/pocket. Quirynen et al. (2005) recorded the development of the "initial" subgingival biofilm on implants with shallow (<3 mm) and moderate (>3 mm) pockets, to estimate the time needed before a complex subgingival flora could be established with the supragingival area as the single source. The undisturbed subgingival microbiota of neighboring teeth in the same individuals served as controls. Checkerboard DNA-DNA hybridization and culture data revealed a complex microbiota (including several pathogenic species) in the pristine pockets within a week, with a minimal increase in counts up to 4 weeks. The reason for the rapid recolonization in the "pristine" environment is not clear. It is possible that the blood coagulum at the fresh implant sites may favor the colonization and growth of oral species in a fashion similar to that which might occur after mechanical debridement of periodontal pockets. Alternatively, the large number of organisms in saliva and on the oral soft tissues, particularly the tongue, and the rapid multiplication rate of bacteria may be sufficient for many species to establish

and reach sizeable numbers in the absence of the competing microbiota (Quirynen et al. 2005).

Surprisingly, it was recently revealed that periodontal pathogens such as *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* are also clearly present in the samples from edentulous subjects. Microbial profiles in samples from the soft tissue surfaces differed among site locations. Samples from the dorsum of the tongue exhibited the highest bacterial counts followed by the "attached gingiva" and the lateral surfaces of the tongue, while the lowest mean counts were found in samples from the buccal mucosa and labial vestibules. Using cluster analysis of the proportions of the test species, three clusters were formed. The first cluster comprised saliva, supragingival plaque, and the lateral and dorsal surfaces of the tongue. The second cluster comprised the other six soft tissue surfaces. Species on the denture palate formed a third cluster (Sachdeo et al. 2008).

1.1.2 Microbial Composition of Dental Plaque in Relation to Periodontal Health or Disease

1.1.2.1 Periodontal Pathogens Associated with Health

Healthy gingivae have been associated with a very simple supragingival plaque composition: few (1–20) layers of predominantly gram-positive cocci (*streptococcus* spp.: *Streptococcus mutans, Streptococcus mitis, Streptococcus sanguinis, Streptococcus oralis; Rothia dentocariosa; Staphilococcus epidermidis*), followed by some gram-positive rods and filaments (*actinomyces* spp: *Actinomyces viscosus, Actinomyces israelis, Actinomyces gerencseriae; corynebacterium* spp.) and very few gram-negative cocci (*Veillonella parvula; neisseria* spp.). The latter are aerobic or facultative aerobic bacteria, able to adhere to the nonexfoliating hard surfaces; initial adhesion is promoted by surface free energy, roughness and hydrophilia, and is mediated by long- and short-range forces (Sbordone and Bortolaia 2003). In older subjects, the microbiota of healthy sites with no prior history of gingivitis, have shown a predominance of gram-negative species including *Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia, Campylobacter rectus, Eikenella corrodens, leptotrichia* and *selenomonas* sp. (Newman et al. 1978).

1.1.2.2 Periodontal Pathogens Associated with Gingivitis

Dental plaque formation increases during inflammation of the gingival margin. It increases in terms of both thickness and the tooth surface area covered. The mechanisms underlying this observation are, however, not fully understood. It has been suggested (1) that enhanced gingival crevicular fluid during inflammation increases the supply of nutrients for plaque-forming bacteria and (2) that the inflammatory edema of the gingival margin constitutes an anatomic shelter for growing plaque. Yet another explanation could be the increased amounts of plasma protein in the pellicle which may affect the bacterial composition of dental plaque. This is consistent with the findings of more gram-negative species, as well as rods and filamentous organisms, on tooth surfaces next to an inflamed gingival margin compared with a healthy one (Rüdiger et al. 2002).

Clinical gingivitis is associated with the development of a more organized dental plaque. Such biofilms are characterized by several cell layers (100–300), with bacteria stratification arranged by metabolism and aerotolerance; besides the gram-positive cocci, rods and filaments associated with healthy gingivae, the number of gram-negative *cocci*, rods and filaments increases and anaerobic bacteria appear (*Fusobacterium nucleatum, Campylobacter gracilis, Tannerella forsythia, capnocytophaga* spp.). The species involved vary depending on local environmental characteristics, but the colonization pattern is always the same (Sbordone and Bortolaia 2003). The severe forms of gingivitis have been associated with subgingival occurence of the black-pigmented asaccharolytic *Porphyromonas gingivalis* (White and Mayrand 1981).

In pregnancy gingivitis, an association has been observed between high levels of *Prevotella intermedia* and elevations in systemic levels of estradiol and progesterone (Kornman and Loesche 1980).

Microbial studies in acute necrotizing ulcerative gingivitis (ANUG) indicates high levels of *Prevotella intermedia* and *Treponema pallidum* – related *spirochetes. Spirochetes* are found to penetrate necrotic tissue as well as apparently unaffected connective tissue (Loesche et al. 1982; Riviere et al. 1991).

1.1.2.3 Periodontal Pathogens Associated with Periodontitis

The etiologic role of bacteria in periodontal disease is clearly established (Socransky 1977). According to the nonspecific plaque hypothesis, it appears that different combinations of indigenous bacteria, rather than just a single species, can produce the pathogenic potential necessary to cause progression from gingivitis to destructive periodontitis (Theilade 1986). Microbiological studies have revealed that some of the infections in periodontal pockets are multibacterial (Rodenburg et al. 1990; Moore et al. 1991; Söder et al. 1993; Colombo et al. 1998). On the other hand, according to the specific plaque hypothesis, one or several bacterial species cause the initiation and progression of destructive periodontal disease (Slots 1979; Socransky 1979; Socransky and Haffajee 1992; Loesche 1982) (Fig. 1.4). However, it can be found that not only an increase in the total microbial load (10^5–10^8 microorganisms), but, with a high probability, certain species, such as *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia* and *Treponema denticola*, are also major etiological agents in destructive periodontal disease (Van der Weijden et al. 1994). *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* fulfil, at least partly, the modified Koch's criteria for defining a periodontal pathogen (Haffajee and Socransky 1994).

Plaque accumulation leads to gingivitis, but the shift to periodontitis depends on both host factors and the selection of virulent bacteria. Periodontitis is not a single disease, but rather a collection of pathologies with similar patterns and symptoms. Though many classifications have been proposed, during the 1999 International Workshop for Classification of Periodontal Diseases and Conditions, the previously accepted terms "early-onset periodontitis" and "adult periodontitis" were replaced by "aggressive periodontitis" and "chronic periodontitis." Thus, age and microbiological features no longer represent the primary classification criteria, but rather, clinical behavior and laboratory findings are used to distinguish the two forms (Sbordone and Bartolaia 2003).

The common features of localized and generalized forms of *Aggressive Periodontitis* are (Armitage 1999):

- Healthy patients, except for the presence of periodontitis.
- Rapid attachment loss and bone destruction.
- Familial aggregation.
- Secondary features that are generally, but not universally present are:
- Amounts of microbial deposits inconsistent with the severity of periodontal tissue destruction.
- Elevated proportions o*f Aggregatibacter actinomycetemcomitans* and, in some populations, *Porphyromonas gingivalis*.
- Phagocyte abnormalities.
- Hyper-responsive macrophage phenotype, including elevated levels of PGE_2 and IL-1β.
- Progression of attachment loss and bone loss may be self-arresting.

Generally the term "*chronic periodontitis*" replace the term "adult periodontitis" (Armitage 1999). The chronic periodontitis is defined as an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment, and bone loss. It is

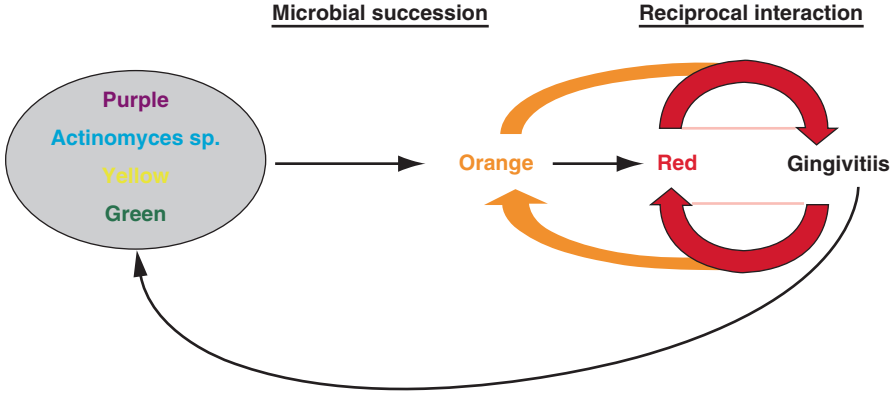

Fig. 1.4 *A hypothesized relationship between the addition of species during microbial succession leading to the development of gingival inflammation*. In turn, the increased inflammation would result in increased growth of colonizing species (Socransky and Haffajee 2005) (with permission from Wiley-Blackwell Publishing)

characterized by pocket formation and/or gingival recession. It is recognized as the most frequently occurring form of periodontitis. Its onset may be at any age, but is most common in adults. The prevalence and severity of the disease increase with age. It may affect a variable number of teeth and it has variable rates of progression.

The microbiota of slight chronic periodontitis in adults and adolescents has been associated with *Porphyromonas gingivalis* and *Tannerella forsythia,* using rapid immunofluorescence (Riviere et al. 1996; Clerehugh et al. 1997; Hamlet et al. 2004), PCR (Tanner et al. 2007) and DNA probe methods (Tran et al. 2001). In a longitudinal study to detect progressing slight periodontitis, a combination of anaerobic culture and DNA hybridization assays associated *Tannerella forsythia, Campylobacter rectus, Selenomonas noxia* and *Prevotella intermedia* with inter-proximal progressing slight (initial) chronic periodontitis, compared with health or gingivitis (Tanner et al. 1998, 2006).

The major species associated with moderate and advanced chronic adult periodontitis were originally detected using cultivation-based methods and include *Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Treponema denticola* and *Aggregatibacter actinomycetemcomitans* (Moore et al. 1991; Kamma et al. 1995; Haffajee et al. 1998; Mombelli et al. 1998; Machtei et al. 1999; van Winkelhoff et al. 2002; Dogan et al. 2003; Kumar et al. 2003; Socransky and Haffajee 2005; Tanner et al. 2007). More recently, the range of bacterial species detected in periodontitis has expanded following the use of noncultural molecular techniques (Kroes et al. 1999; Sakamoto et al. 2000; Paster et al. 2001; Kumar et al. 2005; Aas et al. 2007) and included additional periodontitis associated species: *Filifactor alocis, Porphyromonas endodontalis, Eubacterium saphenum, Eubacterium nodatum* in addition to not-yet cultivated phylotypes (Kumar et al. 2003; Dahlen and Leonhardt 2006; Haffajee et al. 2006 a,b; Tanner et al. 2006).

The composition of the bacterial population in the active, destructive phase differs slightly from that during the remission period, adding support to the theory of the high specificity of pathogenic plaque; a preponderance of *Tannerella forsythia, Porphyromonas gingivalis, Treponema denticola, Campylobacter rectus, Prevotella intermedia* is associated with increasing probing depth and bleeding on probing (Sbordone and Bartolaia 2003; Tanner et al. 2007).

1.1.2.4 Periodontal Pathogens Associated with Peri-Implantitis

Peri-implantitis, i.e., chronic progressive marginal infection, is defined as an inflammatory reaction affecting the tissues surrounding osseointegrated dental implants resulting in loss of supporting bone (Mombelli and Lang 1998; Esposito et al. 1999). It has also been described as "a site-specific infection yielding many features in common with chronic adult periodontitis" (Hultin et al. 2002). Peri-implantitis can be considered the "twin-sister" of periodontitis, even though some important differences between natural teeth and dental implants must clearly be borne in mind, the most important being that implants are not surrounded by a periodontal ligament and therefore, present different biomechanics and defensive cell recruitment (Sbordone and Bartolaia 2003). More factors can be associated with biological failures of oral implants: medical status of the patient, smoking, bone quality, bone grafting, irradiation therapy, parafunctions, operator experience, degree of surgical trauma, bacterial contamination, lack of preoperative antibiotics, immediate loading, nonsubmerged procedure, number of implants supporting a prosthesis, implant surface characteristics and design (Esposito et al. 1998a, b).

The colonization of the implant sulcus is different in partially edentulous patients in comparison to fully edentulous patients. Early bacterial colonization of peri-implant pockets in *edentulous subjects* is characterized by an increase of facultative anaerobic streptococci, whereas gram-negative strict anaerobic rods are usually isolated infrequently in low proportions (Mombelli et al. 1988; van Winkelhoff et al. 2000). Long-term results on colonization of the peri-implant area showed a decrease in the proportions of facultative *streptococci* and an increase in the percentage of gram-positive facultative rods and gram-negative strict anaerobic rods, e.g., *fusobacterium* spp. and *prevotella* spp. (Mombelli and Mericske-Stern 1990; van Winkelhoff et al. 2000). Peri-implant infection in edentulous subjects is associated with bacteria that are found in adult periodontitis, however, with the exception of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* (Mombelli et al. 1987; van Winkelhoff et al. 2000).

In contrast to fully edentulous patients, colonization of peri-implant pockets in *partially edentulous patients* is characterized by rapid appearance of *spirochetes*. Samples from partially edentulous subjects also contained more black-pigmenting gram-negative

anaerobes than samples from fully edentulous subjects (Mombelli 2002). Takanashi et al. (2004) investigated the colonization by black-pigmented anaerobic bacteria that occurs between the time before fixture installation and 6 months after inserting superstructures in implant treatment in partial edentulous cases. Dental plaque was serially collected from around the natural teeth and implants in 12 patients in whom a dental implant was indicated, and *Porphyromonas gingivalis* and *Prevotella intermedia* were detected using polymerase chain reaction. One month after connecting the abutment, the detection rate of *Porphyromonas gingivalis* per site from around the implants was 63.7% and that of *Prevotella intermedia* was 50.8%. Six months after superstructure setting, the detection rate per site of *Porphyromonas gingivalis* from around the implants was 56.8% and that of *Prevotella intermedia* was 41.1%. When chromosomal DNA segmentation patterns in the isolated *Porphyromonas gingivalis* and *Prevotella intermedia* were compared using pulsed field gel electrophoresis (PFGE), the patterns in the natural teeth were in accordance with those in the implants in three of four cases (75.0%) in *Porphyromonas gingivalis,* and all cases in *Prevotella intermedia*. Similar findings were obtained by Koka et al. (1993), Kohavi et al. (1994) and Leonhardt et al. (1993), suggesting that bacterial colonization around implants occurred early after the implant region was exposed to the intraoral cavity and that the bacteria were transmitted from the area around the natural teeth. *Aggregatibacter actinomycetemcomitans* and *Actinomyces viscosus* were, however, more frequent in the supragingival plaque of teeth than of implants.

The occurrence of peri-implantitis may be dependent on distinct individual susceptibility factors, e.g., immuno inflammatory factors, interacting with molecular processes that are similar to periodontitis. Hence, it is important to ascertain *whether patients with an increased susceptibility to periodontitis would have an increased susceptibility to peri-implantitis and implant loss* (i.e., decreased survival or success rate of implants), even in partially dentate patients who have been treated for periodontitis. This is relevant because periodontitis is one of the leading causes of tooth loss, and dental implants are increasingly used to replace missing teeth in such patients. Consequently, a history of past periodontitis may act as a prognostic factor for the future survival and success of dental implants (Ong et al. 2008).

Conversely, there are some studies that have shown successful osseo-integration in patients with different types of periodontitis (Nevins and Langer 1995; Ellegaard et al. 1997; Quirynen et al. 2001). However, in a long term study, Karoussis et al. (2003) demonstrated lower survival rates and more biological complications, than patients with implants replacing teeth lost due to reasons other than periodontitis, during a 10-year maintenance period. Three systematic reviews were performed to determine implant outcomes in partially dentate patients who have been treated for periodontitis compared with periodontally healthy patients. Van der Weijden et al. (2005) concluded that the outcome of implant therapy in periodontitis patients may be different compared with individuals without such a history in terms of loss of supporting bone and implant loss. Schou et al. (2006) revealed that the survival of the supra-structures and the implants was not significantly different in individuals with periodontitis-associated and nonperiodontitis-associated tooth loss. However, significantly increased incidence of peri-implantitis and significantly increased peri-implant marginal bone loss were revealed in individuals with periodontitis associated tooth loss. More recently, Ong et al. (2008) reported that there is some evidence, that patients treated for periodontitis may experience more implant loss and complications around implants than nonperiodontitis patients. Evidence was stronger for implant survival than implant success. This is probably caused by the presence of periodontal pockets that serve as a reservoir for these bacteria (van Winkelhoff et al. 2000).

Microbiological studies of dental implants with clinically healthy marginal peri-implant tissues (Lee et al. 1999; Hultin et al. 2002; Renvert et al. 2007) have demonstrated a scattered, sub-mucosal microbiota dominated by facultative gram-positive cocci and rods. In contrast, a peri-implant pocket of *diseased implants* seems to harbor a microbiota similar to that found in periodontal disease, such as *Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens, Tannerella forsythia, Campylobacter rectus* and *Aggregatibacter actinomycetemcomitans*, especially serotype b (Mombelli et al. 1987; Mombelli 2002; Tanner et al. 1997; Shibli et al. 2008; Quirynen et al. 2002). Organisms not primarily associated with periodontitis, such as *staphylococcus* spp., *enterics* and *candida* spp. have also been found in peri-implant infection (Leonhardt et al. 1999).

It seems that proper periodontal infection control may help to prevent early bacterial complications in implant dentistry. Infection control should involve suppression of commensal periodontal bacteria below certain thresholds and elimination of putative exogenous periodontal pathogens, i.e., *Porphyromonas gingivalis*. This may be of special importance in patients with a history of periodontitis. Microbiological testing in partially edentulous subjects with a history of periodontitis may be one measure to prevent peri-implantitis by employing appropriate antimicrobial therapy before placing the dental implants (van Winkelhoff et al. 2000).

Such findings have relevance for the planning of immediate postextraction implants, especially if tooth loss is determined by periodontal disease. A wait of at least one month after extraction was suggested to allow for the elimination of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* from the extraction socket. The same rules apply when Guided Tissue Regeneration (GTR) and Guided Bone Regeneration (GBR) procedures are performed: membrane exposure and bacterial colonization impair the outcome in terms of tissue regeneration. Exposure is more likely in patients presenting periodontitis, peri-implantitis or residual deep pockets: the smallest degree of attachment and bone gain occur when *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Tannerella forsythia* and *capnocitophaga* spp. are detected on the infected barriers. It can be concluded that implant and GTR/GBR procedures achieve the best results in those subjects that comply with domestic plaque control routines and maintenance protocol schedules (Sbordone and Bortolaia 2003).

1.1.3 Dental Plaque Formation

The formation of bacterial plaque is initiated by the adhesion of micro-organisms to the tooth surface, and is the first step in the development of periodontal infections (Newman et al. 1978).

Until now, no uniform theory has been developed to explain the fundamental mechanisms of cell adhesion. Moreover, it would be impossible and erroneous to conclude that one single mechanism dictates the adhesive tendency of microorganisms because the situation is too complex (Quirynen and Bollen 1995).

The process of plaque formation can be divided into several phases:

1.1.3.1 Adsorption of Host and Bacterial Molecules to the Tooth Surface

This conditioning film (*the acquired pellicle*) forms immediately following eruption or cleaning and directly influences the pattern of initial microbial colonization (Marsh 2004). Dental pellicles mediate many of the interactions that take place at intraoral surfaces. The term pellicle is used to describe a thin, continuous membrane or cuticle, composed primarily of salivary components deposited on a cleaned tooth surface (Al-Hashimi and Levine 1989). All surfaces of the oral cavity, including all tissue surfaces (Bradway et al. 1989) as well as surfaces of teeth-enamel (Al-Hashimi and Levine 1989), cementum (Fisher et al. 1987) and fixed, and removable restorations (Edgerton and Levine 1992; Edgerton et al. 1996) are coated by dental pellicle.

Pellicles contain salivary components, constituents from gingival crevicular fluid, microbial, and cellular sources (Scannapieco 1995). Enamel pellicle formation is driven by a combination of physical forces (ionic, hydrophobic, hydrogen loading and van der Waals) between molecules in saliva and the tooth surface (Scannapieco et al. 1995).

To study the acquired enamel pellicle, it is convenient to examine the freshly extracted teeth (Listgarten 1976) or by placing plastic strips or epoxy crowns in the oral cavity as analogs to the tooth (Brecx et al. 1981; Scannapieco 1995).

Early pellicle, formed within 2 h, contains both proteins and glycoproteins. Pellicle contains components of salivary origin, like mucins (Kajisa et al. 1990; Fisher et al. 1987; Al-Hashimi and Levine 1989), α-amylase (Al-Hashimi and Levine 1989; Scannapieco et al. 1995), s-IgA (Al-Hashimi and Levine 1989; Orstavik and Kraus 1973), lysozyme (Orstavik and Kraus 1973), cystatins (Al-Hashimi and Levine 1989), proline-rich proteins (PRPs) (Bennick 1987), as well as albumin originating from gingival crevicular fluid, (Al-Hashimi and Levine 1989; Kajisa et al. 1990; Edgerton and Levine 1992), and bacterial products such as the glucosyltransferase *of Streptococcus mutans* (Schilling and Bowen 1992).

1.1.3.2 Passive Transport of Oral Bacteria to the Tooth Surface

Weak, long-range physicochemical interactions between the microbial cell surface and the pellicle-coated tooth create a weak area of net attraction that facilitates reversible adhesion. Subsequently, strong, short-range interactions between specific molecules on the bacterial cell surface (adhesions) and complementary receptors in the pellicle can result in irreversible attachment and can explain microbial tropisms for surfaces. Some of the adhesions that have been identified on subgingival species include fimbriae (Cisar et al. 1984; Sandberg et al. 1988) and cell- associated proteins (Socransky and Haffajee 1992). Adhesions are often lectins which bind to saccharide receptors, but some adhesions are thought to bind to proteinaceous receptors (Gibbons 1989). *Receptors on tissue surfaces* include galactosyl residues, sialic acid residues (Murray et al. 1986), proline-rich proteins or statherin and Type I and IV collagen (Socransky and Haffajee 1992). Oral bacteria generally possess more than one type of adhesion on their cell surface and can participate in multiple interactions both with host molecules and similar receptors on other bacteria (coadhesion) (Marsh 2004; Quirynen and Bollen 1995).

1.1.3.3 Coadhesion of Later Colonizers to Already Attached Early Colonizers

This stage also involves specific interbacterial adhesion-receptor interactions (often involving lectins) and leads to an increase in the diversity of the biofilm and to the formation of unusual morphological structures, such as corn-cobs and rosettes (Marsh 2004; Kolenbrander 2000). *Coaggregation* (interactions between the suspended micro-organisms in a fluid phase) between oral microbial pairs as well as its role in the sequential colonization of the tooth surface has been studied extensively (Kolenbrander et al. 1994; Cisar et al. 1997). However, *coadhesion* (interactions between suspended and already -adhering microorganism to a surface) may well be equally important (Bos et al. 1996). Bacteria engage in a range of antagonistic and synergistic biochemical interactions (Marsh and Bradshaw 1995). The efficiency of metabolic interactions among bacteria in food chains may be enhanced if they are brought into close physical contact. Likewise, the coadhesion of obligate anaerobic bacteria to oxygen-consuming species can ensure their survival in overt aerobic oral environments (Marsh 2004).

The analysis of the coaggregation profiles of hundreds of subgingival isolates has provided evidence that *coaggregation* might be important for subsequent plaque development. Certain *streptococci* (for example, *Streptococcus oralis*), which bear receptors are coaggregation partners of members of several genera. Early colonizing partners of receptor-bearing streptococci include *Streptococcus gordonii, Actinomyces naeslundii, Eikenella corrodens, Veillonella atypica, Prevotella loescheii* and *Haemophilus parainfluenzae*, as well as *Capnocytophaga ochracea*. It is worth noting that these coaggregating partners of the initial colonizing *Streptococcus oralis, Streptococcus sanguinis* and *Streptococcus mitis* are almost all gram-negative, which correlates with the 40-year-old reports of a temporal shift from gram-positive to gram-negative bacterial flora. The dominant species in initial dental plaque were *Streptococcus oralis* that are receptor-bearing cells, indicating that receptor-bearing *streptococci* are an abundant surface readily available for recognition by gram-negative bacteria expressing complementary adhesions which recognize receptor polysaccharides. Possibly, receptor polysaccharides on the early colonizing streptococci are a prerequisite for the shift from gram-positive to gram-negative flora accompanying the shift from health to gingivitis (Kolenbrander et al. 2006) (Fig. 1.5).

1.1.3.4 Multiplication of the Attached Micro-Organisms

Cell division leads to confluent growth and, eventually, a three-dimensional spatially and functionally organized, mixed-culture biofilm. Polymer production results in the formation of a complex extracellular matrix made up of soluble and insoluble glucans, fructans and heteropolymers. Such a matrix is a common feature of biofilms and makes a significant contribution to the known structural integrity and general resistance of biofilms; the matrix can be biologically active and retain nutrients, water and key enzymes within the biofilm. Endogenous substrates (derived from saliva or gingival crevicular fluid) are the main source of nutrients for oral bacteria, but their catabolism requires the concerted and sequential action of groups of microbes

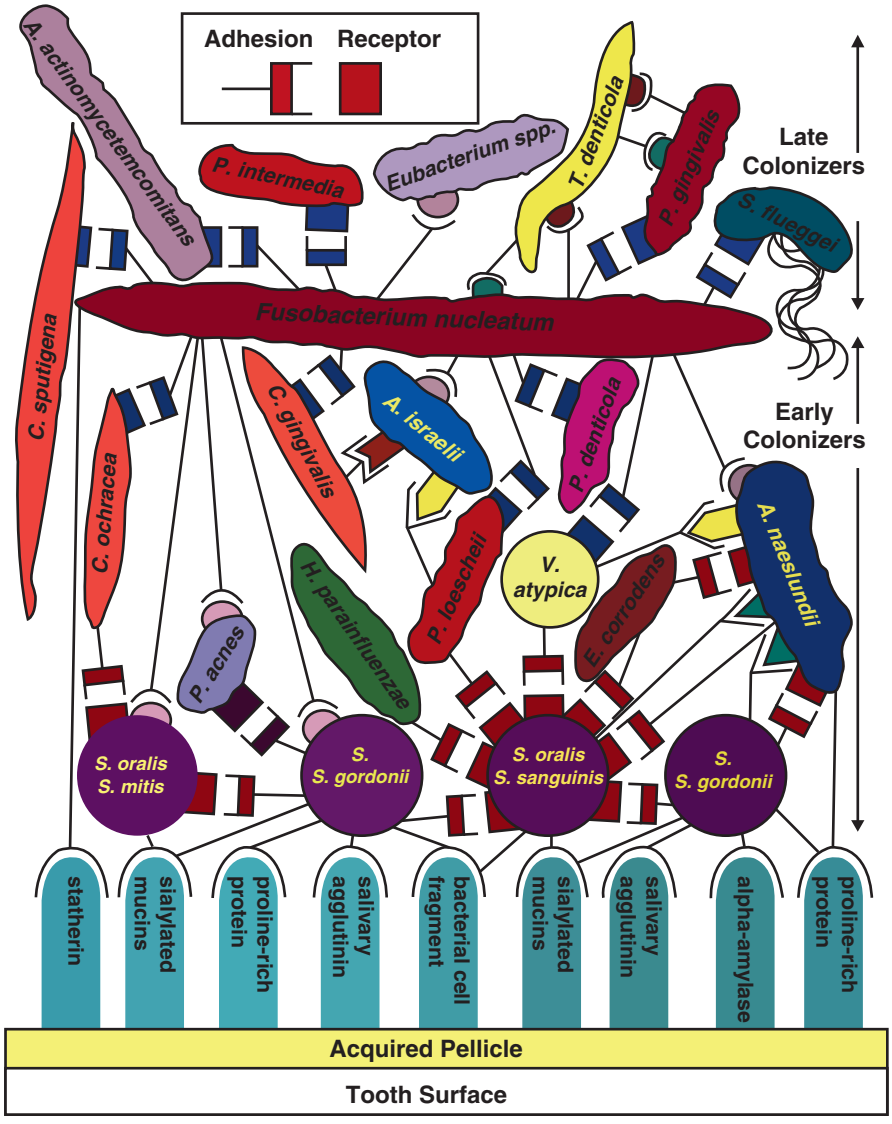

Fig. 1.5 *Spatiotemporal model of oral bacterial colonization, showing recognition of salivary pellicle receptors by early colonizing bacteria, and coaggregations between early colonizers, fusobacteria, and late colonizers of the tooth surface*. Each coaggregation depicted is known to occur in a pairwise test. Collectively, these interactions are proposed to represent the development of dental plaque. Starting at the bottom, primary colonizers bind via adhesions (*round-tipped black line symbols*) to complementary salivary receptors (*blue–green vertical round-topped columns*) in the acquired pellicle coating the tooth surface. Secondary colonizers bind to previously bound bacteria. Sequential binding results in the appearance of nascent surfaces that bridge with the next coaggregating partner cell. Several kinds of coaggregations are shown as complementary sets of symbols of different shapes. One set is depicted in the box at the top. Proposed adhesins (symbols with a stem) represent cell-surface components that are heat inactivated and protease sensitive; their complementary receptors (symbols without a stem) are unaffected by heat or protease. Identical symbols represent components that are functionally similar but may not be structurally identical. Rectangular symbols represent lactose-inhibitable coaggregations. Other symbols represent components that have no known inhibitor. The bacterial species shown are *Actinobacillus actinomycetemcomitans, Actinomyces israelii, Actinomyces naeslundii, Capnocytophaga gingivalis, C. ochracea, C. sputigena, Eikenella corrodens, eubacterium* spp., *Fusobacterium nucleatum, Haemophilus parainfluenzae, Porphyromonas gingivalis, Prevotella denticola, P. intermedia, P. loescheii, Propionibacterium acnes, Selenomonas flueggei, Streptococcus gordonii, S. mitis, S. oralis, S. sanguinis, treponema* spp., and *Veillonella atypical* (Kolenbrander et al. 2006) (with permission from Wiley-Blackwell Publishing)

with complementary enzyme profiles, i.e., plaque functions as a true microbial community (Marsh 2004).

1.1.3.5 Active Detachment

Once established, the resident plaque microflora remains relatively stable over time and is of benefit to the host. The resident microflora of all sites plays a critical role in the normal development of the physiology of the host and also reduces the chance of infection by acting as a barrier to colonization by exogenous (and often pathogenic) species ("colonization resistance"). Mechanisms contributing to colonization resistance include more effective competition for nutrients and attachment sites, the production of inhibitory factors, and creation of unfavorable growth conditions by the resident microflora. Thus, treatment should attempt to control rather than eliminate the plaque microflora (Marsh 2004).

1.1.4 Impact of Surface Characteristics and/or Surface Topography on Biofilm Development

Bacterial accumulation on dental materials is determined by various surface characteristics. The adhesion of bacteria is significantly affected by *high surface roughness* values because of a reduction of shear forces on initially attaching bacteria. The impact of surface roughness on the biofilm formation can be explained by several factors:

- The initial adhesion of bacteria preferably starts at locations where they are sheltered against shear forces, so that they find time to change from reversible to irreversible attachment.
- Roughening of the surface increases the area available for adhesion by a factor 2–3.
- Rough surfaces are difficult to clean, resulting in a rapid regrowth of the biofilm by the multiplication of remaining species, rather than by recolonization (Teughels et al. 2006).

Materials with *high surface free energy* values are known to increase adhesion of bacteria (An and Friedman 1998; Taylor et al. 1998). Furthermore, the *bacterial adhesion process is influenced by the chemical composition of the material, surface hydrophobicity, and the zeta potential*. (An and Friedman 1998; Taylor et al. 1998; Quirynen and Bollen 1995; Carlen et al. 2001).

1.1.5 Bacterial Colonization on Tooth Surfaces and Dental Materials

Differences in the amount of adherent plaque are observed in various materials (Siegrist et al. 1991) and tissues (Nyvad and Fejerskov 1987; Carrassi et al. 1989).

The pattern of microbial colonization in vivo is determined by the surface structure of the *tooth;* on enamel surfaces the first bacteria appeared in pits and surface irregularities followed by proliferation along the perikymata, while on root surfaces bacterial colonization is characterized by a haphazard distribution (Nyvad and Fejerskov 1987). It was also observed that within the initial 24-h period, root surfaces were more heavily colonized than were enamel surfaces (Nyvad and Fejerskov 1987).

Different types of soft mucosal and hard dental surfaces may constitute various prerequisites for bacterial colonization (Gibbons 1989).

There are also more ecological differences in the supra- and subgingival environment which are of importance when bacterial adhesion is considered. Supragingivally, bacteria can adhere to the enamel surface or, to a lower extent, to the desquamating oral epithelium. Subgingivally, more niches are available for bacterial survival: adhesion to the root cementum, adhesion to the desquamating pocket epithelium, swimming in the crevicular fluid, invasion in the soft tissue or invasion into the hard tissue via the dentine tubules (Quirynen 1994).

The ultrastructural pattern of early plaque formation was studied on various *dental materials*: amalgam, casting alloys, titanium, ceramics, glass polyalkenoate cement, composite resins, unfilled resins, and bovine enamel (Hannig 1999). Because only less pronounced variations could be detected in the ultrastructural appearance of the early plaque formed on the different material surfaces, it was concluded that early plaque formation on solid surfaces is influenced predominantly by the oral environment rather than by material-dependent parameters. These findings may be ascribed to the presence of the pellicle layer, which apparently

masks any difference among materials, with regard to surface properties and biocompatibility.

Similar results were obtained by Leonhardt et al. (1995) who evaluated qualitative and quantitative differences in bacterial colonization on titanium, hydroxyl-apatite, and amalgam surfaces in vivo. No significant differences among the materials regarding colonization of investigated bacteria were found during the study period.

The different composition of materials only slightly affects plaque colonization. The amount of the early plaque colonization seems to be related more to the roughness degree than to material composition (Siegrist et al. 1991).

Materials used for dental restorations may also have antibacterial properties *per se*. Several studies have shown that amalgam alloys have a bacteriostatic effect (Glassman and Miller 1984). Titanium has been shown to inhibit plaque growth in vitro, particularly in the early stages, probably due to the antimicrobial effect of metal ion release (Joshi and Eley 1988).

Fixed or removable orthodontic appliances also impede the maintenance of oral hygiene, resulting in plaque accumulation (Batoni et al. 2001; Jordan and LeBlanc 2002). Plaque retention surrounding orthodontic appliances leads to enamel demineralization caused by organic acids produced by bacteria in the dental plaque (Arends and Christofferson 1986; O'Reilly and Featherstone 1987). Recently, fluoride-releasing elastomeric modules (Wiltshire 1999; Banks et al. 2000; Mattick et al. 2001) and chlorhexidine varnish (Beyth et al. 2003) were suggested for reducing plaque accumulation and decalcification (Türkkahraman et al. 2005).

Fixed orthodontic appliances create new retention areas, which are suitable for bacterial colonization and lead to an increase in the absolute number and percentage of *Streptococcus mutans* and *lactobacilli* (Türkkahraman et al. 2005; Forsberg et al. 1991; Balenseifen and Madonia 1970; Corbett et al. 1981; Mattingly et al. 1983; Scheie et al. 1984; Diamanti-Kipioti et al. 1987; Lundström and Krasse 1987; Sinclair et al. 1987; Svanberg et al. 1987; Rosenbloom and Tinanoff 1991; Chang et al. 1999). As revealed by Türkkahraman et al. (2005) and Faltermeier et al. (2008), a lot of studies have evaluated the effect of fixed orthodontic appliances on microbial flora and periodontal status (Balenseifen and Madonia 1970; Corbett et al. 1981; Scheie et al. 1984; Sinclair et al. 1987; Rosenbloom and Tinanoff 1991; Chang et al. 1999; Pender 1986; Huser et al. 1990; Glans et al. 2003), but their sample sizes were relatively low, and generally no additional periodontal evaluation was performed.

Osseo-integrated titanium dental implants have been proven to provide highly reliable restoration of function in totally and partially edentulous patients (Adell et al. 1990). One of the most important causes of dental implant failure appears to be bacterial plaque colonization (Mombelli and Lang 1994). The microbiology of failing implants seems to be similar to that of the natural dentition in advanced stages of adult periodontitis (Becker et al. 1990).

Bacterial colonization seems to be promoted by surface-free (Van Dijk et al. 1987), roughness (Quirynen and Bollen 1995) and the presence of specific molecules adsorbed from the saliva onto the titanium surface (Wolinsky et al. 1989).

The titanium surface with $Ra < 0.088$ mm and $Rz < 1.027$ mm strongly inhibits accumulation and maturation of plaque at the 24-h time period (Rimondini et al. 1997).

The sequential appearance of microbial morphotypes during maturation of supra- and subgingival plaque on natural tooth structure (enamel and cementum surfaces) and implant materials (titanium and plasma spray-coated titanium and hydroxyl-apatite) showed similar results regardless of the surface (Gatewood et al. 1993). In both supra and subgingival plaque, depending on time interval, cocci, rods of various lengths, filamentous organisms, fusiforms, *spirochetes* and corn cob formation were observed.

It has to be noted that the microflora of implants in partially and totally edentulous mouths differ (Bauman et al. 1992). It is also suggested that implants in edentulous mouth have less chance of peri-implant infection than those in the partially edentulous mouth (Gatewood et al. 1993).

Bacterial colonization of both bio-resorbable and non-resorbable *periodontal membranes* used in guided tissue regeneration surgery has been extensively reported in the literature (Zucchelli et al. 1997, 1998; Nowzari et al. 1995; Tempro and Nalbandian 1993; Selvig et al. 1990; DeSanctis et al. 1996; Grevstad and Leknes 1993).

A negative relationship was observed between the amount of microorganisms present on the membrane surfaces and the clinical attachment gain following surgical procedure (Nowzari et al. 1995). It is conceivable that early bacterial accumulation on membrane materials can prevent fibrin organization on the membrane and thus reduce its integration with connective

tissue and consequently the outcome of surgery (Zucchelli et al. 1997). The exposure of bio-absorbable membranes may also reduce the capability of the body to resorb the material (DeSanctis et al. 1996).

Systemic antibiotics and local application of chlorhexidine do not prevent bacterial colonization of exposed membranes (DeSanctis et al. 1996).

Quantitative differences in early plaque accumulation on various membranes (expanded polytetrafluoroethylene, polyglactin 910 and polylactic acid) seem to be related to the textural and structural characteristics of the surface, which is not adequately represented by the surface Ra value measured with a profilometric instrument (Zucchelli et al. 1998). The 4-h results indicated a statistically significant difference in the proportion of bacteria-positive fields among the three membranes; a greater amount of bacteria was demonstrated on the ePTFE membrane compared to the other two membranes. At 24 h, the difference in the proportion of bacteria-positive fields was statistically significant; a lesser amount of bacterial plaque was present on the polylactic acid membrane compared to the ePTFE and polyglactin 910 membranes. No difference in the proportion of rod/bacteria-positive fields was demonstrated among the three membranes at either 4 or 24 h. It was concluded that quantitative differences in early plaque accumulation on various membranes seem to be related to the textural and structural characteristics of the surface. (Zucchelli et al. 1998).

1.1.6 Assessment of Dental Plaque

A summary of most used dental plaque indices is presented in Table 1.2 (Fig. 1.6).

1.1.7 Baterial Plaque Models

Various *supragingival plaque biofilm models* have been employed for the study of plaque formation, structure and antimicrobial susceptibility. Guggenheim et al. (2001, 2004) have described a defined multispecies model designed to mimic the composition of the supragingival plaque and used this model to study the structure and antimicrobial susceptibility. Several investigators have utilized in-mouth splints in healthy subjects in which supragingival plaque formed over time on the splints (Auschill et al. 2001; Wood et al. 2000; Zaura-Arite et al. 2001). Wimpenny et al. (1999) have described several different laboratory biofilm models that make use of a constant depth film fermenter using a plaque inoculum. The constant depth film fermenter models have been used to study the structure (Netuschil et al. 1998; Pratten et al. 2000; Wood et al. 2000) and spatial distribution (Auschill et al. 2001; Hope et al. 2002) of viable and nonviable supragingival plaque bacteria.

Attempts to obtain realistic subgingival plaque biofilms have been made by placing various insert materials into the periodontal pockets of periodontitis patients and then analyzing the bacterial components that colonized the inserts (Takeuchi et al. 2004; Wecke et al. 2000). Recently, Hope and Wilson (2006) have described the development of subgingival plaque on hydroxyapatite disks in a constant depth film fermenter. This model used a plaque inoculum and reached a steady state after 4 days. Although this is an excellent model for the study of subgingival plaque structure and viability, the apparatus for maintaining an anaerobic constant depth film fermenter is somewhat complex.

Numerous biofilm models have been described for the study of bacteria associated with the supragingival plaque. However, there are fewer models available for the study of subgingival plaque. One major challenge in understanding bacterial interactions in *subgingival biofilms* is the acquisition of undisturbed samples in which spatial relationships between bacteria are maintained, and for which the orientation within the oral cavity is known. So far, an analysis of the subgingival microbiota relied on sampling of bacteria either by paper points or by mechanical debridement. Both sampling procedures, however, disrupt the organization of biofilms. However, it has been indicated recently by Teles et al. (2008) that the use of curettes provided a reliable and reproducible method to obtain subgingival samples.

Biofilm formation has also been extensively studied using in vitro models, like flow chambers or chemostats. However, these studies might not necessarily reflect the situation in a periodontal pocket, and clearly have limitations regarding fastidious and so far uncultured microorganisms. The only method to study subgingival plaque, available so far, required the extraction of teeth (Wecke et al. 2000). Such samples have been useful in pioneering studies that map subgingival plaque structure on a macro scale using immunohistochemical approaches. Electron microscopy has also been applied to these samples to

Table 1.2 A summary of most used plaque indices

Index, authors	Scoring criteria
Simplified oral hygiene index – oral debris (Greene and Vermillion 1964)	The surface area covered by debris is estimated by running the side of an explorer along the tooth surface being examined. The following scoring system is used *0* no debris or stain present *1* soft debris covering not more than one third of the tooth surface being examined or the presence of extrinsic stains without debris regardless of surface area covered *2* soft debris covering more than one third but not more than two thirds of the exposed tooth surface *3* soft debris covering more than two thirds of the exposed tooth surface
Plaque index (Ramfjord 1959)	The following teeth were selected as indicators of the periodontal condition within the dentition: maxillary right first molar 16, maxillary left central incisor 21, maxillary left first bicuspid 24, mandibular left first molar 36, mandibular right central incisor 41 and mandibular right first bicuspid 44. Record plaque after application of disclosing solution *P0* no plaque present *P1* plaque present on some but not on all of the interproximal and gingival surfaces of the tooth *P2* plaque present on all interproximal and gingival surfaces, but covering less than one half of the entire clinical crown *P3* plaque extending over all interproximal and gingival surfaces but covering more than one half of the entire clinical crown
Plaque index (Quigley and Hein 1962)	The examiner made a quantitative estimate of the amount of stained plaque on the buccal, labial and lingual surfaces of the teeth as shown below *0* no plaque present *1* flecks of stain at gingival margin *2* definite line of plaque at gingival margin *3* plaque extending on gingival third of surface *4* plaque extending on two thirds of surface *5 P*laque extending greater than two thirds of surface Evaluation of the data consisted of statistical analysis based on the average amount of plaque per tooth surface per person
Plaque index (Turesky et al. 1970)	*Disclosed plaque is scored from 0 to 5 for each facial and lingual nonrestored surface only of all the teeth except third molars, as follows. An index for the entire mouth was determined by dividing the total all plaque scores by the number surfaces examined* *0* no plaque present *1* separate flecks of plaque at the cervical margin of the tooth *2* a thin continuous band of plaque (up to 1 mm) at the cervical margin of the tooth *3* a band of plaque wider than 1 mm but covering less than one-third of the crown of the tooth *4* plaque covering at least one-third but less than two-thirds of the crown of the tooth *5* plaque covering two-thirds or more of the crown of the tooth
Plaque index (Silness and Löe 1964)	The teeth which were examined were: maxillary right first molar 16, maxillary right lateral incisor 12, maxillary left first bicuspid 24, mandibular left first molar 36, mandibular left lateral incisor 32, and mandibular right first bicuspid 44 Assessment of soft deposits was made according to the plaque index system *0* no plaque *1* a film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the tooth surface *2* moderate accumulation of soft deposits within the gingival pocket, or on the tooth and gingival margin which can be seen with the naked eye *3* abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin

Table 1.2 (continued)

Index, authors	Scoring criteria
	Each of the four surfaces of the teeth (buccal, lingual, mesial and distal) is given a score from 0 to 3, the plaque index for the area. The scores from the four areas of the tooth are added and divided by four in order to give the plaque index for the tooth. The indices for the teeth (incisors, premolars and molars) may be grouped to designate the index for the group of teeth. By adding the indices for the teeth and dividing by six, the index for the patient is obtained. The index for the patient is thus an average score of the number of areas examined
Plaque control record (O'Leary et al. 1972)	This index was developed to give the therapist, hygienist or dental educator a simple method of recording the presence of plaque of individual tooth surfaces (mesial, distal, facial, lingual). The form also allows the patient to visualize his own progress in learning plaque control. After rinsing the stained solution, each stained surface is examined with an explorer for soft accumulations at the dentogingival junction. When found, they are recorded by making a dash in the appropriate spaces on the record form. No attempt is made to differentiate between varying amounts of plaque on the tooth surfaces. After all the teeth are examined and scored, an index can be derived by dividing the number of plaque-containing surfaces by the total number of available surfaces. The same procedure is carried out at subsequent appointments to determine the patient's progress in learning and carrying out the prescribed oral hygiene procedures
Navy plaque index (Grossman et al. 1973; Hancock et al. 1977)	This index is one part of the navy periodontal screening examination. This system of scoring stained plaque is applied on six teeth shown to be representative of the total mouth condition: 16, 21, 24, 36, 41 and 44
	The tooth is separated into three major zones, the occlusal, the middle, and the gingival zone. By assigning all areas a score of one, more emphasis is placed on plaque adjacent the gingival tissues since the surface area is much smaller. The scoring is as follows:
	Area A, B, C score 1 a thin line of stained plaque approximately 1 mm or less adjacent to the gingival tissue, both facial and lingual
	Area D, E, F score 1 the stained plaque extends further into the gingival zone
	Area G and H score 1 the mesial and distal halves of the middle zone area, both facial and lingual
	Area I score 1 the occlusal area
	Score facial and lingual areas. The total score for each tooth is the sum total of all areas of stained plaque on that tooth
Rustogi et al. modified navy plaque index (Rustogi et al. 1992)	Disclosed plaque is scored in each tooth area as present (scored as 1) or absent (scored as 0) and recorded in each of the nine areas of the buccal and lingual tooth surfaces. The index assesses the amount of plaque in the tooth area bounded by the tooth contact, the free gingival margin, and mesial or distal line angles. The use of this new index enables the examiner to evaluate and record both the gumline (or marginal areas) and interproximal areas of the tooth, thus giving these anatomical areas an increased importance
New method of plaque scoring (NMPS) (Dababneh et al. 2002)	According to the NMPS, the visible facial or lingual surface of the tooth is divided horizontally into two major zones – the gingival one-third (zone A) and the remaining coronal two-thirds of the surface, T, which is further subdivided into three vertical thirds: mesial (zone B), distal (zone C) and middle (zone D). Each of zones A, B and C is given a score ranging from 0 to 3 depending on subjective evaluation of the proportional area, in thirds, of disclosed plaque on the relevant zone, i.e., *0* no plaque, *1* up to one-third coverage, *2* more than one-third and up to two-thirds coverage, *3* more than two-thirds coverage. The middle third zone, D, is scored on the basis of presence or absence of stained plaque as 1 or 0, respectively. This gives a score ranging from 0 to 10 per buccal or lingual surface. The aim of the NMPS was to emphasize the plaque scoring at the gingival and proximal regions of the tooth surface

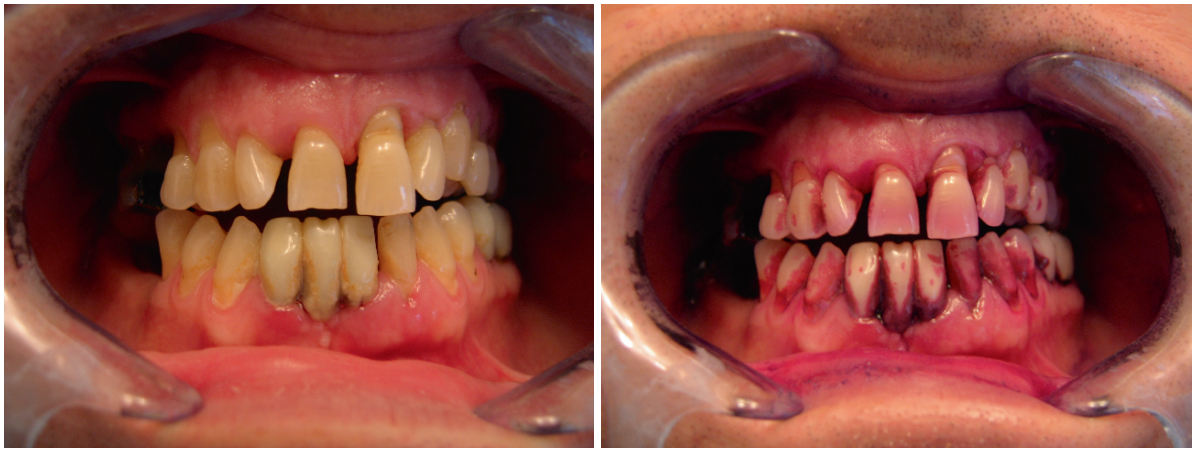

Fig. 1.6 Heavy deposits of dental plaque

examine the biofilm on a finer scale. Sectioning of samples prior to microscopy makes standard microscopy approaches cumbersome and time-consuming; the use of confocal microscopy would relieve investigators from the need to section their samples extensively prior to examination (Kolenbrander et al. 2006).

Walker and Sedlacek (2007) have developed and validated a model that closely mimicked the composition of the subgingival flora: calcium hydroxylapatite disks were coated overnight with 10% sterile saliva, placed in flat-bottomed tissue culture plates containing trypticase-soy broth, directly inoculated with a small aliquot of dispersed subgingival plaque, incubated anaerobically, and transferred to fresh medium at 48-h intervals until climax (steady-state) biofilms were formed (approximately 10 days). The biofilm model obtained closely reproduces the composition of the cultivable subgingival plaque, both, in the species present and in their relative proportions, and was also used for testing resistances in subgingival biofilm communities to antibiotics, commonly used as adjuncts to periodontal therapy: tetracycline, doxycycline, minocycline, amoxicillin, metronidazole, amoxicillin/clavulanate, and amoxicillin/metronidazole (Sedlacek and Walker 2007).

Attempts to obtain realistic subgingival plaque biofilms have been made by placing various insert materials into the periodontal pockets of periodontitis patients and then analyzing the bacterial components that colonized the inserts (Takeuchi et al. 2004; Wecke et al. 2000) (Fig. 1.7). The noninvasive human model system, described by Wecke et al. (2000) included *a small rod surrounded by a plastic membrane that is inserted into the periodontal sulcus* of a human volunteer. The carrier was fixed supragingivally to the tooth surface by using cyanoacrylic glue. The construction was guided by the assumption that the carrier positioned in the pocket might be colonized from both, the tooth and the soft tissue side. After 3 or 6 days of exposure, carriers were removed from the periodontal pockets. Only those carriers that kept their stable position during the exposure period can be used for further investigations. After removal, the membrane is embedded and minimally sectioned prior to examination by confocal microscopy or electron microscopy. Fluorescence in situ hybridization has been used to stain these samples, but other approaches, such as immunofluorescence, are also possible. Knowledge of sample orientation allowed these investigators to conclude that *spirochetes* and gram-negative bacteria predominated in deeper regions of the pocket, whereas streptococci were abundant in the shallow regions.

Another noninvasive human model system, such as the retrievable *enamel chip model* carried in intraoral acrylic stents has been described by Palmer et al. (2001b, 2003). Briefly, enamel pieces (2 by 2 by 1 mm [length by width by thickness]) were cut from extracted, unerupted human third molars. Chips were cleaned in an ultrasonic bath (1510; Branson; Danbury, Conn.) for 20 min, sterilized with ethylene oxide, and affixed in custom-fabricated acrylic stents using red dental wax. Two bilateral mandibular stents (spanning the posterior buccal surface from the first premolar to first molar), each of which contained three chips, were worn by each volunteer. In certain experiments, visible plaque was first removed and teeth were polished prior to stent insertion (prophylaxis). In certain experiments, a series of 30-s sucrose rinses (20 mL of filter-sterilized

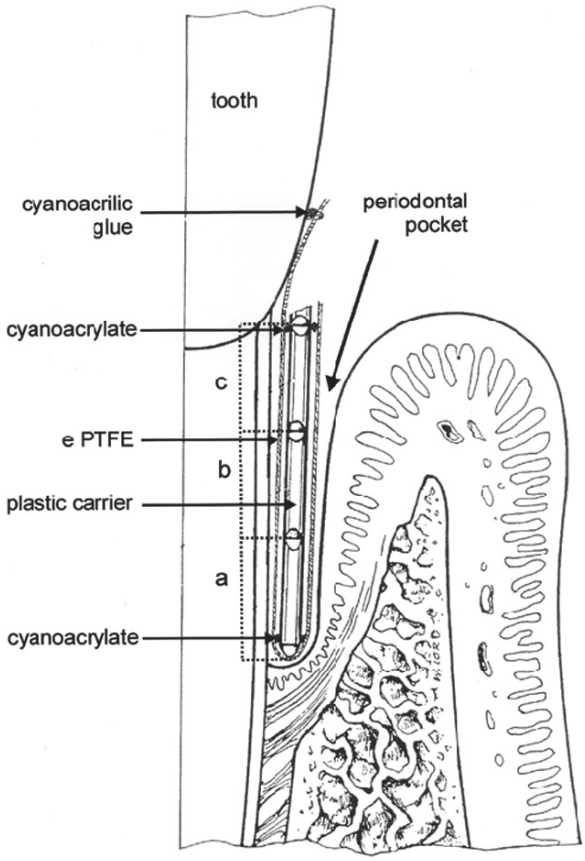

Fig. 1.7 Positioning of the e-PTFE carrier in a periodontal pocket; gold foil was inserted accordingly. (modified from Wecke et al. 2000) (with permission from Wiley-Blackwell Publishing)

10% sucrose) took place at 90-min intervals, beginning immediately after stent insertion. One stent was worn for 4 h and the other for 8 h. The appliance is removed at the proper time and the enamel chips are retrieved and processed for microscopy without disturbance to spatial relationships within the native biofilm. A second method for mounting enamel pieces or other materials may also be employed, in which a removable orthodontic appliance is fitted to each subject (Removable Orthodontic Retainer Method). In this case, after formation of a continuous bacterial layer on the material of choice, the pieces are mounted in Registration Material, which is integrated with the appliance as before, leaving only the facial surface of the enamel piece exposed. Four pieces located just facial to the upper first molars and premolars are carried per appliance. These are relatively short-term experiments, because the enamel pieces are on a removable appliance and eating is not possible for the subjects unless the retainer is stored in a holding medium while the subjects eat (Palmer et al. 2001a).

Wood et al. (2000) have described an in situ device for the in vivo generation of intact dental plaque biofilms on natural tooth surfaces in human subjects. Briefly, two suitable free buccal surfaces were chosen on the first or second upper molars. The enamel surface was lightly etched with 10% maleic acid for 15 s, rinsed with water, and dried. A drop of adhesive was placed on the etched surface, and the devices were bonded into place by means of Herculite composite resin. Devices were left in place for 4 days and were then debonded by means of an orthodontic bracket remover so that the rings would be retained intact with the plaque in situ. Immediately upon removal from the mouth, the intact, undisturbed biofilms were imaged by the noninvasive technique of confocal microscopy in both reflected light and fluorescence mode (Wood et al. 2000).

Short-term plaque regrowth studies are perhaps the most commonly used clinical experiments to screen chemical oral hygiene products. They have the advantage of assessing the chemical action of the formulation divorced from the indeterminate variable of toothbrushing. Typically, plaque regrowth from a zero baseline under the influence of the test agent is recorded. Originally used for mouthrinses, the method has been modified for toothpaste by delivering the formulation in a tray applied to the teeth, or as a slurry rinse. Studies are usually crossover, following many formulations to be evaluated against suitable controls. Study periods range from 24 h to several days, usually 4–5 days. A negative control or placebo, such as water, and a positive control, such as chlorhexidine, may be used. These help to position the activity of the test formulations between the extremes. Also, because the results from these controls can be predicted, their use tends to confirm, or otherwise, the conduct of these blind randomized study designs (Addy 1995; Chilton and Fleiss 1986).

1.2 Dental Calculus

1.2.1 Dental Calculus: Localization

Calculus is mineralized by dental plaque which forms both above (supragingival) and below (subgingival) the gumline. Once highly mineralized, calculus can become

cement-like, in terms of both physical hardness (Vickers Hardness averaging 30–40 U, maximum observed = 190) and adhesive strength. From a topographical point of view, calculus is classified into two categories: supragingival and subgingival calculus. While the substrate for subgingival calculus is limited to root surfaces, supragingival calculus can occur on enamel surfaces, dentin or cementum, depending upon exposure of the latter two surfaces through recession or attachment loss (White 1997). Supragingival calculus and subgingival calculus generally occur together, but one may be present without the other. It has been suggested that the initial deposits in a supragingival location might have created conditions for subgingival calculus formation. This does not imply that supragingival calculus is a prerequisite for subgingival calculus formation (Ånerud et al. 1991). When the gingival tissues recede, subgingival calculus becomes exposed and is classified as supragingival. Thus, supragingival calculus can be composed of both supragingival and subgingival types.

Supragingival calculus is located coronal to the gingival margin and therefore is visible in the oral cavity. It is usually white or whitish yellow, has a hard clay-like consistency, and is easily detached from the tooth surface. After removal, it may recur rapidly especially in the lingual area of the mandibular incisor (Fig. 1.8).

The color of supragingival calculus is affected by contact with substances such as tobacco and food pigments. It may localize on a single tooth or group of teeth, or it may be generalized throughout the mouth. Supragingival calculus occurs most frequently and in greatest quantity, on the buccal surfaces of the maxillary molars opposite Stenon's duct and on the lingual surfaces of the mandibular anterior teeth, particularly the centrals, opposite Wharton's duct. In extreme cases, calculus may form a bridge-like structure over the interdental papilla of adjacent teeth or cover the occlusal surface of teeth without functional antagonists (Corbett and Dawes 1998). The proportion of supragingival calculus on the lingual surface of the six lower anterior teeth has been reported to range from 63 to 88%; the amount of calculus on the lateral incisors and canines is 70.2 and 44.5%, respectively, of that on the central incisors. Several factors seem to explain why supragingival calculus forms most readily on the lingual surface of the lower anterior teeth. First, because plaque is thin in that region, any acid formed will diffuse out easily. Second, when sugar is ingested, its concentration is lowest there and is cleared most rapidly. Third, salivary

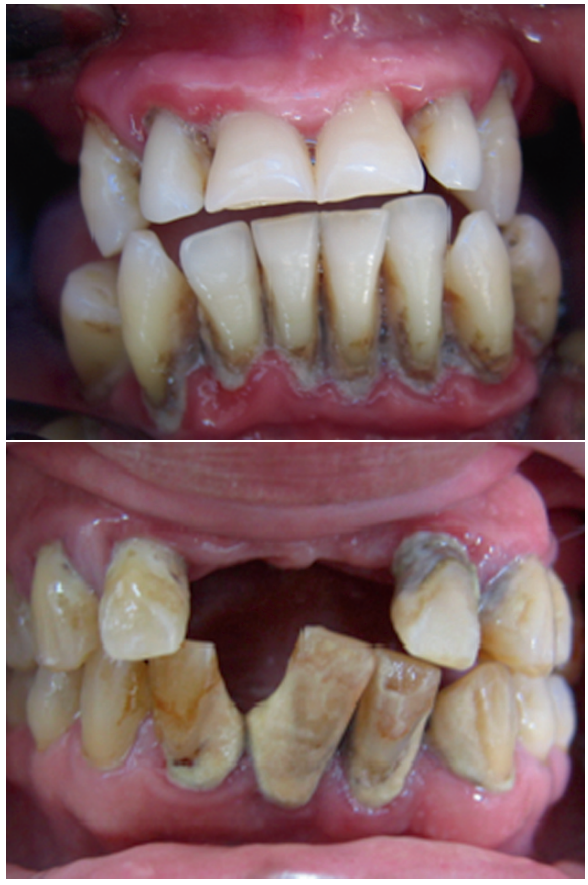

Fig. 1.8 Heavy deposits of dental calculus

film velocity is highest in that region, which promotes acid clearance from plaque. When sugar is ingested, all of these factors lead to the development of Stephan curves that are shallow and of short duration and, because the pH of the plaque lingual to the lower anterior teeth will be above the critical level for much longer than in other regions of the mouth, calculus will deposit there most readily. In addition, the high salivary film velocity will bring more urea to that region, leading to an elevated plaque pH (Dawes 2006).

A extensive prevalent study performed by Ånerud et al. (1991) on two samples of populations, between 1970 and 1985 in Sri Lanka, and between 1969 and 1988 in Norway revealed:

In populations with regular oral hygiene and regular access to professional care (Western Model):

- Supragingival calculus forms in a vast majority of adults (>50–100%).

- Supragingival calculus is observed in the early teen years, and the rate of accumulation does not increase significantly with age.
- Supragingival calculus is restricted *primarily* to "volpe-manhold index (VMI)" teeth. – mandibular lingual surfaces of anterior teeth and buccal surfaces of maxillary molars.
- Subgingival calculus is also widely prevalent (>50–100%).
- Subgingival calculus occurs throughout the dentition – particularly on proximal surfaces.
- In populations without access to regular professional care and/or those who do not practice regular hygiene (Non-Western Model):
- Supragingival and subgingival calculus is found in 100% of subjects.
- Supragingival and subgingival calculus formation occurs throughout the dentition.
- Supragingival calculus formation starts soon after tooth eruption and continues to a maximum at age 30.
- Subgingival calculus is formed within a decade of tooth eruption and both, affected teeth and quantity of calculus increase with age – until age 30.

Both the prevalence and the amount of calculus seen on the permanent teeth of children are low, but they increase with age, and many adults are seen to form supragingival calculus. Studies indicate that supragingival calculus formation is progressive with time, reaching a plateau after several months, after which in any one individual, there is little or no further increase in the actual amount of calculus present on the teeth (Volpe et al. 1969).

Subgingival calculus is located below the crest of the marginal gingival, and therefore is not visible on routine clinical examination. It is usually dense, dark brown or greenish black, and hard or flint-like in consistency, and is firmly attached to the tooth surface.

A study performed by Richardson et al. (1990) with an aim to evaluate the relationship between apical calculus position and the depth and morphology of the intrabony defect showed that calculus has not been found apical to the groove in any histologic section. The mean distance measured clinically between the base of the calculus and the base of the defect was found to increase with the depth of the defect. This relationship did not vary with either tooth type or the number of remaining osseous walls in the defect.

Subgingival calculus is first formed on the interproximal root surfaces either as a subgingival continuation of an already existing supragingival deposit, or as a separate and independent subgingival entity. In exams taken among men at approximately 30 years of age, however, subgingival calculus was seen on all root surfaces of all types of teeth without any special pattern of predilection (Ånerud et al. 1991).

Determination of the location and extent of subgingival calculus requires careful examination with an explorer.

1.2.2 Composition of Dental Calculus

The composition of calculus and adhesive aspects are influenced by the location of its formation as well as its age. The mineral component of dental calculus predominates in formed, hardened deposits (White 1997). Dental calculus consists of inorganic or mineral phase and organic phase.

1.2.2.1 Inorganic Phase

The mineral phase usually consists of a mixture of different calcium phosphate (Ca-P) phases which include:

- Brushite or dicalcium phospate dihydrate (DCPD): $CaHPO_4$. $2H_2O$.
- Octacalcium phosphate (OCP): $Ca_8H_2(PO_4)_6.5H_2O$.
- Magnesium substituted tricalcium-phosphate or whitlockite (β-TCMP): $(Ca,Mg)_3(PO_4)_2$.
- Carbonate hydroxyapatite, (CHA): $(Ca,Na,X)_{10}(PO_4, HPO_4,CO_3)_6(OH,Cl)_2$.

The relative abundance of the different calcium phosphate phases depend on the "age" (younger or older) of the calculus and its geographic site, supra- or subgingival. Thus, DCPD, with a Ca/P ratio = 1.0 is predominant in "young" supragingival calculus; OCP (Ca/P ratio = 1.33) occurs frequently in supra- and occasionally in sub-gingival calculi; b-TCMP (Ca/P ratio = 1.50) occurs in supragingival, and consistently in subgingival calculi; and CHA (Ca/P ratio = 1.1–2.3) can be found in supra-gingival calculi (LeGeros et al. 1999). A compositional gradient was also observed in supragingival calculus: the inner layer (closest to the tooth surface) having different type of calcium

phosphate phases than the outer layer (farthest away from the tooth surface) (Sundberg and Friskopp 1985; LeGeros and Shannon 1979; Tsuda et al. 1996).

Besides calcium and phosphorus, the calculus also contains carbon dioxide 1.9%; magnesium 0.8%; and trace amounts of sodium, zinc, strontium, bromine, copper, manganese, tungsten, gold, aluminum, silicon, iron, and fluorine (McDougall 1985; Knuuttila et al. 1979, 1980, 1981, 1983; Kodaka et al. 1988).

In addition to the mineral components, the calculus contains a variety of inorganic and organic species of bacterial, salivary and dietary origin. These can be incorporated either during mineralization or following calcification, as calculus is quite porous (White 1997).

1.2.2.2 Organic Phase

The organic component of calculus consists of a mixture of protein-polysaccharide complexes, desquamated epithelial cells, leukocytes, and various types of microorganisms; 1.9–9.1% of the organic component is carbohydrate, which consists of galactose, glucose, rhamnose, mannose, glucuronic acid, galactosamine, and sometimes arubinose, galacturonic acid, and glucosamine, all of which are present in salivary glycoprotein, except arabinose and rhamnose. Oxalic acid (Wahl and Kallee 1994), porphyrins (Dolowy et al. 1995), osteopontin (Kido et al. 1995), and calprotectin (Kido et al. 1997) have recently been newly identified.

Salivary proteins account for 5.9–8.2% of the organic component of calculus and include most of the amino acids (Eggen and Rolla 1985), while lipids account for 0.2% of the organic content in the form of neutral fats, free fatty acids, cholesterol, cholesterol esters, and phospholipids (Slomiany et al. 1983).

The variability of calculus mineral composition and structure is the likely result of the numerous factors which can affect mineral nucleation and growth. Whatever the mineral composition may be, the surface of mature calculus always remains covered with a matte of dental plaque (White 1997).

1.2.3 Morphology of Dental Calculus

The ultra-structural morphology of supragingival and subgingival calculus was studied with the scanning electron microscope. The external surface was found to be mostly globular or coarse, and on high magnifications and features could be divided into four main groups: (a) amorphic calcified deposits covering extensive areas, (b) other areas covered with crystals in a variety of arrangements, (c) heavy accumulations of calcified rod-like and filamentous like microorganisms, and (d) platelet crystals in juxtaposition to calcified microorganisms in several areas. In most calculi, the split area was found to be laminated (Lustmann and Shteyer 1981).

Another study of scanning electron microscopy on oral, crevicular, and fracture surfaces of dental calculus was performed by Friskopp and Hammarstrom (1980). Both subgingival and supragingival calculus had a heterogenous core covered by a soft, loose layer of microorganisms. On supragingival calculus, this layer is dominated by filamentous microorganisms while subgingival calculus was covered by a mixture of cocci, rods and filaments. The supragingival covering of filaments is oriented with the filaments approximately perpendicular to and in direct contact with the underlying dense calculus. This arrangement was rarely seen subgingivally where there was no distinct pattern of orientation.

Differences in the manner in which calculus is attached to the tooth surface affect the relative ease or difficulty encountered in its removal.

Four modes of attachment have been described by Zander (1953):

- Attachment by means of an organic pellicle.
- Penetration of calculus bacteria in cementum (this mode of attachment is not accepted by some investigators).
- Mechanical locking into surface irregularities, such as resorption lacunae and caries.
- Close adaptation of calculus undersurface depressions to the gently sloping mounds of the unaltered cementum surface.

Calculus embedded deeply in cementum may appear morphologically similar to cementum and has been termed calculocementum.

1.2.4 Mechanisms of Dental Calculus Formation

The formation, development, and dissolution of hard deposits such as calculus are complex processes that

involve numerous calcium phosphate phases, as well as the interaction of these ions with organic molecules (Nancollas and Johnsson 1994).

The steps in calculus formation, at both chemical and ultrastructural level, have been well summarized by White (1997). The soft plaque is hardened by precipitation of mineral salts, which usually starts between the first and the 14th day of plaque formation; however, calcification has been reported to occur in as little as 4–8 h. Calcifying plaques may become 50% mineralized in 2 days and 60–90% mineralized in 12 days (White 1997). Plaque absorbs calcium and phosphate out of saliva, for supragingival calculus, and out of crevicular fluid for subgingival calculus. Scheie (1989) has reviewed the factors that may affect the initial mineral nucleation and subsequent calcium phosphate crystal growth within dental plaque. *Factors that may potentiate initial calcification processes* include the sensitivity of specific bacteria and bacterial proteolipid membrane components to mineralization. Endogenous factors contributing to calculus mineralization may include salivary mineral ion levels, protein and lipid. Exogenous factors which may affect tartar formation may include dietary components such as silicon, which may act to promote mineral nucleation. While both vital and dying bacterial cells, and by-products, may serve as nucleating centers, numerous salivary and plaque components may counteract calcification by acting as inhibitors of plaque mineralization. These may include salivary phosphoproteins, saliva pyrophosphate and plaque lipoteichoic acid. Several salivary proteins, like statherin and the proline-rich protein, PRPs, are able to inhibit the precipitation and dissolution of calcium phosphate minerals by adsorbing at active sites on the crystallite surfaces (White 1997; Nancollas and Johnsson 1994).

In addition to specific inhibition of mineralization, enzyme systems in plaque and saliva, including phosphatases and proteases may contribute to the degradation of protective inhibitor species (White 1997). The location of acid and alkaline phosphatases in dental plaque was revealed by LoStorto et al. (1992). Both phosphatases had intra- and extramicrobial localization. In the extracellular matrix, phosphatases were associated with small vesicles of bacterial origin, or were freely scattered in the matrix without apparent connection with microbial structures. Intracellularly, alkaline and acid phosphatases were observed in gram-negative and gram-positive bacteria, showing a different localization: the alkaline phosphatases are mainly located in the periplasmic space, while acid phosphatases had a double preferential localization: along the outer surface of the cell wall and in the periplasmic space. Less frequently, an intracellular phosphatase reaction was seen in the cytoplasm (White 1997).

The supersaturation of plaque fluid is a prerequisite to the mineralization of partially soluble calcium phosphate minerals within the calculus (Driessens et al. 1985; White 1997). The degree of supersaturation of plaque fluid increases when its pH is high. This occurs in patients who are tube fed, as their plaque is not exposed to fermentable carbohydrates. It also occurs in patients on dialysis for renal disease, as their salivary urea levels are high and the urea can be converted by plaque bacteria to ammonia, which increases plaque pH. Both classes of patients are very susceptible to calculus deposition (Dawes 2006).

Calcification entails the binding of calcium ions to the carbohydrate-protein complexes of the organic matrix, and the precipitation of crystalline calcium phosphate salts. Crystals form initially in the intercellular matrix and on the bacterial surfaces and, finally, within the bacteria (Friskopp and Hammarstrom 1980).

Calcification begins along the inner surface of the supragingival plaque (and in the attached component of subgingival plaque) adjacent to the tooth in separate foci that increase in size and coalesce to form solid masses of calculus. From surface observations, calculus is apparently composed of two components with distinguishable patters of calcification. One component is formed by the precipitation of minute calcific crystals on microorganisms and intermicrobial substances (plaque matrix). Such calcified masses, often spherical in shape, have a sponge-like appearance with empty spaces representing the former sites of degenerated organisms. Thus, intracellular calcification is not evident at this stage of calculus development. The other component, although having at least one common calcification front with the former, does not appear to be directly associated with microbial calcification. It exhibits a configuration of generally larger crystal growths of varying shapes and sizes. These two calcification patterns are comparable, both in distribution and size, to what has been observed by means of the transmission electron microscope, and what Schroeder has designated as "types A and B centers of mineralization," respectively. The calcific precipitation in type A centers have been identified by X-ray diffraction as hydroxyapatite. It is, therefore, speculated that the

crystal patterns in type B centers might represent other known forms of calcium phosphates present in calculus, such as octacalcium phosphate, whitlockite and brushite (Lustmann et al. 1976). The calcification process may be accompanied by alterations in the bacterial content and staining qualities of the plaque. With the occurrence of calcification, filamentous bacteria increase in number. In the calcification foci, there is a change from basophilia to eosinophilia; the staining intensity of groups exhibiting a positive periodic acid-Schiff reaction and of sulfhydryl and amino groups is reduced, and staining with toluidine blue, which is initially orthochromatic, becomes metachrommic and disappears. Calculus is formed in layers, which are often separated by a thin cuticle that becomes embedded in the calculus as calcification progresses (White 1997).

Rate of Formation and Accumulation of Dental Calculus. The starting time and the rates of calcification and accumulation of calculus vary from person to person, in different teeth, and at different times in the same person. On the basis of these differences, persons may be classified as heavy, moderate, or slight calculus formers or as noncalculus formers. The average daily increment in calculus formers is from 0.10 to 0.15% of dry weight (White 1997).

Calculus formation continues until it reaches a maximum, after which it may be reduced in amount. The time required to reach the maximal level has been reported as 10 weeks, 18 weeks, and 6 months. The decline from maximal accumulation (reversal phenomenon) may be explained by the vulnerability of bulky calculus to mechanical influences, and it is difficult to separate these effects on calculus and wear from food, and from actions of the cheeks, lips, and tongue (White 1997).

Several reported observations have suggested that individuals taking certain systemic medications (e.g., antidepressants, anticholinergics, beta-blockers, diuretics, thyroid hormone) found less supragingival calculus than those who did not take these medications. Calculus reduction in medicated subjects was observed, despite the fact that similar mean plaque levels were present in both the medicated and nonmedicated groups. The mechanism of action by which these drugs affect supragingival calculus formation needs clarification; it is possible that the mechanism of reduced calculus formation is different for the various drugs (McClain et al. 1991; Turesky et al. 1992; Breuer et al. 1996).

1.2.5 Association of Calculus with Disease Pathology

1.2.5.1 Clinical Implications of Supragingival Calculus

The location of supragingival calculus precludes its direct participation in advanced periodontal disease. Researchers have, however, considered whether calculus deposits can enhance gingivitis along the gingival margin as a precursor toward more advanced periodontal disease (White 1997).

A mixed-longitudinal study carried out to determine the prevalence of plaque, calculus, gingival bleeding, and type of tooth cleaning device, amongst school children in Morogoro, Tanzania, (Frencken et al., 1991) showed that the prevalence of calculus increased with increasing age, while the gingival bleeding was not age-dependent. Ånerud et al. (1991) demonstrated that supragingival calculus produced little effect on local attachment loss.

Despite the various epidemiological results, it is difficult to separate the effects of dental plaque vs. calculus as etiological factors in disease initiation and progression. A study with an aim to establish the disease promoting potential of supragingival calculus vs. that of dental plaque was conducted by Gaare et al. (1990). For comparison, they used a population of Indonesian soldiers, 20–25 years of age, none of whom had pathological pockets (CPITN less than or equal to two), but all had large amounts of calculus. In one half of the subjects was performed a careful professional prophylaxis (group A), while in the other half, toothbrushing was the sole oral hygiene aid (group B). Gingival health in both groups improved after 2 months: from 63 to 34% bleeding points in group A, and from 61 to 36% in group B. There was thus no obvious benefit from the professional prophylaxis received by group A. The results are particularly relevant for populations in which professional prophylaxis is not normally available. However, they were obtained in a group of young, healthy individuals and may not be extrapolated to older and less healthy populations, or to individuals with deep periodontal pockets. The improvement of gingival health through toothbrushing, in spite of the presence of calculus, supports the contention that plaque, rather than calculus, as a non-inflammatory scale, provides the pathogenic potential.

However, in populations with heavy calculus formation, these deposits are not without consequence. The significant correlation of supragingival calculus formation with the incidence of gingival recession in the adjacent tissues has been reported by various authors. Rustogi et al. (1991) performed a survey on a sample of 260 children and teenagers, aged 10–17 years, in Thailand. Subjects with slight calculus had more mild recession, whereas medium or heavy calculus formers had more moderate or extensive total gingival recession scores. A significant association between calculus presence and gingival recession was reported also by Joshipura et al. (1994) and van Palenstein Helderman et al. (1998).

Several authors have evaluated the use of anticalculus dentifrices not only in preventing calculus accumulation but also in reducing gingival recession. Rustogi et al. (1991), conducted a 1-year, double-blind clinical study in a population of Thai children and showed that the subjects using the anticalculus dentifrice containing 1.3% soluble pyrophosphate and 1.5% of a copolymer, had 57.9% less supragingival calculus, and 16.6% less gingival recession, than the subjects who continued their customary oral hygiene procedures. In a similar study performed by Triratana et al. (1991), the use of an anticalculus dentifrice containing 3.3% soluble pyrophosphate and 1.0% of a copolymer determined after one year of use, a reduction of 34.9% supragingival calculus, and 30.0% gingival recession.

1.2.5.2 Clinical Implications of Subgingival Calculus

Data produced by a number of contemporary studies demonstrated that subgingival calculus is invariably associated with a loss of periodontal attachment and pocket formation.

From the analyses presented by Ånerud et al. (1991) of the longitudinal relationship between subgingival calculus formation and loss of attachment, in a population in which calculus formation occurred without interference or interruption, it was observed that subgingival calculus is associated with higher rates of progression of the periodontal lesion. Christersson et al. (1992) observed that race and age are factors that are correlated to both subgingival calculus formation and periodontal status. Albandar et al. (1996, 1998) suggested a significant association between gingival inflammation and subgingival calculus and the development and progression of early-onset periodontitis, and early periodontal breakdown. An extended epidemiologic survey on the prevalence, extent, and severity of periodontal diseases in the United States performed by Oliver et al. (1998) reported that one risk factor for extensive and severe periodontitis is subgingival calculus.

The aim of a study performed by Martinez-Canut et al. (1999) was to determine the association between the quantity of subgingival calculus and the following factors: type and severity of periodontal disease, age, gender and tobacco consumption. They studied a sample of 622 periodontal patients. A statistically significant association was found between the absence/presence of subgingival calculus and the type and severity of periodontal disease ($P < 0.001$), tobacco consumption ($P = 0.0049$) and age ($P < 0.001$). The quantity of radiographically-detectable subgingival calculus increased with increasing age and severity of the disease. However, the reverse association was found in smokers, which presented more surfaces free of calculus ($P = 0.037$) and less surfaces exhibiting deposits equal or greater than 1 mm. The amount of subgingival calculus decreased as the quantity of tobacco consumed increased ($P = 0.012$), and such differences were more significant in those smoker patients with severe periodontitis ($P = 0.006$). An explanation is presented to justify these latter findings, since most literature supports the fact that the presence of calculus is higher in smokers. According to the results of this study, more radiographically-detectable subgingival calculus in proximal root surfaces was found with increasing severity of the disease, with increasing age and with the absence of tobacco consumption.

However, the exact role of subgingival calculus in the initiation and progression of the periodontal lesion is still open to the conjecture, basically because of the confounding effect of the live bacteria in the plaque covering the surface of subgingival calculus (Mandel and Gaffar 1986; Ånerud et al. 1991).

1.2.6 Assessment of Calcified Deposits

A summary of most calculus indices are presented in Table 1.3.

1.2.7 Anticalculus Agents

Traditional studies for evaluating the effects of chemotherapeutic agents on calculus formation require long time intervals and large populations. Currently, the most widely used clinical design for testing tartar control agents consists of a 2–3-month pretrial period and another 3–6-month trial period. Because of wide variations in calculus formation between individuals, large or carefully selected populations are required in order to discriminate between treatments. Consequently, the traditional, long-term clinical design is expensive and time-consuming to conduct, which usually restricts the evaluation and comparison to one or two experimental formulations vs. a control (Santos et al. 1999).

Some major strategies have been investigated to find an efficacious and acceptable chemical means of inhibiting calculus: (1) dissolve or soften the mature

Table 1.3 A summary of most used calculus indices

Index, authors	Scoring criteria
Oral calculus index (OCI) (Greene and Vermillion 1964)	It is component of the oral hygiene index. An explorer is used to estimate the surface area covered by supragingival calculus and to probe for the subgingival calculus. Scores are assigned according to the following criteria *0* no calculus *1* supragingival calculus covering no more than one-third of the exposed tooth surface *2* supragingival calculus covering more than one-third but not more than two-thirds of tooth surfaces *3* supragingival calculus covering more than two-thirds of exposed tooth surfaces and/or a continous band of subgingival calculus After the scores for debris and calculus are recorded, the Index values are calculated. For each individual, the debris scores are totalled and divided by the number of surfaces scored
Calculus index – CI (Ramfjord 1959)	The scores on calculus for each individual tooth examined are added and the sum divided by the number of teeth examined to yield the index on calculus. The following teeth were selected as indicators of the periodontal condition within the dentition: maxillary right first molar 16, maxillary left central incisor 21, maxillary left first bicuspid 24, mandibular left first molar 36, mandibular right central incisor 41 and mandibular right first bicuspid 44. Calculus recording *0* no calculus *1* supragingival calculus extending only slightly below the free gingival margin (not more than 1 mm) *2* supragingival calculus covering more than one-third but not more than two-thirds of tooth surfaces *3* supragingival calculus covering more than two-thirds of exposed tooth surfaces and/or a band of subgingival calculus
Calculus surface index (CSI) (Ennerver et al. 1961)	CSI assesses the presence or absence of calculus on the four surfaces of the four mandibular incisors. Each surface is given a score of 1 for the presence of calculus or 0 for the absence of calculus. Maximum score for each subject is 16. In applying the scoring method, calculus was considered to be present in any amount, supragingival or subgingival, and it could be detected either visually or by touch. If the examiner was uncertain about the presence of calculus on a given surface, the surface was called calculus free
Calculus rating (Volpe and Manhold 1962)	Calculus formation in vivo is performed using a coloured periodontal probe placed against the lingual surface of the anterior tooth that will be scored with the probe and placed at the most inferior border of any calculus present (supra- or subgingival). With the different colours at the probe end representing units, the amount of calculus present can be measured as *0 U* no calculus *1 U* 1 mm of calculus *2 U* 2 mm of calculus *3 U* 3 mm of calculus *4 U* 4 mm of calculus
Marginal line calculus index (MLC-I) (Muhlemann and Villa 1967)	Calculus recording *0* no calculus *1* calculus observable, but less than 0.5 mm in width and/or thickness *2* calculus not exceeding 1 mm in width and/or thickness *3* calculus exceeding 1 mm in width and/or thickness

deposit by removing the inorganic portion; (2) affect the calculus matrix, i.e., to change the "skeleton" around which calculus is deposited; (3) alter the attachment of the calculus to the tooth surface; (4) prevent plaque from forming; (5) inhibit crystal growth and thereby prevent the development of mineralized plaque (Fairbrother and Heasman, 2000).

Research directed at finding suitable chemical anticalculus agents has paralleled that which has helped to clarify the physiology and biochemistry of calculus formation. Agents used for softening the mature calculus deposit are acids, alkalis, chelating agents, enzymes, urea, antimicrobials (Penicillin, Cetylpyridinium chloride, Niddamycin), metals (Bisphosphonates, Vitamin C, Pyrophosphates, Polymers and co-polymers) (Fairbrother and Heasman 2000). Although multiple mineralization inhibitors have been proven to be clinically effective, their precise mechanism of action is not completely understood. All effective inhibitors studied to date have in common, the ability to inhibit calcium phosphate nucleation and /or crystal growth processes, and the transformation of precursor calcium phosphate mineral phases into more stable calcium phosphates. These agents also show the ability to slow down initial plaque mineralization in biofilm models (Gaffar et al. 1987, 1990; Stephen et al. 1987, Rykke and Rolla 1990a, b; White 1997; LeGeros et al. 1999).

1.2.7.1 Dentifrices

In all studies, the VMI (Volpe and Manhold 1962) was used for calculus assessment. The standard VMI is based on clinical measurement of calculus deposits on the lingual surfaces of the six anterior mandibular teeth. Another system for clinical assessment of calculus is the marginal-line-calculus index (MCLI) (Muhlemann and Villa 1967).In all studies, rapid calculus formers were selected after a pretest period. However, small amounts of calculus are often not detectable, and relatively little is known about the calculus formation and distribution, after using antitartar dentifrices (Gaengler et al. 1993).

Recently, Netuveli and Sheiham (2004) assessed the evidence on the effectiveness of commercially available anticalculus dentifrices. It was revealed that random effect model for 3-month studies showed an effect size of −0.6 for all comparisons. The effect sizes varied from −0.3 for dentifrices with zinc chloride 0.5 to −1.1%, for pyrophosphate 1.3%, and copolymer 1.5% dentifrices. Meta-analysis of all the studies with a 6-month follow-up gave an effect size of −1.1 (−1.5 to −0.8) and for 12-month follow-up, the effect size was −13.6 (−21.4 to −5.8). It was concluded that anticalculus dentifrices containing pyrophosphates, zinc compounds and/or copolymers were effective in significantly reducing calculus scores evaluated with the Volpe-Manhold Index.

Besides this beneficial effect on soft tissues, several authors have reported *mucosal reactions and other soft tissue lesions* with the use of these anticalculus dentifrices. Beacham et al. (1990) observed after a use of a tartar control dentifrices, circumoral dermatitis and cheilitis: a moderately severe perioral erythema with fissuring of the angles of the mouth and scaling erythematous patches, separated by normal skin lateral to the initial erythema. After a double-blind clinical trial on 92 dental hygienists for the evaluation of the effects of different toothpaste formulations on soft tissues, Kowitz et al. (1990) reported that the tartar control toothpastes resulted in statistically significant, higher rates of mucosal reactions (e.g., ulceration, sloughing, erythema, migratory glossitis) than the nontartar control toothpastes. The tartar-control toothpaste can have a contributory role in the onset of other oral lesions like the superficial mucocele (Navazesh 1995), perioral dermatitis (Ferlito 1992), burning mouth syndrome, and higher rates of intraoral mucosal reactions: ulceration, sloughing, erythema, migratory glossitis (DeLattre 1999).

A possible explanation of adverse oral manifestations that may occur when pyrophosphates are added to a dentifrice can be: First, tetrasodium pyrophosphate in a dentifrice forms a slightly alkaline solution upon oral use which could irritate oral membranes. Second, increased concentrations of flavoring agents, known to be sensitizers, are needed to mask the strong bitter taste of pyrophosphates. Third, increased concentrations of detergents, capable of producing hypersensitivity reactions, are necessary to allow the pyrophosphates to become soluble in the dentifrice. Fourth, a preexisting condition of reduced salivary flow may augment hypersensitivity to tartar control toothpastes. While pyrophosphates have been approved as additives in dentifrices, these compounds along with increased concentrations of flavorings and detergents, and their higher intraoral alkalinity are strongly implicated as the causative factor in certain hypersensitivity reactions (DeLattre 1999).

The incorporation of specific anticalculus ingredients into toothpaste formulations does not seem to interfere with other desirable effects such as anticaries or antiplaque (Stephen et al. 1990; Disney et al. 1989; Ripa et al. 1990).

The anticalculus dentifrices can also have other possible secondary effects on hard tissues, being used

as antihypersensitivity agents. The in vitro models of dentin sensitivity showed that the tartar control dentifrices gave reductions in fluid flow rates through the dentin discs, comparable to those obtained with specific antisensitivity toothpastes. Additionally, tartar control dentifrices did not remove microcrystalline debris (smear layers) from the surfaces of dentin in vitro (Mason et al. 1991). Using an in vitro model that simulates in vivo conditions, Miller et al. (1994b) demonstrated that a dentifrice containing 5% potassium nitrate, 1.3% soluble pyrophosphate, 1.5% polyvinylmethylether maleic acid (PVM/MA) copolymer and 0.243% sodium fluoride in a silica base (Sensitive/Tartar Control) allows a rapid penetration of potassium nitrate through the dentine matrix, reduces hydraulic conductance by occluding dentine tubules with a mixed surface deposit of copolymer and silica. The results indicate that this new dentifrice should provide multiple clinical therapeutic benefits including controlling tooth decay and tartar formation, and reducing and preventing dentinal hypersensitivity.

Different results have been revealed by other researchers. In a double-blind clinical pilot study, Lavigne et al. (1997) assessed the effects of a tartar-control dentifrice (containing tetrapotassium pyrophosphate, PEG-6, disodium pyrophosphate, and tetrasodium pyrosulphate in a 5.0% soluble pyrophosphate formulation) on tooth sensitivity and revealed that patients may experience dentinal hypersensitivity when using the tartar-control toothpaste. A possible explanation in some aspects of hypersensitivity of teeth, observed in some individuals using dentifrices containing pyrophosphate was given by Rykke and Rolla (1990a, b) who have shown that this component desorbed the acquired enamel pellicle in vivo.

1.2.7.2 Mouthrinses

Mouthrinses constitute a simple and commonly used delivery system for antimicrobial agents. Schaeken et al. (1996) has showed the efficacity of mouthrinses containing 0.4% zinc sulphate and 0.15% triclosan on plaque accumulation, development of gingivitis, and formation of calculus in a 28-week clinical test. Other studies have also revealed the efficacy of delmopinol (Claydon et al. 1996, Lang et al. 1998), pyrophosphates (Mandel 1992), zinc sulphate with triclosan (Singh et al.1989, Triratana et al. 1995) and essential-oil/$ZnCl_2$ (LeGeros et al. 2003; Charles et al. 2001) mouthrinses in the control of calculus formation.

1.2.7.3 Chewing Gum

Studies on the relationship between gum-chewing and calculus formation have produced contradictory results, and it is not clear whether frequent use of chewing gum promotes or inhibits calculus formation (Edgar and Geddes 1990). The in vitro study indicated that the use of a sugar-free chewing gum would most probably promote calcium deposition (Dawes and MacPherson 1993), while the in vivo studies revealed that the frequent use of sugar free chewing gum, neither promotes nor inhibits calculus formation (Fure et al. 1998; Macpherson et al. 1995; Lingström et al. 2005). However, recently Porciani et al. (2003) revealed in a 12-week clinical study that a chewing gum containing tripolyphosphate and pyrophosphate reduced calculus formation by 37.6%, compared to a no gum treatment.

1.2.7.4 Other Products with Effect on Formation of Calculus

Hidaka et al. (1993) have described the calculus inhibitory efficacy of different traditional Chinese medicines. Four of them (TJ-41, TJ-77, TJ-1 20 and TJ-1 35) showed an inhibitory effect on the formation of amorphous calcium phosphate. Twelve of these medicines showed a significant reduction in the rate of hydroxylapatite induction time. These results suggest a potential for these medicines to be included as anticalculus agents in toothpastes or mouthwashes.

In a clinical study, Kleber et al. (1998) compared the effect of a dental floss containing 0.25 mg tetrasodium pyrophosphate per cm and a placebo floss on supragingival calculus formation using a 6-week, partial-mouth toothshield model. The final results demonstrated that the pyrophosphate floss significantly inhibited calculus formation between teeth (mesial-distal scores) by 21%, and on labial surfaces by 37% relative to the placebo floss.

In addition to the study of new agents for the chemical prevention of tartar development, and new benefits for existing tartar control formulations, research has also reported on the attempts toward the convenient in-office techniques to assist in softening dental calculus, thus facilitating professional removal. A commercial scaling gel, SofScale®, has been developed. Components of the gel include calcium chelators and surfactants (White 1997). Initial published studies reported efficacy of the gel in making calculus easier to remove from the teeth and in extracting

endotoxin from calculus, possibly rendering deposits less pathogenic (Jabro et al. 1992). More recent studies, including human and animal studies, showed no benefits of the scaling gel in facilitating calculus removal (Arends et al. 1996; Smith et al. 1994; Maynor et al. 1994; Miller et al. 1994a; Nagy et al. 1998; Koshy et al. 1999).

Anticalculus agents are now ubiquitous in dentifrices and mouthwashes. These so-called antitartar formulations now very often contain more than one active agent. It has clearly been shown in randomized controlled studies that these formulations are efficacious in reducing calculus formation, although the long-term clinical benefit is yet to be determined. Furthermore, comparative studies have failed to show, consistently and unequivocally, that any one formulation has superiority in this field. Indeed, it may well be understood that, unless a new active agent is discovered, such superiority may not be forthcoming.

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Periodontal Microbiology

2

Alexandrina L. Dumitrescu and Masaru Ohara

There is a wide agreement on the etiological role of bacteria in human periodontal disease. Studies on the microbiota associated with periodontal disease have revealed a wide variety in the composition of the subgingival microflora (van Winkelhoff and de Graaff 1991).

The search for the etiological agents for destructive periodontal disease has been in progress for over 100 years. However, until recently, there were few consensus periodontal pathogens. Some of the reasons for the uncertainty in defining periodontal pathogens were determined by the following circumstances (Haffajee and Socransky 1994; Socransky et al. 1987):

1. *The complexity of the subgingival microbiota*. Over 300 species may be cultured from the periodontal pockets of different individuals, and 30–1,000 species may be recovered from a single site.
2. *Sample taking*. The physical constrains of a pocket make it difficult to obtain a representative sample from that pocket: that is, a sample that contains the pathogen and low number of contaminating species. If the sample is too large, the pathogen(s) would be diluted by noncontributory "contamining" species. If the sample were too small or taken from the wrong place, one might miss the pathogen entirely.
3. *Difficulties in cultivation, characterization and identification of micro-organisms of plaque*. Many of the species in pockets are difficult or impossible to culture and difficult to identify. No single medium or environment is capable of recovering all of the organisms, which currently can be isolated from subgingival plaque. Many subgingival bacteria cannot be placed into recognized species. Some isolates are fastidious and are easily lost during characterization. Others are readily maintained but provide few positive results during routine characterization, and thus require special procedures for their identification.
4. *Mixed infections*. Not only single species are responsible for disease. If disease is caused by a combination of two or more microbial species, the complexity increases enormously. Mixed infections will not be readily discerned unless they attract attention by their repeated detection in extreme or problem cases.
5. *Opportunistic microbial species*. The opportunistic species may grow as a result of the disease, taking advantages of the conditions produced by the true pathogen. Changes in the environment such as the release of required substrates from damaged tissues or deepening of the periodontal pocket Could be selected by certain opportunistic species. Their levels may increase concomitant with or after those of the true pathogens, and they may thus be difficult to distinguish experimentally.
6. *Disease activity*. Periodontal disease appears to progress with periods of exacerbation and remission. Ideally, a plaque sample should be taken at the peak of the disease activity. Failure to detect the peak of activity may lead to an underestimate of the contribution of a pathogen(s) to a given lesion.
7. *Multiple periodontal diseases in different subjects*. There appear to be multiple destructive periodontal diseases that, for the most part, cannot be differentiated on a clinical basis. Thus, disease types may be misclassified and inappropriately pooled.
8. *The possibility of multiple diseases in a subject*. Differences observed in clinical symptoms in different parts of the mouth may be explained by differences in levels of the pathogen or the stage of the

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destructive process. Disease might have occurred in shallow lesions due to one species and in deepening lesions by a succession of other species. Disease occurring in one site in the mouth could be due to an agent that is different from the one inducing destruction at a second site at the same time.

9. *The carrier state*. Pathogens may be carried in low numbers in mouths that are free of destructive periodontal diseases (the so-called carrier state), making their role in disease more difficult to evaluate.
10. *Virulent factors*. Strains of putative pathogens may differ in virulence. A virulent clonal type might be detected in periodontally healthy subjects, whereas virulent clonal types might be present in subjects with periodontal disease. An inability to distinguish virulent from virulent clonal types would impede understanding.
11. *Genetic virulence elements*. It has been suggested that more virulent strains may harbor bacteriophages or plasmids. Bacterial plasmids are known to code for several virulence factors like invasiveness, adherence, and antimicrobial resistance as well as the production of toxins and noxious products. Several strains of *A.actionomycetemcomitans* isolated from periodontal lesions of a rapidly destructive periodontitis patients have been described to have identical profiles consisting of four plasmids (Olsvik and Preus 1989). *A.actionomycetemcomitans* phages were isolated from recently active periodontal sites in a patient suffering from prepubertal periodontitis, suggesting an association between periodontal breakdown and phage infection of *A.actinomycetemcomitans* (Preus et al. 1987).

The task of defining the etiological agents of periodontal disease and subsequently the development of improved methods of classification, diagnosis, and treatment is clearly a cyclical process with continual revaluation and refinement (Socransky et al. 1987).

The criteria for defining pathogens in destructive periodontal diseases were initially based on Koch's postulates. These postulates were: (1) the agent must be isolated from every case of the disease (2) it must not be recovered from cases of other forms of disease or nonpathogenically, and (3) after isolation and repeated growth in pure culture, the pathogen must induce disease in experimental animals. These postulates have been amended and extended in recent years, the criteria including association (the species should be found more frequently and in higher numbers in cases of an infection than in individuals without overt disease or with different forms of disease), elimination (elimination of a species should be accompanied by a parallel remission of disease), host response (if a species, or its antigens, gains access to underlying periodontal tissues and causes damage, it seems likely that the host will produce antibodies or a cellular immune response that is directed specifically at those species; thus, the host response could act as a pointer to the pathogens), virulence factors (potentially damaging metabolites produced, or properties possessed by certain species may be suggestive that that species could play a role in the disease process), animal studies (experimentally induced disease in dogs or monkeys, which can be manipulated to favor selection of single or subsets of species that may or may not induce pathology), and risk assessment (prospective studies are performed in which the risk of periodontal disease progression conferred by the presence of an organism at given levels may be assessed). The discrimination of a pathogen from a nonpathogenic species is not based on a single criterion but rather on a "weight of evidence" evaluation (Haffajee and Socransky 1994).

2.1 Virulence Factors of Periodontal Pathogens

The identification of pathogen(s) of an infectious disease, including periodontal diseases, leads inevitably to the question "how do these organisms cause the disease?" Thus, the study of *potential virulence factors* produced by oral species including periodontal pathogens is a very active area of research.

The term virulence is generally defined as the relative ability of an organism to cause disease or to interfere with a metabolic or physiological function of its host. The word derives from the Latin, "virulentus" or "full of poison." Thus, virulence refers to the ability of a microbe to express pathogenicity (e.g., virulent), which is contrasted with nonpathogenic or avirulent organisms. Thus, virulence is not a separate property of the microbe, but is a complex interaction between the microbe and its host; this interaction being dependent upon many extrinsic factors of the environment. The characteristic endproducts of bacterial metabolism; the chemical composition of bacterial components; the ability of the intact bacterium or its parts to overwhelm host

defense mechanisms, its invasiveness, and of course its ability to kill were all used to characterize and distinguish a virulent microbe from an avirulent one (Holt and Ebersole 2005). Poulin and Combs (1999) defined the concept of virulence in terms of the type of molecules being produced by the microbe. As such, they defined virulence in terms of "virulence factors," that is, components of a microbe, which when present harm the host, but when absent (i.e., mutation) impair this ability. This mutation does not affect the ability of the microbe to grow and reproduce. Thus, virulence factors can have a multitude of functions (Holt and Ebersole 2005):

- The ability to induce microbe–host interactions (attachment).
- The ability to invade the host.
- The ability to grow in the confines of a host cell.
- The ability to evade / interfere with host defenses.

A great number of studies have provided a global overview of the microbial factors that are thought to cause virulence in bacterial infections and have described specific examples of such factors that are produced by periodontal species, in particular, *A.actinomycetemcomitans (*van der Reijden et al. 2008; Dileepan et al. 2007; Venketaraman et al. 2008; Fine et al. 2006) and red complex species *P. gingivalis, Tannerella forsythia*, and *Treponema denticola* (Holt and Ebersole 2005; Holt et al. 1999; Imamura 2003; Kadowaki et al. 2000; Nakayama 2003; Potempa et al. 2003).

2.2 Pathogens Suspected Currently in Destructive Periodontal Diseases

Several suspected pathogens have been identified to be involved in the destructive periodontal disease.

2.2.1 Aggregatibacter actinomycetemcomitans (Formerly Actinobacillus actinomycetemcomitans)

A.actinomycetemcomitans is small, nonmotile, gram-negative, saccharolytic, capnophilic, round-ended rod (Haffajee and Socransky 1994). During the last two decades, it has been shown that *Aggregatibacter actinomycetemcomitans* can be regarded as a major pathogen in destructive periodontal diseases (Slots et al. 1990a; van der Reijden et al. 2008; Slots and Ting 1999). The species is represented by six serotypes (a–f). Serotype b has been found more frequently and detected in higher numbers in active periodontitis lesions, whereas serotypes a and c have a stronger association with periodontal health (van der Reijden et al. 2008). Serotype b was significantly found more often in aggressive than in chronic periodontitis. They also found serotype b more frequently in periodontitis subjects under the age of 18 years (60.9%) in comparison to subjects older than 35 years (29%). The global distribution of the different *A. actinomycetemcomitans* serotypes is not homogeneous, which implies that the association between serotype and periodontal status may depend on the geographical location and/or ethnical status of the study population (van der Reijden et al. 2008; Fine et al. 2007).

2.2.1.1 Distribution

Aggregatibacter actinomycetemcomitans was first identified as a possible periodontal pathogen in 1975 in studies of localized juvenile periodontitis, now known as localized aggressive periodontitis (LAP) (Newman et al. 1976). Destructive periodontal disease in children is frequently associated with *A. actinomycetemcomitans. Prepubertal periodontitis* and other types of early onset periodontitis yield the organism in prevalence rates of 40–100% (Slots and Ting 1999). The close relationship between *A. actinomycetemcomitans* and *early-onset periodontitis* incriminates the organism in the development of many cases of the disease. *Localized juvenile periodontitis* is the most notorious disease associated with *A. actinomycetemcomitans*. Despite uncertainty about clinical diagnosis and prior periodontal therapy, studies have isolated *A. actinomycetemcomitans* from 75–100% of localized juvenile periodontitis lesions (Slots and Ting 1999).

A. actinomycetemcomitans is also associated with periodontitis lesions of Papillon-Lefèvre syndrome patients. Papillon-Lefèvre patients exhibit decreased function of monocytes, neutrophils and lymphocytes, which in part may be due to cytomegalovirus infection. It was hypothesized that the virally mediated host defense impairment may set the stage for overgrowth of subgingival *A. actinomycetemcomitans* (Slots and Ting 1999).

It was also showed that 30–40% and higher proportions of *adult periodontitis* patients exhibit the organism. In addition, the proportion of the subgingival microbiota comprising *A. actinomycetemcomitans* increases considerably with increasing periodontal probing depth. Also, *A. actinomycetemcomitans* has been detected four times as frequently in periodontal lesions with angular than with horizontal alveolar bone loss (Slots and Ting 1999). *A.actinomycetemcomitans* was also found to occur in *periodontal sites undergoing active breakdown* at levels 100-fold greater than those of the nonactive sites (Mandell 1984). Defining the "active" or "progressing" disease as a loss of connective tissue attachment of >2 mm during a 37-day monitoring period, Mandell et al. (1987) reported that 90% of the progressing sites (18/20) harbored *A.actinomycetemcomitans,* whereas only 44% of the stable or nonprogressing sites (7/16) harbored the organism ($P < 0.05$). Similar results were reported by Slots et al. (1986) who examined the occurrence of *A.actinomyctemcomitans, P.gingivalis* and *P.intermedia* in 235 sites, including 104 from 61 untreated patients. Progressive lesions revealed a high prevalence of *A.actinomyctemcomitans* (50.0%), while nonprogressive sites demonstrated a significantly low prevalence of *A.actinomyctemcomitans* (4.8%). *A. actinomycetemcomitans* seems to be a particularly frequent organism in *refractory periodontitis lesions*, possibly due to the organism's ability to invade gingival tissue and thereby evade the cleaning efforts of the dentist and the patient (Slots and Ting 1999).

A.actinomycetemcomitans can be found also in individuals with no history of destructive periodontal disease. Periodontally healthy children below 11 years of age exhibit an occurrence of *A. actinomycetemcomitans* from 0 to 26%. Adolescents with healthy periodontium or minimal disease exhibit less than 15% subgingival *A.actinomycetemcomitans* occurrence, while young adults with minimal periodontal disease reveal subgingival *A.actinomycetemcomitans* in a frequency of about 15%, although higher frequencies of occurrence have also been reported (Slots and Ting 1999).

In periodontitis patients, *A.actinomycetemcomitans* has been isolated not only from subgingival sites but also from extracrevicular locations in the mouth. Correlation analysis revealed significant positive association between the incidence of *A.actinomycetemcomitans* in infected individuals for deep and normal periodontal sites, periodontal pockets and cheek, tongue and saliva, and cheek and saliva (Muller et al. 1995).

In general, studies investigating successful and infected implants reveal differences in composition of the associated microbiota. Successful implants are reported to be populated with gram-positive coccoid cells, very few rods, a low ratio of anaerobe/aerobes and a low number of gram-negative anaerobes. *Infected and failing implants* show greater proportions of periodontal pathogens, including gram-negative anaerobe rods, motile rods, fusiform bacteria, and spirochetes, than nonfailing implants. These include large numbers of *Fusobacterium ssp*. and *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*, *Peptostreptococcus micros, Campylobacter rectus, Capnocytophaga spp., P. intermedia*, and *P. gingivalis*. Other bacterial species such as *Pseudomonas aeruginosa, Enterobacteriaceae spp., Candida albicans, Staphylococcus epidermidis*, and *S. aureus* have also been identified around implants, but may reflect an opportunistic colonization of the plaque secondarily to antibiotic treatments (Norowski and Bumgardner 2009).

2.2.1.2 Virulence Factors

A.actinomycetemcomitans has been showed to possess a myriad of virulence factors that enhance its survival in the oral cavity and enable it to circumvent the host's protective strategies (Fives-Taylor et al. 1999). Many of these virulence factors may be involved in the pathogenesis of periodontitis (Table 2.1). They include:

1. Factors that promote colonization and persistence in the oral cavity: Adhesins, Invasins, Bacteriocins, Antibiotic resistance.
2. Factors that interfere with the host's defenses: Leukotoxin, Chemotactic inhibitors, Immunosuppressive proteins, Fc-binding proteins.
3. Factors that destroy host tissues: Cytotoxins, Collagenase, Bone resorption agents, Stimulators of inflammatory mediators.
4. Factors that inhibit host repair of tissues: Inhibitors of fibroblast proliferation, Inhibitors of bone formation.

Adhesion of *A. actinomycetemcomitans*

Most *A. actinomycetemcomitans* strains that have been tested adhere strongly to epithelial cells. Binding

Table 2.1 Virulence factors or antigens of *A. actinomycetemcomitans* studied that are associated with periodontal disease immunity (modified from Teng 2006) (with permission from Sage Publications)

Virulence factor/antigen	Functional or immune characteristics
SF1 (14 kDa)	Immuno-suppression of Th cells and down-regulate cytokine production
Cdt	Cell-cycle G2 arrest and apoptosis of human T-cells; stimulate pro-inflammatory cytokine production; CdtB-mediated nuclear transport in host cells; induce RANKL release in periodontal ligament cells
TadA and Flp-1	Bacterial colonization, nonspecific adherence, and fibrils assembly; immune IgG protection of alveolar bone loss in a rat oral challenge model
Leukotoxin	Lysis of monocytes/PMN, T-cells, NK cells, and HeLa cells (via β_2-integrin and LFA-1) in a dose-dependent apoptosis or necrosis manner; stimulate human IgG response
CagE-homologue	Induce apoptosis of epithelia, endothelia, osteoblasts, and lymphocytes; activate; CD4+ T-cell-mediated immune response associated with osteoclastogenic activity
OMP-1	Induce IgG and CD4 + T-cell-mediated immunity and associated with osteoclastogenic activity
OPM-100	Bacterial adhesin, invasin, and serum resistance factor; induce host cytokine production
Surface proteins (SAM: 14–79 kDa)	Stimulate protective immunity in s.c. mouse lesion model (via IgG activity)
GroEL (Hsp60)	Stimulate pro-inflammatory cytokine release; modulate antibacterial immunity; stimulate bone resorption in vitro (via osteoclast activity)
65-kDa protein	Modulate immune response by binding to IL-10R
LPS	Stimulate cytokine IL-1, TNF-α, IL-6, IL-8, and PGE_2 release from host cells; induce bone osteoclastic activity

occurs very rapidly, reaching saturation levels within 1 h after infection (Fives-Taylor et al. 1999). Cell surface entities that mediate adherence include fimbriae, extracellular amorphous material and extracellular vesicles (Fives-Taylor et al. 1999). It was suggested that fimbriae most probably function in adherence of rough variants, whereas nonfimbrial components (such as vesicles) are probably involved in adherence of smooth, highly invasive strains (Meyer and Fives-Taylor 1994a, b). *A. actinomycetemcomitans* also produces poly-*N*-acetylglucosamine (PGA), a surface polysaccharide that mediates intercellular adhesion, biofilm formation and detergent resistance (Venketaraman et al. 2008).

In order to initiate disease in extraoral sites (such as endocarditis and osteomyelitis), *A. actinomycetemcomitans* must bind to the extracellular matrix, the complex network of proteins and polysaccharides that is secreted by, and underlies epithelial and endothelial cells and surrounds connective tissue. The major component of the extracellular matrix is collagen (Fives-Taylor et al. 1999). Mintz and Fives-Taylor (1999) showed that multiple strains of *A.actinomyctemcomitans* bind to several types of connective tissue collagen and fibronectin, but not to the plasma protein, fibrinogen. Binding, therefore is highly specific. All the collagen types were demonstrated to be substrates for the binding of *A.actinomyctemcomitans* strains. A degree of specificity in the binding of *A.actinomyctemcomitans* SUNY465 to various collagen molecules was demonstrated by the almost complete lack of binding to type IV collagen, which is only found in basement membranes (Mintz and Fives-Taylor 1999). Outer membrane proteins on the bacterial cell surface are essential for binding. The binding of *A. actinomycetemcomitans* to the insoluble form of proteins that are major structural components of the extracellular matrix must aid the organism in its spread and colonization, not only at oral sites but at extraoral sites as well (Fives-Taylor et al. 1999).

Antibiotic Resistance

Roe et al. (1995) examined 18 clinical isolates of *A.actinomyctemcomitans* from 16 patients with periodontitis. Eighty-two percent of the *A.actinomyctemcomitans* isolates were resistant to tetracyclines, and frequently employed antibiotic used as an adjunct to mechanical debridement in the treatment of localized juvenile periodontitis, and carried the Tet B resistance determinant. It was also that the TetB determinant transferred at frequencies of 3.5×10^{-5}–2.5×10^{-5}per *A.actinomyctemcomitans* recipient and 1×10^{-8}–6×10^{-8}per *H.influenzae* recipient. Marked reduction of subgingival *A.actinomyctemcomitans* associated with the resolution of clinical signs of localized juvenile periodontitis after 7 days course with a combination of systemic metronidazole and amoxicillin was reported (Christersson et al. 1989).

Bone Resorption

A characteristic feature of periodontal disease is the loss of bone supporting the teeth. *A. actinomycetemcomitans* has been shown to stimulate bone resorption by several different mechanisms: lipopolysaccharide, proteolysis-sensitive factor in microvesicles and surface-associated material (Fives-Taylor et al. 1999; Wilson et al. 1985). Surface-associated material has recently been identified as the molecular chaperone, GroEL. The chaperone appears to act in a direct way with the major bone-resorbing cell population, the osteoclast (Meghji et al. 1992, 1994; Fives-Taylor et al. 1999).

Collagenase

As previously stated, collagen is the most abundant constituent of the extracellular matrix. A major feature of periodontal disease is a marked reduction in gingival collagen fiber density. Collagenase activity is associated with *A. actinomycetemcomitans* (Fives-Taylor et al. 1999).

Cytotoxins

One of the most important cell types within the gingival connective tissue is the fibroblast. Fibroblasts are a major source of collagen and confer structural integrity to the tissue. Many oral bacteria express toxins that inhibit human fibroblast proliferation, but the heat-labile cytotoxin produced by *A. actinomycetemcomitans* is especially cytotoxic. The toxin is considered a virulence factor due to its impact on fibroblast viability (Fives-Taylor et al. 1999).

Extracellular Membranous Vesicles

Almost all strains of *A. actinomycetemcomitans* examined extrude membrane vesicles from their surface. The vesicles associated with SUNY 465 grown on agar are fibrillar membranous extensions with knob-like ends. These vesicles often contain leukotoxin, endotoxin, bone resorption activity and a bacteriocin. *A. actinomycetemcomitans* vesicles must also contain adhesins, since their addition to a weak adherent or nonadherent strains significantly increases the ability of those strains to attach to epithelial cells (Fives-Taylor et al. 1999).

Bacteriocins are proteins produced by bacteria that are lethal for other strains and species of bacteria. These toxic agents can confer a colonization advantage for the bacterium by lessening the ecological pressures associated with competition by other organisms for both nutrients and space (Fives-Taylor et al. 1999). Hammond et al. (1987) has showed that it enhances its chance to colonisation by producing in vivo an extracellular factor, actinobacillin, that is directly toxic to two major plaque formers that primarily colonize the tooth surface, *S.sanguinis* and *Actinomyces viscosus*. It was also showed that bacteriocin produces alterations in the cell permeability of target bacteria, with resultant leakage of RNA, DNA, and other essential intracellular macromolecules and cofactors (Fives-Taylor et al. 1999).

Leukotoxin

One of the best studied *A. actinomycetemcomitans* virulence factors is leukotoxin, a 114-kDa secreted lipoprotein that belongs to the RTX family of pore-forming bacterial toxins. *A. actinomycetemcomitans* leukotoxin has been shown to kill polymorphonuclear leukocytes (PMNs) and macrophages isolated specifically from humans and Old World primates. Human subjects harboring highly leukotoxic strains of *A. actinomycetemcomitans* are more likely to develop periodontitis than subjects harboring minimally leukotoxic strains. These findings suggest that leukotoxin may play a role in host cell killing and immune evasion in vivo (Kolodrubetz et al. 1989; Venketaraman et al. 2008; Balashova et al. 2006; Diaz et al. 2006).

Fc-Binding Proteins

Bacterial immunoglobulin-binding proteins, or Fc receptors, are proteins that bind to the Fc portion of Igs. These receptors are postulated to interfere with complement- or antibody-dependent host immune mechanisms, as well as certain immune functions. If other proteins compete for binding to this region of PMNs, binding of the antibody may be inhibited, and

thereby inhibit phagocytosis (Fives-Taylor et al. 1999). Mintz and Fives-Taylor (1994) demonstrated the presence of Ig Fc receptors on the surface cells of several *A.actinomyctemcomitans* strains. The murine monoclonal antibodies of unrelated specificity were of the IgG subclass of Igs. It was proposed that Fc receptors may be another factor that aids in the persistence of *A.actinomyctemcomitans* at extracellular sites during the disease process. Tolo and Helgeland (1991) showed that release of Fc-binding components from bacteria may interfere with the phagocytic activity (a 90% phagocytosis reduction was noted), complement function and down-regulation of B-cell proliferation in the periodontal infiltrates.

Lipopolysaccharide

Lipopolysaccharides (endotoxins) have a high potential for causing destruction of an array of host cells and tissues. Tissue destruction is a key feature of periodontal diseases; thus, the lipopolysaccharide of *A. actinomycetemcomitans* has been extensively characterized (Low concentrations of *A. actinomycetemcomitans* lipopolysaccharide stimulate macrophages to produce interleukins (interleukin-lα, interleukin-lβ) and tumor necrosis factor (TNF), cytokines involved in tissue inflammation and bone resorption. These data suggest that macrophages that migrate to gingival sites of *A. actinomycetemcomitans* infection will be stimulated to produce these cytokines, which may then be involved in gingival inflammation and alveolar bone resorption (Fives-Taylor et al. 1999; Rogers et al. 2007).

Immunosuppressive Factors

Host defense mechanisms play a major role in controlling concentrations of bacterial communities in dental plaque. *A. actinomycetemcomitans* has been shown to elaborate many factors capable of suppressing these host defense mechanisms (Fives-Taylor et al. 1999). It was showed that *A.actinomyctemcomitans* also produces *a 60-kDa protein, which down regulates both T- and B-cell responsiveness through the activation of a subpopulation of B lymphocytes* (Shenker et al. 1990). Now, this factor is known as cytolethal distending toxin (CDT), which induces apotosis to lymphocytes (Ohara et al. 2004). It was also found that the bacteria produce a novel 14-kDa substance (*designated suppressive factor 1*), which suppresses cell proliferation and cytokine production by mouse splenic T cells (Kurita-Ochiai and Ochiai 1996). SF1 is a 14 kDa protein, which inhibits T-cell proliferation and production of Th1 (IL-2 and IFN) and Th2 (IL-4 and IL-5) cytokines by ConA-stimulated splenic T cells. Therefore, this molecule could affect the induction of humoral and/or cell-mediated immune responses through the modulation of the T-cell responses, including cytokine production (Kurita-Ochiai and Ochiai 1996). A distinct decrease in the helper-to-suppressor T-cell ratio of patients with either the juvenile or the rapidly progressive forms of early-onset periodontal disease was showed by Kinane et al. (1989). Separate analysis of patients with either the juvenile or rapidly progressive types of early-onset periodontal disease showed both to have reduced CD4+/CD8+ ratios (means 0.91 and 0.92) relative to their controls (means 1.57 and 1.45), which were both statistically significant ($P < 0.05$) (Fig. 2.1).

Inhibitors of Polymorphonuclear Leukocyte Function

The host's first line of defense against invading bacteria is the recruitment of phagocytes to the area. The ability to disrupt chemotaxis permits the invading organism to survive this major challenge from the host. *A. actinomycetemcomitans* secretes a low-molecular-weight compound that inhibits polymorphonuclear leukocyte (PMN) chemotaxis. The inhibitory activity is abrogated by treatment with proteinase K, suggesting that the compound is proteinaceous in nature. Another activity of PMNs is the killing of bacteria by a wide variety of potent antibacterial agents that are gained when the PMNs fuse with lysosomes. *A. actinomyceterncomitans* has been shown to be capable of inhibiting PMNs from producing some of these compounds, and it is intrinsically resistant to others. A heat-stable protein in *A. actinomycetemcomitans* inhibits the production of hydrogen peroxide by PMNs, and many strains are naturally resistant to high concentrations of hydrogen peroxide. Furthermore, *A. actinomycetemcomitans* has been shown to be resistant to several of the cationic peptides, known as defensins, which are found in neutrophils (Fives-Taylor et al. 1999).

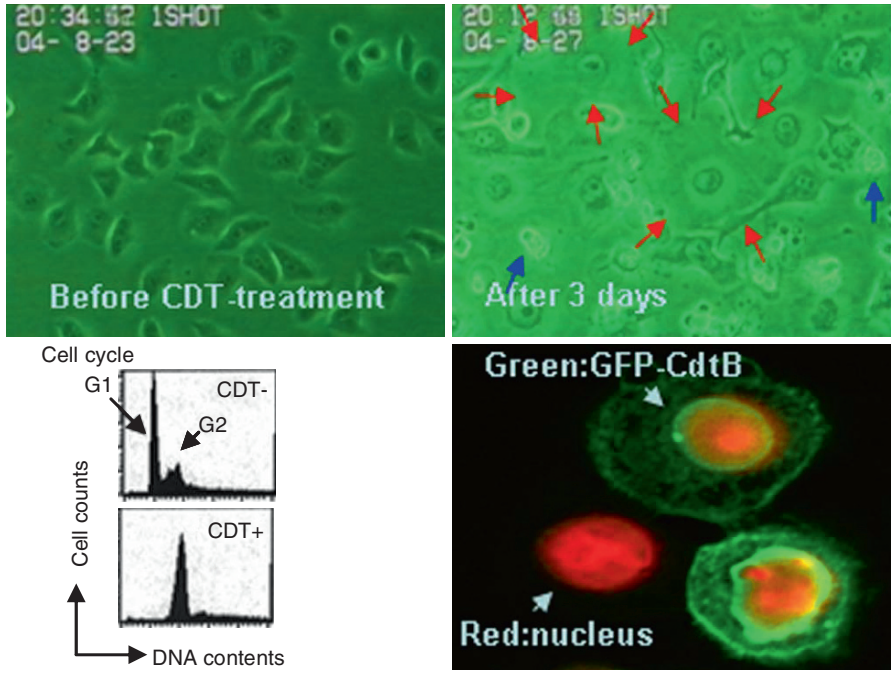

Fig. 2.1 *Effect of Cytolethal distending toxin (CDT).* CDT-intoxicated HeLa cells show cellular and nuclear distension (*red arrows*, *upper panels*). Several cells commit suicide by CDT-induced apoptosis (*blue arrows*). CDT also induces cell cycle block at G2/M phase (*lower left panel*). The active subunit, CdtB targets to nucleus to damage host chromatin (*lower right*). Provided by Prof. M. Sugai, Hiroshima University

Penetration of Epithelial Cells

It was showed that *A.actinomycetemcomitans has the ability to penetrate the gingival epithelium*. An in vitro model of infection of the human KB cell line was developed to investigate the interaction of *A. actinomycetemcomitans* with epithelial cells. The results showed that the degree of invasion by *A. actinomycetemcomitans* was greater in KB cells than in cells originating from nonoral sources. TEM analysis revealed that the *A.actinomycetemcomitans* occurred singly in vacuoles, and it was suggested that colonial morphology may be involved in determining the invasive capability of this organism (Meyer et al. 1991). Saglie et al. (1986) reported that Gram-stained sections from diseased sites (advanced and localized juvenile periodontitis) contained large numbers of bacteria in the oral epithelium and adjacent connective tissue. 87% of the sections showed more than 20 bacteria in the oral epithelium and 6% of the sections showed between 10 and 20 bacteria.

Immunofluorescence examination of frozen gingival section from patients with localized juvenile periodontitis with pooled antisera to *A.actinomycetemcomitans* revealed staining in subepithelial tissues, intracellularly and extracellularly in 80% of the lesions. Staining for *A.actinomycetemcomitans* antigens was detected extracellularly, in the connective tissue, in 69% of the biopsies. This finding indicates penetration by, or transfer of, bacteria or bacterial antigens into the connective tissue (Christersson et al. 1987a). The numbers of *A.actinomycetemcomitans* cultivable from pockets infected with this bacterium correlated with tissue infiltration by this microorganism when assessed by both immunofluorescence microscopy ($P = 0.013$) and by culture from the minced gingival biopsy ($P = 0.002$) (Christersson et al. 1987b).

2.2.1.3 A.actinomycetemcomitans and Systemic Diseases

It was recently revealed that organisms of the HACEK group (*Haemophilus spp., A. actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens* and *Kingella kingae*), and *A. actinomycetemcomitans*, in particular, are associated with systemic diseases distant from the oral cavity (Fine et al. 2006). Nonoral manifestations of periodontal infections merit attention, especially in light of the recent discovery of putative relationships between periodontal disease and coronary heart disease, preterm birth and cerebral infarction. Most likely, systemically healthy individuals are at low risk of becoming ill from dental focal infections. In

immunocompromised patients, the oral cavity may constitute a significant reservoir for serious pathogens. Periodontal disease, odontogenic abscesses and endodontic infections increase the likelihood of dissemination of oral microorganisms to nonoral sites. As *A. actinomycetemcomitans* is an organism that avidly attaches to both soft and hard tissues of the tooth, and positions itself adjacent to the permeable junctional and pocket epithelium, it should come as no surprise that *A. actinomycetemcomitans* has been isolated from a number of organs distant from the oral cavity and is capable of causing serious infections in humans. Such infections include fascial plane infection, heart infection, endocarditis, pericarditis, lung infection, necrotizing pneumonia, mediastinitis, mediastinal abscess, transdiaphragmatic infection, endophthalmitis, skin infection, vertebral osteomyelitis, cervical lymphadenitis, submandibular space abscess, and urinary tract infection (Fine et al. 2006 Kaplan et al. 1989).

2.2.2 *Porphyromonas gingivalis*

Porphyromonas (P.) gingivalis is the second intensively studied probable periodontal pathogen. Isolates of this species are gram-negative, anaerobic, nonmotile asaccharolytic rods that usually exhibit coccal to short rod morphologies. *P.gingivalis* is a member of the much investigated black-pigmented Bacteroides group (Haffajee and Socransky 1994).

2.2.2.1 Prevalence in Periodontal Disease

Most authors agree that *periodontally healthy* children and adolescents harbor few or no *P. gingivalis* in the subgingival microbiota. *P. gingivalis* has been described in 37–63% of *localized juvenile periodontitis* patients; however, the organism is rarely found at the debut of the disease and tends to comprise only a small part of the microbiota in early disease stages. In contrast, *P. gingivalis* is a predominant organism in *generalized juvenile periodontitis* and may assume pathogenetic significance in the disease. Adults having a healthy and minimally diseased periodontium reveal subgingival *P. gingivalis* in less than 10% of study sites. On the other hand, 40–100% of *adult periodontitis* patients may yield the organism. Furthermore, *P. gingivalis* comprises a considerably higher proportion of the subgingival microbiota in *deep than in shallow periodontal pockets* (Slots and Ting 1999; Ready et al. 2008), and in *progressive deep periodontal lesions* than in nonprogressive sites (31.6 vs. 7.4%) (Slots et al. 1986) *Infected and failing implants* show greater proportions of periodontal pathogens, including gram-negative anaerobe rods, motile rods, fusiform bacteria, and spirochetes, than nonfailing implants. These include large numbers of *Fusobacterium ssp*. and *Prevotella intermedia*, *A.actinomycetemcomitans*, *Peptostreptococcus micros, Campylobacter rectus, Capnocytophaga spp., P. intermedia*, and *P. gingivalis* (Norowski and Bumgardner 2009).

Several studies suggested that the outcome of *periodontal treatment* is better if particular suspected pathogens, notably *P. gingivalis* and *A. actinomycetemcomitans*, can no longer be detected after therapy (Slots and Rosling 1983; Christersson et al. 1985; Kornman and Robertson 1985; Haffajee et al. 1988a, b; Rodenburg et al. 1990), and that positive sites are at greater risk for further break down (Slots et al. 1986, Bragd et al. 1987; Slots and Listgarten 1988; Fine,1994; Rams et al. 1996; Brochut et al. 2005). However, despite the fact that non-surgical, mechanical periodontal treatment as well as self-performed plaque control is effective in reducing the numbers of P. gingivalis, P. intermedia and A. actinomycetemcomitans at periodontal sites, these organisms re-establish themselves rapidly in most subjects once adult periodontitis is present, indicating that effective plaque control is constantly required (Doungudomdacha et al. 2001; Johnson et al. 2008).

2.2.2.2 Virulence Factors

Lipopolysaccharide

Identical to Gram-negative prokaryotes, *P.gingivalis* synthesizes a lipopolysaccharide. The cell envelope of the gram-negative bacterium consists of two distinct membranes: the inner (cytoplasmic) membrane; and the outer membrane. The outer membrane of gram-negative bacteria lies external to the peptidoglycan and is attached to it by selected lipoproteins. These lipoproteins or murein lipoproteins attach by both covalent and noncovalent bonds to protein units within *P. gingivalis* and to the outer membrane by their lipid moieties.

The outer membrane of gram-negative bacteria is asymmetric, the outer leaflet of which contains the lipopolysaccharide. The lipopolysaccharide is a very large molecule, with estimates ranging from 10 kDa and larger. Its amphipathic character is a result of one end of the molecule, the hydrophilic end consisting of the polysaccharide or 0-specific (somatic) antigen, which is exposed to the environment on the exterior surface of the outer membrane, and the core region, buried within the outer leaflet, which connects the *O*-antigen to the hydrophobic end of the molecule or lipid A. This complex lipid is embedded in the lipid portion of the outer membrane leaflet (Holt et al. 1999). Chemical dissection of the lipopolysaccharide into its component parts (0-antigen, core, lipid A) has permitted the determination of the biologically active components of the parent molecule (Yoshimura et al. 2009) (Fig. 2.2) (Table 2.2).

LPS does function as a significant cytotoxin as well as inducer of several host derived cyto- and chemokines. The lipopolysaccharide of *P. gingivalis* is chemically different from that found in the well studied and benchmark enteric lipopolysaccharide. These chemical and structural differences more than likely reflect the functional differences between the two molecules and may relate to their role in the pathogenesis of periodontal disease. The low biological activity of *P. gingivalis,* especially its very low endotoxicity, may reflect the organisms' ability to colonize and grow in sterile tissue undetected by the host (Holt et al. 1999).

Adhesion and Coaggregation

As part of the repertoire of *P. gingivalis* virulence factors, it has been shown to possess distinct molecules/structures that are essential to interactions with the host. Specifically, this species has been shown to be capable of adhering to a variety of host tissues and cells, and to invade these cells and multiply (Holt and Ebersole 2005).

Coaggregation is a phenomenon that describes the specific interaction of pairs of oral bacteria via cognate binding. Many species of oral bacteria have been shown to demonstrate this function, presumably related to the development of the complex biofilms of the oral cavity. Thus, intergeneric coaggregation clearly contributes to the characteristics of the complex microbial ecology of biofilms established in the multiple habitats of the oral cavity. *P. gingivalis* is capable of coaggregating with *Actinomyces naeslundii two (Actinomyces viscosus), Streptococcus gordonii, S. mitis* and fimbriated *Streptococcus salivarius*. This interaction is altered by heat treatment, various sugars, amino acids, cation chelation, and protease treatment, suggesting a specific ligand–receptor interaction (Holt and Ebersole 2005).

The initial event in the pathogenicity of *P. gingivalis* is its interaction (adherence) in the oral cavity. To accomplish this, *P. gingivalis* employs several bacterial components: fimbriae, proteases, hemagglutinins, and lipopolysaccharide (Holt and Ebersole 2005). Fimbriae or pili are proteinaceous, filamentous

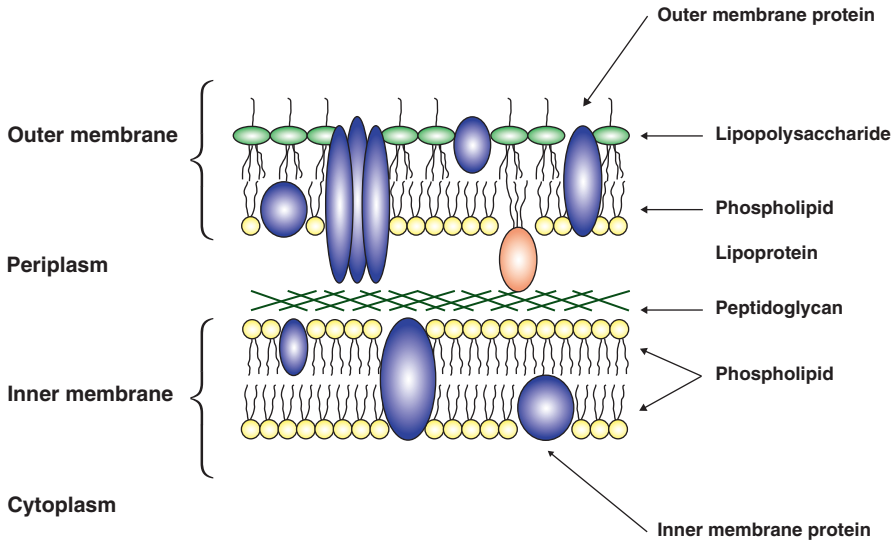

Fig. 2.2 *Schematic illustration of the surface structure of gram-negative bacteria.* The asymmetric outer membrane contains lipopolysaccharides, phospholipids, lipoproteins and outer membrane proteins (modified from Yoshimura et al. 2009) (with permission from Wiley-Blackwell Publishing)

Table 2.2 Virulence factors or antigens of *P. gingivalis* that are associated with periodontal immunity (modified from Teng 2006) (with permission from sage publications)

Virulence factor/antigen	Functional or immune characteristics
dpp	Abscess formation and lethality
HagA (hemagglutinin)	Tissue/cell invasion
HagB (hemagglutinin)	Stimulate strong IgG and Th immune responses; induce immune protection
Gingipains (RgpA, RgpB, Kgp)	Tissue destruction and alter cytokine/chemokine and Igs bio-activity (i.e., IL-12, TNFα, C3 and C5, IgG/A)
	Stimulate protease-activated receptors (PAR-1 and -4) or platelet-activating factor associated with Th1 and IgG2 immune responses
Gingipain-R1 (Rgp-A and -B) (RgpA is critical for protection)	Stimulate immune protection in a murine oral challenge and s.c. abscess model, respectively
Cysteine proteinases (Arg- and Lys-)	Induce RANKL production; disrupt PMN function
Rgp-Kgp/adhesin-based peptide complex, or Kgp-DNA vaccine	Induce protection in mouse s.c. abscess model or rat oral challenge model (via IgG activity); protection via IgG activity; immune protection related to IgG4/Th2 response; mAb to RgpA inhibit P.g. colonization in the experimental human subjects
Hemoglobin-binding domain	Stimulate immune protection in a rat model (via both IgG and Th2/Th1-ratio-driven responses)
Fimbriae (FimA, etc.)	Bacterial colonization, induce host IgA, IgG, and Th1 immune responses; stimulate pro-inflammatory cytokine release; stimulate CD14, TLR2 and 4, CD11a/CD18
	Endothelial atherosclerotic change; induce periodontal bone loss in rats and mice (Immunization induces protective IgG/A immunity in a guinea pig subcutaneous lesion model and a germ-free rat model, and T-cell epitope mapped.)
	A 20-mer P8-peptide (T- and B-cell epitope) induces immune protection in a mouse s.c. lesion model
LPS	Escape immune recognition; innate hypo-responsiveness
	Activate APC's immuno-suppressive effects (i.e., increase ILT-3 and B7-H1 release); change CD14 and LTR expressions
	Modulate various cytokine expressions
ClpP	Bacterial invasion into epithelium
GroEL (Hsp60)	Modulate TNF-α and host cytokine production
LPS: Lipid-A	Stimulate TLR2 and -4
Outer membrane proteins (PG32 and PG33, or OMP40/41)	Stimulate immune protection in a mouse s.c. abscess model (via IgG activity); stimulate PBMC T-cells to produce IL-17
Capsular PS	Stimulate immune protection in mouse oral challenge model (via IgG activity)
Ag53 (53 kDa)	Stimulate strong IgG2 and Th1 immune responses; both B-cell epitopes and dominant T-cell epitope (hu-HLA-DRB1 restricted) mapped

appendages that protrude outwards from the bacterial cell surface and play a crucial role in virulence by stimulating bacterial attachment to host cells or tissues (Holt and Ebersole 2005).

The first fimbriae are called major, long, or *FimA* fimbriae, and the second ones are referred to as minor, short, or *Mfa1* fimbriae (Yoshimura et al. 2009). Major fimbriae are filamentous components on the cell surface, and their subunit protein, fimbrillin (FimA) reportedly acts on bacterial interactions with host tissues by mediating bacterial adhesion and colonization in targeted sites. Major fimbriae are capable of binding specifically to and activating various host cells such as human epithelial cells, endothelial cells, spleen cells, and peripheral blood monocytes, resulting in the release of cytokines including interleukin-1 (IL-1), IL-6, IL-8, and tumor necrosis factor-α (TNF-α), as well as cell adhesion molecules including intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and P- and E-selectins. In addition, *P. gingivalis* major fimbriae have been shown necessary for bacterial invasion to host cells (Amano et al. 2004).

Minor fimbriae were shown to be short fimbria like appendages in an *fimA* (major fimbria-deficient) mutant of strain ATCC 33277. A subunit protein of a minor fimbriae (*Mfa*1) encoding the *mfa1* gene was shown to be different in size (67 kDa in contrast to 41 kDa of major fimbria subunit) and antigenicity from that of major fimbriae. Although a *fimA* mutant revealed a significant reduction of adhesive potential to saliva-coated

hydroxyapatite, gingival epithelial cells, and fibroblasts, as well as bone adsorption capability, in an orally infected rat model, minor fimbriae purified from *P. gingivalis* ATCC 33277 markedly induced IL-1α, IL-β, IL-6, and TNF-α cytokine expression in mouse peritoneal macrophages (Amano et al. 2004).

Proteinases

One of the potential virulence proprieties displayed by *P. gingivalis* is the high proteolytic activity associated with these organisms. The primary function of proteases and peptidases secreted by asaccharolytic bacteria such as *P. gingivalis* is, most likely, to provide nutrients for growth. At least eight secreted proteinases have now been described for *P. gingivalis* and their concerted activities, in addition to providing amino acids, peptides and hemin for growth, including processing of essential cell surface components and provision of substrates for bacterial cell adhesion. The proteinases are also involved directly in tissue invasion and destruction by bacteria, and in evasion and modulation of host immune defenses. Specific examples of tissue degradation and attenuation of host defense mechanisms include: the degradation of extracellular matrix proteins; activation of matrix metalloproteinases (MMPs); inactivation of plasma proteinase inhibitors; cleavage of cell-surface receptors; activation or inactivation of complement factors and cytokines; activation of the kallikrein-kinin cascade; stimulation of apoptotic cell death; and disruption of PMN functions (Holt et al. 1999; Holt and Ebersole 2005; Kadowaki et al. 2000).

The adhesive and enzymatic functions of *P. gingivalis* proteinases are intricately interconnected. *P. gingivalis* cells bind to and degrade human plasma fibronectin, laminin, fibrinogen and collagen. The adhesion and degradation processes involve the activities of fimbriae and of the Arg-X-specific and Lys-X-specific cysteine proteinases. Hydrolysis of fibronectin or other matrix proteins such as collagen by the *P. gingivalis* Arg-X proteinases RgpA and RgpB enhances the binding of fimbriae to these substrates. The proteinases are able to expose sequences within host matrix protein molecules that carry *C*-terminal Arg residues, thus promoting adhesion of the organism through a fimbrial–arginine interaction. This may represent one mechanism by which initial gingivitis progresses to more severe periodontitis. Increased proteolytic activity associated with infection of the gingival sulcus could expose previously hidden receptors that would then enhance colonization by *P. gingivalis* (Lamont and Jenkinson 2000).

The best studied are the cysteine proteinases, or the "*gingipains,*" with specificities for cleavage after arginine and lysine residues (Curtis et al. 2001; Holt et al. 1999; Travis et al. 1997). Gingipains, originally considered "trypsin-like proteases," actually comprise a group of cysteine endopeptidases that have been reported to account for at least 85% of the general proteolytic activity displayed by *P. gingivalis*, and 100% of the expressed "trypsin-like activity." The gingipains, both soluble and cell-associated, are the products of three genes, *rgpA, rgpB*, and *kgp*, encoding these cysteine proteinases. The product of the *kgp* gene (Lys-gingipain, gingipain K) cleaves polypeptide chains exclusively after lysine residues. The products of the *rgp*A and *rgp*B genes (Arggingipain, gingipain R) are proteinases specific for arginine residues (Holt and Ebersole 2005).

Gingipains contribute to the virulence potential of *P. gingivalis* in a multifactorial way, especially by influencing the binding of the bacterium to host tissues. These proteinases may play a role in binding to host cells, either by binding to a cognate receptor or by exposing cryptitope receptors. *P. gingivalis* strains with high levels of trypsin-like protease activity (Arg-gingipain activity) adhere better to human epithelial cells than do strains with lower levels of such activity. The mature forms of Arg-gingipain A and Lys-gingipain possess a catalytic domain and three or four hemagglutinin/adhesin (HA) domains (HA1 to HA4) linked by strong noncovalent bonds. The HA domains of Arg-gingipain A and Lys-gingipain share a high degree of homology (over 97%) and have been implicated in the adherence of *P. gingivalis* to gingival epithelial cells. Gingipains have been shown to play important physiological roles, more particularly in controlling the expression of virulence factors and the stability and/or processing of extracellular and cell-surface proteins (Andrian et al. 2006).

Other proteases of *P. gingivalis* are less well studied than the gingipains with regard to their participation in infection by this microorganism and the inflammatory events of periodontitis. However, several of them might play some role in the host destructive events. Genes coding for collagenase, a protease-hemagglutinin gene, a broadspectrum protease, an endothelin converting like enzyme, a dipeptidyl peptidase, and a reported protease called periodontain have all been isolated and

partially studied (Holt et al. 1999; Holt and Ebersole 2005).

Inavasion of Oral Epithelial Cells

P. gingivalis has developed adaptive strategies to invade gingival epithelial cells and overcome the protective defense mechanisms of epithelial cells. *P. gingivalis* adheres to and invades epithelial cells by targeting specific host receptors, modulating host signaling events and deregulating the host cytokine network. Interactions between *P. gingivalis* and epithelial cells lead to the activation of several complex signaling cascades, which ultimately regulate the transcription of target genes that encode effectors and regulators of the immune response. Effectors of the innate immune system, proinflammatory cytokines, chemokines, MMPs, and antimicrobial peptides are up-regulated and may have a direct impact on disease progression and the inflammation processes, which may contribute to bacterial persistence and the progression of chronic manifestations of periodontal diseases (Andrian et al. 2006; Yilmaz 2008).

2.2.3 *Tannerella forsythia* (Formerly Bacteroides forsythus)

The second member of the red complex of Socransky et al. (1998) is *T. forsythia*. The original isolate, identified as a "fusiform Bacteroides," was first reported in the literature by Tanner et al. in 1979. *T. forsythia* is described as a gram-negative anaerobic fusiform isolated from the human oral cavity. Because of its unique growth requirements (hemin, menadione, L-cysteine, and *N*-acetylneuraminic acid) and the fact that it is a somewhat difficult to grow, its precise role in the severe bone and tissue destruction at sites from which it can be isolated, remains to be determined (Tanner and Izard 2006).

2.2.3.1 Distribution

The microorganism was frequently isolated along with *P. gingivalis* from cases of active chronic periodontitis, and has been frequently associated with severe periodontal disease compared to healthy controls. Periodontitis that progresses posttherapy, "refractory" periodontitis, represents a particularly aggressive form of disease, which has also been associated with detection of *T. forsythia*. Moreover, *T. forsythia* was frequently associated with *P. gingivalis* colonization and was elevated in older groups of patients. Detection of *T. forsythia* was associated with early periodontitis in a comparison of subgingival and tongue samples from healthy subjects and those with early periodontitis. Detection associated with bleeding on probing (BOP) or attachment loss in adolescents has also been recorded, further suggesting an association with early periodontitis, which reveals a relationship to existing disease and potentially active disease sites (Holt and Ebersole 2005). Risk factors for periodontitis have also been linked with increased detection of *T. forsythia*. Detection of *T. forsythia* was associated with subjects who were smokers, positive for aspartate aminotransferase activity, or interleukin-1 genotype (PST test). Systemic disease is frequently associated with lowered resistance to infection, including periodontal infections. *T. forsythia* was associated with viral diseases, subjects infected with Human immunodeficiency virus (HIV), diabetes, and Papillon–Lefèvre syndrome (Tanner and Izard 2006).

2.2.3.2 Virulence Factors

T. forsythia expresses a robust enzymatic repertoire related to its asaccharolytic physiology. *T. forsythia* produces an enzymatic peptidase activity that degrades benzoyl-DL arginine-naphthylamide (BANA), the activity of which appears related to sites of periodontal tissue destruction, and that was originally described as a trypsin-like protease. *T. forsythia* produces lipoproteins (BfLP) that were shown to activate gingival fibroblasts to produce elevated levels of interleukin-6 and TNF-α. Interleukin-6 is known to function in the induction of several acute phase proteins in liver cells involved in cytotoxic T-cell differentiation, as well as the growth of myeloma /plasmacytoma cells. It is also capable of inducing B-cells. Interleukin-6 also induces bone resorption by osteoclast formation with soluble interleukin-6 receptor. In addition, as described above, it is known that *T. forsythia* synthesizes a sialidase and a trypsin-like enzyme, which are thought to be involved in host-cell virulence (Holt and Ebersole 2005).

Moreover, it was demonstrated that *T. forsythia* had the ability to induce apoptosis. Functionally, this activity

of *T. forsythia* might be considered part of the progression of periodontitis. *T. forsythia* appears to invade the periodontal pocket along with *P. gingivalis* (and *T. denticola*), and these species could be attacked by the host's white blood cells. The apoptotic-inducing activity could result in the elimination of host immune or preimmune cells; loss of these host immune cells from the developing periodontal pocket would support bacterial colonization of the pocket and the potential rapid progression of the disease (Holt and Ebersole 2005).

2.2.4 Treponema denticola

T. denticola is but one member of the oral treponemes. These rapidly motile, obligatory anaerobic gram-negative bacteria have been estimated to account for approximately 50% of the total bacteria present in a periodontal lesion. *T. denticola* increases to large numbers in adult periodontitis but is almost undetectable in oral health (Holt and Ebersole 2005; Sakamoto et al. 2005). Treponemes, as a group, often localize at the forefront of periodontal infections, in chronic periodontitis and necrotizing ulcerative gingivitis (NUG), in both invasive disease and when confined to the pocket (Ellen and Galimanas 2005).

Socransky and coworkers' studies have emphasized the cohabitation of oral bacterial species and have demonstrated a pattern of bacterial flora succession, emphasizing a relationship of the various clusters with periodontal disease progression and severity. The cluster composed of highly proteolytic species *P. gingivalis*, *T. forsythia*, and *T. denticola*, the so-called "red" cluster, has the strongest relationship to advanced and progressive periodontitis (Ellen and Galimanas 2005).

Tissue invasion is a hallmark of spirochetal infections, and many oral treponemes have the capacity to penetrate the gingiva as individual species in experimental models or as cohabitants of natural mixed infections. The known virulence factors that determine their invasiveness are the various proteins involved in the synthesis and energetics of flagellar motility, chemotaxis proteins, and the chymotrypsin-like protease, dentilisin (Ellen and Galimanas 2005). *T. denticola* possesses several peptidases associated with its outer sheath. One of these, a prolyl-phenylalanine specific protease, also called chymotrypsin-like protease, appears to be important in *T. denticola* virulence. Isolated in its native form, it appears to exist as a complex protein with a molecular mass of approximately 100 kDa. When denatured, the 100 kDa native protein was separated into three peptides, with molecular masses of 72, 43, and 38 kDa. The 72 kDa protein has been named dentilisin (Holt and Ebersole 2005).

Periodontal pathogens in the proteolytic complex have the ability to degrade human matrix components, which would enhance their tissue penetration and those of neighboring species.*T. denticola* binds extracellular matrix proteins and proteoglycans, elaborates an enzyme that degrades hyaluronic acid and chondroitin sulfate, while its subtilisin family serine protease, dentilisin, has a wide range of protein substrates including fibronectin, laminin, and fibrinogen (Ellen and Galimanas 2005).

T. denticola also synthesizes two low-iron induced outer membrane proteins, HbpA and HbpB that bind hemin. These proteins appear to be necessary for efficient iron utilization, although this microorganism has the ability to replace the function of these proteins by accessing a variety of sources of host iron for nutrition (Ellen and Galimanas 2005).

Elevated levels of *T. denticola* were identified along with nearly a dozen other species in sulfide-positive compared with sulfide-negative sites. The results suggested that the sulfide levels in the pockets reflected the proportion of bacteria, whose metabolism resulted in the production of sulfide as an end-product (Ellen and Galimanas 2005).

2.2.5 Fusobacterium nucleatum

Fusobacterium nucleatum, which refers to a group of three subspecies (nucleatum, vincentii, and polymorphum), is a gram-negative anaerobic bacterium associated with gingivitis and chronic periodontitis. This periodontopathogen has also been implicated in a variety of nonoral infections such as pleuropulmonary infections, urinary tract infections, endocarditis, and intra-amniotic infections (Grenier and Grignon 2006; Boldstad et al. 1996).

The ability of *F. nucleatum* to coaggregate with many plaque bacteria suggests that it acts as a *microbial bridge*

between early and late colonisers. In addition to its ability to coaggregate with many oral bacteria, *F. nucleatum* has also been described as an important initiator organism by promoting physico-chemical changes in the gingival sulcus, allowing pathogenic successors to establish and proliferate. An important change associated with the onset of periodontal disease is the increased alkalinisation of the gingival sulcus. Ammonia produced by the metabolism of amino acids found in gingival crevicular fluid and released by the breakdown of host tissues, leads to an increase in pH above 8.0, thereby promoting the proliferation of acid-sensitive pathogenic bacteria. It was also reported that *F. nucleatum* alters its gene expression according to environmental pH. The ability to form a biofilm and coadhere could be an important virulence mechanism, and may explain the finding in a study on alkali-resistant bacteria in root canal systems that *F. nucleatum* is capable of surviving at pH 9.0 (Zilm and Rogers 2007). It was recently also suggested that *F. nucleatum* facilitates invasion of host cells by *P. gingivalis* (Saito et al. 2008).

Apart from its metabolic versatility, its cell-surface properties enable it to attach to epithelial cells, collagen, gingival epithelial cells and other bacterial genera, but not with other *Fusobacteria*. However, recently it was reported that *F. nucleatum* has been shown to coadhere and form a biofilm, which may be important in the organism's persistence during the transition from health to disease in vivo (Zilm and Rogers 2007).

F. nucleatum was demonstrated to be a significant marker for destructive periodontal disease in adult subjects (van Winkelhoff et al. 2002; Papapanou et al. 2002; Mosca et al. 2007), was identified more often in active sites than in inactive sites (Dzink et al. 1988), and was associated with higher pocket sulfide levels in chronic periodontitis subjects (Torresyap et al. 2003). It has demonstrated high IL-1 and TGF-β production by gingival mononuclear cells extracted from adult periodontitis tissues after stimulation with the putative periodontopathic bacteria, *F. nucleatum* (Gemmell and Seymour 1993).

2.2.6 Prevotella intermedia

P. intermedia, a black-pigmented gram-negative obligate anaerobic nonmotile rod, has received a considerable interest as it was reported to be an important periodontal pathogen, and it was significantly prevalent in patients with chronic periodontitis, aggressive periodontitis, destructive periodontitis, puberty-associated gingivitis and acute NUG (Socransky et al. 1998; Dahlén et al. 1990; Dzink et al. 1983; Moore et al. 1985). In an Australian population, Hamlet et al. (2001) revealed that the odds of a site being *P. intermedia* positive were marginally greater (1.16) for pockets deeper than 3 mm. When Kook et al. (2005) performed microbial screening for predicting the outcome of periodontal treatment in Koreans using a polymerase chain reaction, a close association was revealed between the presence of BOP and the presence of *Prevotella intermedia*. Furthermore, the sites harboring both *T. forsythia* and *P. intermedia* at the baseline had a poorer response to treatment than the sites where these two species were not detected.

Additionally, in vitro invasion of *Prevotella intermedia* to human gingival epithelial cells has been observed (Dorn et al. 1998), and intracellular division of *Prevotella intermedia* in cultured human gingival fibroblasts has been observed by Dogan et al. (2000). Prevotella intermedia induced proinflammatory cytokine expression in human gingival epithelial cells (Sugiyama et al. 2002) and human periodontal ligament (hPDL) cells (Yamamoto et al. 2006; Guan et al. 2006). Pelt et al. (2002) also demonstrated that *P. intermedia* induced pro-MMP-2 and pro-MMP-9 expression in fetal mouse osteoblasts.

In *P. intermedia*, several proteases have been described, among them being trypsin-like serine proteases, a dipeptidyl peptidase IV, CPs (Shibata et al. 2003; Guan et al. 2006; Deschner et al. 2003) and a new cysteine protease from the cysteine-histidine-dyad class, interpain A (Mallorquí-Fernández et al. 2008). *Prevotella intermedia* also possess various types of fimbriae (surface appendages). Some of these surface structures mediate the adherence of the organism to several mammalian erythrocytes, resulting in the agglutination of the erythrocytes (Leung et al. 1999).

2.2.7 Campylobacter rectus

Campylobacter rectus was previously called *Wolinella recta*; it was renamed by Vandamme et al. (1991). *Campylobacter rectus*, a gram-negative, microaerophilic,

round ended, straight, nonglycolytic and motile bacterium has been proposed to play a pathogenic role in human periodontitis. *Campylobacter rectus* has often been detected in large numbers in deeper subgingival pockets (Dzink et al. 1985; Grenier and Mayrand 1996; Tanner et al. 1981; Gmür and Guggenheim 1994; Lai et al. 1992; Listgarten et al. 1993; Lopez et al. 1995; van Steenbergen et al. 1993c; Ihara et al. 2003; Moore et al. 1983; Rams et al. 1993 Macuch and Tanner 2000), and has been implicated in adult periodontitis, rapidly advancing periodontitis and periodontitis associated with certain conditions (pregnancy) (Yokoyama et al. 2005, 2008) and diseases such as AIDS and diabetes (Zambon et al. 1988, 1990). It has been reported that, in adult periodontitis, these organisms were detected more frequently than *P. gingivalis* or *A. actinomycetemcomitans* by using PCR methods, and correlate with clinical parameters, including probing depth and BOP (Ihara et al. 2003). Furthermore, *C. rectus* is also found in combination with other suspected periodontopathogens (Socransky et al. 1998; Haffajee et al. 1988b). Longitudinal studies suggest that *C. rectus* is one of the major species that characterizes sites converting from health to disease (Tanner et al. 1998), and its levels are reduced after periodontal treatment (Haffajee et al. 1988a; Bostanci et al. 2007).

Surface components such as the flagellum, surface layer (S-layer), and GroEL-like protein (GroEL) have been reported as possible virulence factors of the microorganism, and can induce the expression of various inflammatory mediators by host cells (Wang et al. 1998; Ishihara et al. 2001; Hinode et al. 1998, 2002; Braun et al. 1999; Miyamoto et al. 1998). More specifically, *C. rectus* lipopolysaccharide stimulates the production of PGE2, interleukin-1b (IL-1β), and IL-6 by gingival fibroblasts, whereas the crystalline surface layer stimulates the secretion of IL-6, IL-8, and TNF-α by HEp-2 cells derived from a human pharyngeal cancer. *C. rectus* GroEL, which is a 64-kDa heat-shock protein, also stimulates the production of IL-6 and IL-8 by human gingival cells (Yokoyama et al. 2008).

2.2.8 Eikenella corrodens

E. corrodens is a gram-negative, facultative anaerobe, capnophilic, saccharolytic, regular, small rod with blut ends, and may also cause extra-oral infections including abscesses, endocarditis, arthritis, osteomyelitis, keratitis, conjunctivitis and cellulitis (Haffajee and Socransky 1994; Chen and Wilson 1992; Fujise et al. 2004; Chang and Huang 2005; Karunakaran et al. 2004).

E. corrodens is found predominantly in subgingival plaque in patients with advanced periodontitis (Nonnenmacher et al. 2001; Noiri et al. 2001; Salari and Kadkhoda 2004). The frequency of cultivable *E. corrodens* from subgingival sulci of healthy, adult periodontitis and juvenile periodontitis patients was reported as 10, 52 and 59%, by Chen et al. (1989). In periodontitis patients, *E. corrodens* was related to disease active sites compared to inactive sites either before or after successful periodontal therapy, emerging as possible periodontal pathogen (Tanner et al. 1987). Additionally, the monoinfection of germ-free rats with *E. corrodens* causes periodontal disease with severe alveolar bone loss (Crawford et al. 1977; Noiri et al. 2001; Cortelli and Cortelli 2003; Apolônio et al. 2007).

It was reported that *E. corrodens* 1,073 has a cell-associated *N*-acetyl-D-galactosamine (GalNAc) specific lectin-like substance (EcLS) that mediates its adherence to various host tissue cell surfaces and oral bacteria, induces ICAM-1 production by human oral epithelial cells, and also stimulates the proliferation of murine B cells. EcLS is a large molecule and is composed of several components including 25-, 45- and 300-kDa proteins. Moreover, it has been shown that soluble products from *E. corrodens* 1,073 induce the secretion and the expression of IL-8 by a human oral epidermoid carcinoma cell line (KB) (Yamada et al. 2002). Nevertheless, despite the many virulence factors exhibited by *E. corrodens*, such as lipopolysaccharides, proteins of the outer membrane, adhesins and the exopolysaccharide layer (Chen and Wilson 1992), antagonistic substances produced by this bacterium have only recently been reported (Apolônio et al. 2007, 2008).

2.2.9 Parvimonas micra (Previously Peptostreptococcus micros or Micromonas micros)

One of the suspected pathogens related to periodontal disease is the gram-positive anaerobic coccus *Peptostreptococcus micros*. Although it is considered a natural commensal of the oral cavity, elevated levels of

this organism are not only associated with chronic, aggressive periodontitis and with active sites of periodontal destruction (Rams et al. 1992a; von Troil-Larsen et al. 1995; Socransky et al. 1998; Choi et al. 2000; Gajardo et al. 2005; van Winkelhoff et al. 2005; Salari and Kadkhoda 2004; Haffajee et al. 2004; Lee et al. 2003; van Winkelhoff et al. 2002), but also with periodontal decline in old adults (Swoboda et al. 2008). Nonnenmacher et al. (2001) evaluated the prevalence of *P.micros* in different periodontal disease groups, and reported a prevalence of 6.3% in rapidly progressive periodontitis, 5.1% in adult (now known as chronic) periodontitis and 2.9% in localized juvenile (now known as localized aggressive) periodontitis. Significantly higher numbers of *P. micros* were present in smokers and associated with moderate and deep pockets (van der Velden et al. 2003; Gomes et al. 2006). When heavy smokers were considered, higher counts of total bacteria, *M. micros*, and *D. pneumosintes* were observed (Gomes et al. 2006). *P. micros* has also been associated with infected dental root canals (Gomes et al. 2004) and dentoalveolar infection (Dymock et al. 1996; Kuriyama et al. 2007).

Van Dalen et al. (1993) reported the existence of two morphotypes (rough and smooth) of *P. micros*, which differ in the presence of cell-associated fibrillike appendages, in the composition of cell wall proteins, the surface hydrophobicity, and in the ability to lyse erythrocytes. Both morphotypes may be recovered from subgingival plaque and are likely acting as opportunistic pathogens, in association with gram-negative bacteria, to contribute to periodontitis (van Dalen et al. 1998; Grenier and Bouclin 2006).

The virulence factors produced by *P. micros*, which may play a role in the pathogenesis of periodontitis, are poorly characterized. *P.micros* is able to adhere to epithelial cells and to other periodontopathogens, including *Porphyromonas gingivalis* and *Fusobacterium nucleatum* (Kremer et al. 1999; Kremer and van Steenbergen 2000). *P.micros* cells have also the ability to bind *A. actinomycetemcomitans* lipopolysaccharide on their surface, thus significantly increasing their capacity to induce TNF-α production by human macrophages (Yoshioka et al. 2005). It was also showed that the *P.micros* cell wall preparation induced intracellular signaling pathways, leading to an increased production of proinflammatory cytokines, chemokines and MMP-9 by macrophages (Tanabe et al. 2007). Recently, Grenier and Bouclin (2006) provided evidence that the proteolytic and plasmin-acquired activities of *P. micros* may facilitate the dissemination of bacterial cells through a reconstituted basement membrane. Kremer et al. (1999) reported the ability of *P. micros*, more particularly the smooth morphotype, to adhere to oral epithelial cells. Gelatinase and hyaluronidase activities produced by *P. micros* have also been reported (Ng et al. 1998; Tam and Chan 1984).

2.2.10 Selenomonas species

Selenomonas sp. are gram-negative, curved, saccharolytic rods that may be recognized by their curved shape, tumbling motility, and in good preparations, by the presence of a tuft of flagella inserted in the concave site. The organisms have been somewhat difficult to grow and speciate (Haffajee and Socransky 1994).

In patients with generalized aggressive periodontitis, *S. sputigena* was the most frequently detected bacterial species, often at high levels of about 20% of the total bacterial population. This gram-negative, multiflagellated, motile, anaerobic rod has also been previously associated with necrotizing ulcerative periodontitis (Gmür et al. 2004), rapidly progressive periodontitis (Kamma et al. 1995), and in smokers with early onset periodontitis (Kamma and Nakou 1997) and active periodontitis lesions (Haffajee et al. 1984; Tanner et al. 1998; Faveri et al. 2008). Other predominant *Selenomonas* sp. are *Selenomonas* sp. oral clone EW084, *Selenomonas* sp. oral clone EW076, *Selenomonas* sp. oral clone FT050, *Selenomonas* sp. strain GAA14, *Selenomonas* sp. Oral clone P2PA_80, and Selenomonas noxia. All of these have been previously associated with oral infections (Kumar et al. 2005; Paster et al. 2001, 2002; Faveri et al. 2008). Selenomonas noxia was found at significantly higher levels in periodontal pocket sulfide levels (Torresyap et al. 2003).

2.2.11 Eubacterium species

Eubacterium nodatum, *Eubacterium brachy,* and *Eubacterium timidum* are gram-positive, strictly anaerobic, small and somewhat pleomorphic rods. They are often difficult to cultivate, particularly on primary

isolation, and appear to grow better in roll tubes than on blood agar plates. The *Eubacterium* sp. appear to be promising candidates as periodontal pathogens; however, difficulty in their cultivation has slowed assessment of their contribution (Haffajee and Socransky 1994).

Moore and Moore (1994) used the roll tube cultural technique to examine the proportions of bacterial species in subgingival plaque samples from subjects with various forms of periodontitis and gingivitis, and in healthy subjects. They found that *E. nodatum* was absent or in low proportions in periodontal health and various forms of gingivitis, but was present in higher proportions in moderate periodontitis (2%), generalized early onset periodontitis (8%), localized juvenile periodontitis (6%), early onset periodontitis (5%) and adult periodontitis (2%). *E. nodatum* was in the top two to 14 species enumerated in these different periodontal states (Haffajee et al. 2006). More recent studies have confirmed an association of *E. nodatum* with periodontitis using molecular techniques. Using species-specific oligonucleotide probes, Booth et al. (2004), revealed that simple comparisons of *E. nodatum* at shallow sites in periodontitis patients and healthy controls and at deep and shallow sites in the patient group suggested that *E. nodatum* was associated with periodontal disease. A strong association of *E. nodatum* and *T. denticola* with periodontitis, whether in the presence or absence of high levels of the consensus pathogens, was recently emphasized (Haffajee et al. 2006). Similar data were reported by Papapanou et al. (2000) and Colombo et al. (2002). *E. nodatum* was found to be significantly higher in current smokers than nonsmokers (Haffajee and Socransky 2001, 2006).

2.2.12 Streptococcus intermedius

Cultural studies of the last decade have suggested the possibility that some of the *streptococcal* sp. are associated with and may contribute to periodontal disease progression. At this time, evidence suggests that *S. intermedius* or closely related species may contribute to disease progression in subsets of periodontal patients (Haffajee and Socransky 1994).

Polymorphisms in the cluster of IL-1 genes have been significantly associated with the severity of adult periodontitis. When microbiological parameters in IL-1 genotype negative and positive adult subjects with a range of periodontitis severities were compared, it was observed that the proportion of IL-1 genotype positive subjects that exhibited mean counts of specific subgingival species above selected thresholds was significantly higher than the proportion of genotype negative subjects. Significantly higher mean counts of *S. intermedius* were detected at periodontal pockets >6 mm in subjects who were genotype positive when compared with genotype negative subjects. The increase was due to increased numbers of cells of these species rather than a major shift in proportion (Socransky et al. 2000). When the mean frequency of detection of *Streptococcus intermedius* in epithelial cell samples from 49 periodontitis subjects was evaluated, the obtained percent was 36% (Colombo et al. 2006).

When subgingival microflora in periodontal disease patients was assessed, the detection rates for *A. actinomycetemcomitans*, *E. corrodens, S. noxia* and *S. intermedius* varied between 58–83% in smokers and 55–82% in nonsmokers. *S. intermedius* was the only species for which the detection rates in smokers and nonsmokers differed more than 10%-U (Boström et al. 2001).

2.2.13 Other Species

Interest has grown in groups of species not commonly found in the subgingival plaque as initiators or possibly contributors to the pathogenesis of periodontal disease, particularly in individuals who have responded poorly to periodontal therapy. Species not commonly thought to be present in subgingival plaque can be found in a proportion of such subjects or even in subjects who have not received periodontal treatment (Haffajee and Socransky 1994).

In order to elucidate the range of species of nonoral, gram-negative, facultatively anaerobic rods in human periodontitis, Slots et al. (1990a, b) has studied 3,050 advanced periodontitis patients and obtained pooled samples from 9,150 deep periodontal pockets. *Enterobacter cloacae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Klebsiella oxytoca* and *Enterobacter agglomerans* were the most frequently isolated species, accounting for more than 50% of all strains. It was suggested that some species of this group of organisms can be cofactors in destructive periodontitis and they should not be given the benefit of doubt in the treatment. These bacteria are often recovered from the subgingival microbiota of patients considered to be clinically refractory to mechanical and antibiotic periodontal treatment.

Preliminary clinical findings indicate that systemic ciprofloxacin administration, but not conventional periodontal therapy, may cure periodontal infections with these organisms (Slots et al. 1990c).

Enterococci are bile-tolerant, facultatively anaerobic, chaining gram-positive cocci that are common inhabitants of the human gastrointestinal and genitourinary tracts as normal commensals. They can cause a variety of diseases in humans, infecting the urinary tract, bloodstream, endocardium, abdomen, biliary tract, burn wounds, and indwelling foreign devices (Jett et al. 1994). Enterococci are also able to colonize a variety of other sites, including the oral cavity, where they have been associated with oral mucosal lesions in immunocompromised patients, periodontitis and root canal infections (Pinheiro et al. 2006; Wahlin and Holm 1988; Rams et al. 1992b). Of the *enterococcal* sp. associated with colonization and infection in humans, Enterococcus faecalis is the most common (Pinheiro et al. 2006).

However, very few studies have evaluated the correlation between the prevalence of *E. faecalis* and periodontal diseases (Rams et al. 1992b; Souto et al. 2006; Gonçalves Lde et al. 2007; Pinheiro et al. 2006). Rams et al. (1992b) detected *E.faecalis* in 1% of early onset periodontitis and 5.1% of chronic periodontitis patients using cultural methods, whereas Souto and Colombo (2008) found a much higher prevalence of this species (80%) in a large number of subgingival biofilm samples from periodontitis patients. In addition, these authors observed that this bacterium was much more prevalent in healthy sites from periodontitis patients as compared to sites in periodontally healthy individuals. Souto and Colombo (2008) showed a significantly higher frequency of *E. faecalis* in saliva (40.5%) and subgingival biofilm samples (47.8%) from periodontitis patients compared to periodontally healthy controls (14.6 and 17.1%, respectively). Similar data were reported by Colombo et al. (2002), who examined the presence and levels of *E. faecalis* in the subgingival microbiota of untreated periodontitis patients and healthy controls using the checkerboard method (Souto and Colombo 2008).

Staphylococcal sp., in particular *Staphylococcus epidermidis* and *S. aureus*, dominate the microbial aetiology of prosthetic valve endocarditis. However, the oral cavity has been established as a source of the organisms for native valve endocarditis (NVE), where *Viridans streptococci* are responsible for 50% of cases (Debelian et al. 1994).

Staphylococci have been isolated from the oral cavity, but they are not considered resident oral bacteria and are generally regarded as transient organisms. While it is not clear whether there is a causal relationship between staphylococci and chronic periodontal disease (Dahlén and Wikström 1995), staphylococci have been isolated from subgingival sites within periodontitis patients (Rams et al. 1990a, b; Slots et al. 1990a, b; Dahlén and Wikström 1995; Murdoch et al. 2004). However, few subgingival plaque samples have been collected from nondiseased sites, and consequently, it has not been possible to determine if the isolation of staphylococci was because of the diseased state of the tissues or whether staphylococci are a feature of all subgingival sites (Murdoch et al. 2004). In a recent study, staphylococci were isolated from 54% diseased subgingival and 43% healthy subgingival sites in over 50% periodontitis patients and from 29% healthy subgingival sites in 54% controls. No significant differences in the frequency of isolation or numbers of staphylococci isolated from diseased and healthy sites were noted. *Staphylococcus epidermidis* was the predominant oral species.

2.3 Ecologic Relationships Among Bacterial Species and Between Bacterial Species and the Host

Periodontitis is caused by mixed bacterial infection in the oral cavity. Pathogenic subgingival microorganisms may be responsible for initiation/progression of periodontal diseases. Among them include *Porphyromonas gingivalis*, *Tannerella forsythia*, *Aggregatibacter* (*Actinobacillus*) *actinomycetemcomitans*, *Prevotella intermedia,* and *Treponema denticola*. These bacteria are usually found in combination in periodontal pockets rather than alone, suggesting that some of the bacteria may cause destruction of the periodontal tissue in a cooperative manner (Yoneda et al. 2005; Haffajee and Socransky 1994).

T. forsythia has been found in subgingival plaques in patients with severe periodontitis along with *P. gingivalis* (Jervøe-Storm et al. 2005; Tanner and Izard 2006; Simonson et al. 1992), and cooperates with *P. gingivalis* to form severe abscesses in rabbits and mice (Yoneda et al. 2001). It was suggested that *P. gingivalis* with either *T. forsythia* or *T. denticola* directly induces synergistic IL-6 protein production and that gingipains play a role in this synergistic effect (Tamai et al. 2009). Sonicated cell extracts from *T. forsythia*

stimulate the growth of *P. gingivalis* (Yoneda et al. 2005), while the outer membrane vesicles produced by *P. gingivalis* enhance the attachment to and invasion of epithelial cells by *T. forsythia* (Inagaki et al. 2006). It was also observed that *P. gingivalis*, *T. denticola*, and *T. forsythia* stimulate the secretion of proinflammatory cytokines (IL-1β, IL-6), chemokines (IL-8, RANTES), PGE_2, and MMP-9 in a macrophage/epithelial cell coculture model. This indicates that these periodontopathogens have a strong potential for activating host-mediated destructive processes. No synergistic effects on cytokine, chemokine, PGE_2, or MMP-9 production were observed for the bacterial mixtures compared to monoinfections by individual bacterial species. This study supports the view that bacterial species of the red complex act in concert to increase the levels of proinflammatory mediators and MMP-9 in periodontal tissues, a phenomenon that may significantly contribute to the progression of periodontitis (Bodet et al. 2006).

Moreover, in a murine abscess model, combinations of *P. gingivalis–Fusobacterium nucleatum*, *P. gingivalis–T. denticola*, and *P. gingivalis–A. actinomycetmcomitans* exhibited enhanced virulence compared to monoinfections (Ebersole et al. 1997; Chen et al. 1996; Kesavalu et al. 1998; Yoneda et al. 2005).

The data from these investigations suggests that different complexes of microbial species reside in different periodontal pockets. The major ecological determinants are probably provided by the interactions that take place between the host and resident subgingival species, and between the different species. These interactions can produce a series of selective pressure, which determine the composition of the microbiota in the subgingival sites (Socransky et al. 1988).

Several prerequisites are necessary for periodontal disease initiation and progression: the virulent periodontal pathogen, the local environment and the host susceptibility.

– *The virulent periodontal pathogen*

It seems unlikely, however, that a single factor alone will be entirely responsible for the virulence of *P. gingivalis*. Several studies have shown, by the use of animal models, that virulent and avirulent strains exist within the species of *P. gingivalis*; differences in hydrophobicity and hemagglutinating activity between pigmented and non-pigmented strains, however, suggest additionally that structural differences also occurred between both groups of strains (Shah et al. 1989; Neiders et al. 1989; Smalley et al. 1989). Besides the virulence strain, another requirement is that the organism possesses all of the necessary genetic elements. Some of these elements might be missing in a strain inhabiting the gingival crevice area but could be received from other strains of that species via bacteriophages, plasmids or tranposons. Thus, periodontally healthy sites might be colonized with periodontal pathogens without a full complement of genes needed to lead to tissue destruction (Haffajee and Socransky 1994).

– *The local environment*

In a complex ecosystem, such as the periodontal pocket, antagonistic and commensal relationships are to be expected. Most of these would have minimal impact on the health of the periodontium. However, such relationships may be causally related to the maintenance of health or the initiation and progression of disease, particularly when putative periodontal pathogens are involved. Certain viridans streptoccocci, by virtue of their ability to produce hydrogen peroxide, appear to promote periodontal health by keeping the numbers of potentially pathogenic organisms below the threshold level necessary to initiate disease. Certain types of periodontal disease may therefore result from an ecological imbalance, which arises from the following sequence of events: first, unknown factors promote the relative outgrowth of an organism such as *A. actinomyctemcomitans,* which produces a factor inhibitory to the growth of certain *streptococcal* sp.; this result in an reduction of local hydrogen-peroxide production, which in turn permits the outgrowth of various periodontal pathogens (Hillman et al. 1985). The local subgingival environment can affect disease pathogenesis in other ways. It has been showed that iron is an essential requirement for the growth of most microorganisms. Pathogenic microorganism has developed specific mechanisms to obtain iron from host protein. The most extensively studied iron uptake system is that used by many aerobic organisms and depends on the production of siderophores. Iron restriction in the environment increases the expression of a number of virulence factors of *P. gingivalis* (Barua et al. 1990).

– *The host susceptibility*

A number of host factors have been suggested to affect the initiation and rate of progression of periodontal diseases. Such factors include defects in PMN

levels or function, a poorly regulated immune response, smoking, diet and various systemic diseases. In HIV-positive and diabetic subjects, it has been shown that the periodontal lesions, for the most part, appeared to be related to already suspected periodontal pathogens and not to some novel species. It seems that altered host susceptibility may change the rate of disease progression in affected individuals, and largly, the periodontal pathogens are likely to be the same as those found in uncompromised subjects (Haffajee and Socransky 1994).

2.4 The Possible Role of Viruses in the Pathogenesis of Periodontal Diseases

Even though specific infectious agents are of key importance in the development of periodontitis, it is unlikely that a single agent or even a small group of pathogens are the sole cause or modulator of this heterogeneous disease (Slots 2005).

2.4.1 HIV Infection

HIV is a retrovirus with special affinity for the CD4 receptor molecule, which is situated on the surface of T-helper lymphocytes. Other infected cell populations include monocytes and macrophages, Langerhans' cells, B lymphocytes, endothelial cells and cells in the brain. As a result of the HIV infection, the number of CD4+ cells decreases. The ratio of T-helper to T-suppressor lymphocytes (the CD4+:CD8+ cell ratio) is increasingly reduced with the progression of disease, and the function of the entire immune system of the host is widely affected. The host thereby becomes susceptible to several infectious diseases and neoplasms (Holmstrup and Westergaards 1998).

A consensus has been reached by the EC-WHO on the classification of the oral manifestations of HIV infection and their diagnostic criteria, based on presumptive and definitive criteria. The former relate to the initial clinical appearance of the lesion and the latter are often the result of special investigations. Candidiasis, hairy leukoplakia, specific forms of periodontal disease [linear gingival erythema (LGE), necrotising-(ulcerative) gingivitis (NG) and necrotising (ulcerative) periodontitis], Kaposi's sarcoma and non-Hodgkin's lymphoma are *strongly associated with HIV infection. Lesions less commonly associated with HIV infection includes:* bacterial infections, melanotic hyperpigmentation, necrotising (ulcerative) stomatitis, salivary gland disease, thrombocytopenic purpura, ulcerations, viral infections, *while lesions seen in HIV infection, but not indicative of the disease*, are: bacterial infections, cat-scratch disease, drug reactions, epitheloid (bacillary) angiomatosis, fungal infection other than candidiasis, neurologic disturbances, recurrent aphtous stomatitis, and viral infections (Table 2.3).

- *Linear gingival erythema*

LGE is a distinct fiery red band along the margin of the gingiva. The amount of erythema is disproportionately intense for the amount of plaque seen. No ulceration is present and, according to the EC-WHO criteria, there is

Table 2.3 Revised classification of oral lesions associated with HIV infection (modified from WHO-EC 1993)

Lesions strongly associated with HIV infection	Lesions less commonly associated with HIV infection includes	Lesions seen in HIV infection, but not indicative of the disease
Candidiasis (erythematous, pseudomembranous)	Bacterial infections (*Mycobacterium avium-intracellulare, Mycobacterium tuberculosis*)	Bacterial infections (*Actinomyces israellii, Escherichia coli, Klebsiella pneumoniae*)
Hairy leukoplakia	Melanotic hyperpigmentation	Cat-scratch disease
Specific forms of periodontal disease [linear gingival erythema, necrotising-(ulcerative) gingivitis and necrotising (ulcerative) periodontitis],	Necrotising (ulcerative) stomatitis	Drug reactions
	Salivary gland disease	Epitheloid (bacillary) angiomatosis
	Thrombocytopenic purpura	Fungal infection other than candidiasis
Kaposi's sarcoma	Ulcerations NOS	Neurologic disturbances
Non-Hodgkin's lymphoma	Viral infections (*Herpes simplex virus* (HSV), *Human papillomavirus, Varicella-zoster virus*)	Recurrent aphtous stomatitis
		Viral infections (*Cytomegalovirus, Molluscum contagiosum*)

no evidence of pocketing or attachment loss. A characteristic feature of this type of lesion is that it does not respond well to improved oral hygiene nor to scaling (Holmstrup and Westergaards 1998). Treatment includes debridement by a dental professional, twice-daily rinses with a 0.12% chlorhexidine gluconate suspension for 2 weeks, and improved home oral hygiene (Reznik 2005).

The prevalence of LGE in HIV infected population varies from 0 to 49%, and this considerable variation can be due to the lack of clear diagnostic standardization and the heterogeneity of the populations studies (Cappuyns et al. 2005).

- *Necrotising-(ulcerative) gingivitis*

Necrotising gingivitis, necrotising periodontitis, and necrotizing stomatitis may be different stages of the same disease (Cappuyns et al. 2005). HIV-related necrotizing gingivitis is defined by ECWHO as destruction of one or more interdental papillae. In the acute stage of the process ulceration, necrosis and sloughing may be seen with ready hemorrhage and characteristic fetor.

The available information about the microbiology of HIV-associated necrotizing gingivitis is limited. The isolated organisms include *Borrelia,* gram-positive cocci, beta-hemolytic streptococci and *C.albicans* (Holmstrup and Westergaards 1998).

- *Necrotizing (ulcerative) periodontitis (NP)*

Although necrotizing gingivitis and necrotizing periodontitis may reflect the same disease entity, they are differentiated by the rapid destruction of soft tissue in the former condition and hard tissue in the latter. Necrotizing ulcerative periodontitis is a marker of severe immune suppression. The condition is characterized by severe pain, loosening of teeth, bleeding, fetid odor, ulcerated gingival papillae, and rapid loss of bone and soft tissue. Patients often refer to the pain as "deep jaw pain" (Holmstrup and Westergaards 1998; Tirwomwe et al. 2007).

The occurrence of *P.gingivalis,* spirochetes and motile eubacteria in periodontitis has been found to be similar in HIV-infected patients and systemically healthy adults. Moreover, the microflora found in HIV-associated periodontitis was similar to that of classical adult periodontitis, except that *P.gingivalis* was more prevalent in conventional periodontitis (Holmstrup and Westergaards 1998; Feller and Lemmer 2008).

It has been suggested that NP may be used as a marker for immune deterioration, with a 95% predictive value that CD4+ cell counts have decreased below 200 cells μl^{-1}). If untreated, the cumulative probability of death within 24 months is 72.9% (Cappuyns et al. 2005). NG/NP, in otherwise systemically healthy individuals, is strongly correlated with HIV infection, with a predictive value of 69.6%. It is recommended that patients presenting with these conditions be encouraged to undergo testing to establish their HIV status for appropriate referrals and management (Shangase et al. 2004).

Necrotizing periodontitis in HIV-infected patients does not always respond to conventional treatment with scaling and improved oral hygiene (Holmstrup and Westergaards 1998). However, treatment includes removal of dental plaque, calculus, and necrotic soft tissues, utilizing a 0.12% chlorhexidine gluconate or 10% povidone-iodine lavage, and institution of antibiotic therapy (Reznik 2005).

- *Necrotizing stomatitis*

Necrotizing stomatitis is described as a localized acute, painful ulceronecrotic lesion of the oral mucosa that exposes underlying bone or penetrates or extends into contiguous tissues. The lesions may extend from areas of necrotizing periodontitis. The lesions are acute, extensively destructive, rapidly progressive, ulcerative, and necrotizing. Usually, the lesions extend from the gingiva into adjacent mucosa and bone causing massive destruction of the oral soft tissues and underlying bone. Like HIV-associated periodontitis, it appears to be related to the immune depletion caused by HIV infection and, importantly, it may be life-threatening. Extensive denudation of bone may result in sequestration. Progression of necrotizing periodontitis to necrotizing stomatitis may subsequently result in progressive osseous destruction with the development of oroantral fistula and osteitis (Holmstrup and Westergaards 1998).

- *Conventional chronic and aggressive periodontitis*

In addition to the specific forms of periodontal disease described below, it should be appreciated that chronic marginal gingivitis and adult periodontitis can occur in patients with HIV infection. The clinical appearances of these conditions may, however, be altered or exagerated as a result of immunosupression (WHO-EC 1993).

The reported prevalence rates of periodontitis among HIV-seropositive patients show considerable variation (5–69%) (Holmstrup and Westergaards 1998). Recently, Kroidl et al. (2005) revealed that compared with data of oral diseases of the pre-HAART (highly active antiretroviral therapy) era, prevalence of HIV-specific lesions was markedly reduced. Among 139

HIV + patients, 86% presented any oral lesions with a prevalence of 76% of any periodontal diseases. Most periodontal lesions were classified as conventional gingivitis (28%) or periodontitis (30%). Prevalence for HIV-specific oral lesions was 29%, with a proportion of 9% of LGE, 3.6% of necrotizing and ulcerative gingivitis or periodontitis, 7% of oral candidiasis, 3.6% of oral hairy leucoplakia and single other lesions. Lack of oral hygiene determined by plaque formation and reduced CD4-counts with pronounced periodontal inflammation could be seen as risk factors for periodontal disease in HIV + patients (Kroidl et al. 2005).

It has been suggested that HIV-infected patients are at risk of advanced periodontal disease with severe gingivitis, gingival recession, and alveolar bone loss (Alpagot et al. 2004). One of the most recent longitudinal studies evaluates periodontal probing depth (PD), clinical attachment level (CAL), and tooth loss from 584 HIV-seropositive and 151 HIV-seronegative women, recorded at 6-month intervals from 1995 to 2002. Adjusted longitudinal analysis showed that CD4 count and viral load had no consistent effects on PD or AL. Among HIV-infected women, a 10-fold increase in viral load was associated with a marginal increase in tooth loss. The progression of periodontal disease measured by PD and AL did not significantly differ between HIV-infected and HIV-uninfected women. The HIV-seropositive women lost more teeth (Alves et al. 2006).

Information on the microbiota associated with periodontitis in HIV-positive patients is controversial as several studies have shown that there is a similar microbial flora in both groups, and no difference in the distribution of black pigmented anaerobes could be observed (Patel et al. 2003; Botero et al. 2007a; Gonçalves Lde et al. 2007). It has been also shown that *Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum, Aggregatibacter (Actinobacillus) actinomycetemcomitans, Eikenella corrodens* and *Campylobacter rectus* may occur in diseased sites of HIV-positive patients and non-HIV-infected subjects with classical periodontitis (Patel et al. 2003). HIV-periodontitis seems also to be associated with elevated occurrence of Epstein–Barr virus type 2 (EBV-2), human herpes virus (HHV)-6 and herpesvirus coinfections compared to periodontitis in non-HIV-patients (Contreras et al. 2001).

Several risk factors for periodontitis in HIV + individuals were identified, including age, smoking pack-years, viral load, *F. nucleatum, P. intermedia, A. actinomycetemcomitans*, neutrophil elastase, and β-glucuronidase in gingival crevicular fluid (Alpagot et al. 2004). It has been also reported that sites with high ginigval crevice fluid levels of MMP-9 and TIMP-1 in HIV-positive patients are at significantly greater risk for progression of periodontitis (Alpagot et al. 2006).

In HIV-1-seropositive chronic periodontitis patients receiving periodontal therapy by conservative scaling and root planning and maintenance care, it has been showed that periodontal inflammatory parameters improved significantly under the immune reconstituting influence of highly active antiretroviral therapy (Jordan et al. 2006) (Table 2.4).

2.4.2 Herpesviruses

Studies during the past 10 years have associated herpesviruses with human periodontitis. The involvement of herpesviruses in the etiology of periodontal diseases is suggested by their presence in gingival tissue, gingival cervicular fluid and subgingival plaque, in the presence of periodontal disease (Cappuyns et al. 2005).

Of the approximately 120 identified different herpesviruses, eight major types are known to infect humans, namely, herpes simplex virus (HSV) type 1 and 2, varicella-zoster virus, EBV, Human cytomegalovirus (HCMV), (HHV)-6, HHV-7, and HHV-8 (Kaposi's sarcoma virus) (Slots et al. 2005). Genomes of HCMV and EBV-1 were detected in severe adult periodontitis, localized juvenile periodontitis, generalized juvenile periodontitis, Papillon-Lefèvre syndrome periodontitis, Down's syndrome periodontitis, periodontal abscesses, HIV-associated periodontitis and acute NUG (Slots and Contreras 2000; Saygun et al. 2004b).

Herpesviruses may cause periodontal *pathogenesis* as a direct result of virus infection and replication, or as a consequence of virally induced impairment of the periodontal immune defense, resulting in heightened virulence of resident bacterial pathogens (Slots 2005). An infectious disease model for the development of periodontitis based on herpesvirus-bacteria–host interactive responses was proposed by Slots (2005). Herpesvirus infection of periodontal sites may be important in a multistage pathogenesis by altering local host responses. Initially, bacterial infection of the gingival causes inflammatory cells to enter gingival tissue, with periodontal macrophages and T-lymphocytes harboring latent HCMV and periodontal B-lymphocytes harboring latent EBV (Contreras et al. 1999). Reactivation of herpesviruses from latency may occur spontaneously or during

Table 2.4 Recent studies evaluating the periodontal conditions in HIV positive patients

Author, year	Country	Study population	Periodontal status	Main findings
Contreras et al. 2001	USA	21 HIV + patients and 14 HIV-negative patients	Chronic periodontitis	HIV-periodontitis seems to be associated with elevated occurrence of EBV-2, HHV-6 and herpesvirus coinfections compared to periodontitis in non-HIV-patients
Patel et al. 2003	South-Africa	20 HIV + patients and 20 HIV-negative patients	Chronic periodontitis	The results showed a significant prevalence of *P.gingivalis* and *Treponema denticola* among HIV-negative patients compared to HIV-positive patients. Odds ratio analysis revealed a statistically significant positive association between three of the 28 possible combinations in the HIV-positive group. They included *Prevotella nigrescens/Campylobacter rectus, P. nigrescens/ P. gingivalis* and *P. nigrescens/T. denticola*
Alves et al. 2006	USA	584 HIV-seropositive and 151 HIV-seronegative women	Chronic periodontitis	The progression of periodontal disease did not significantly differ between HIV-infected and HIV-uninfected women
Aas et al. 2007	USA	14 HIV + patients	Gingivitis, Chronic periodontitis, LGE	The classical putative periodontal pathogens, *Treponema denticola, Porphyromonas gingivalis* and *Tannerella forsythia* were below the limit of detection and were not detected. Species of *Gemella, Dialister, Streptococcus* and *Veillonella* were predominant
Botero et al. 2007a	Columbia	31 HIV + periodontitis patients, 32 HIV-negative periodontitis patients and 32 systemically and periodontally healthy patients	Chronic periodontitis	HIV + patients harbor higher levels of superinfecting microorganisms. *Pseudomonas aeruginosa, Enterobacter cloacae* and *Klebsiella pneumoniae* were identified
Gonçalves Lde et al. 2007	Brazil	72 subjects were distributed into two HIV-seropositive groups (37 chronic periodontitis and 35 periodontally healthy individuals) and two HIV-seronegative groups (49 chronic periodontitis and 51 periodontally healthy subjects)	Chronic periodontitis	Periodontal pathogens including *Tannerella forsythensis, Porphyromonas gingivalis, Prevotella nigrescens, Eubacterium nodatum, Fusobacterium nucleatum,* and *Selenomonas noxia* were more frequently detected in H-CP + subjects compared to H + CP + and controls. In contrast, *Enterococcus faecalis* and *Acinetobacter baumannii* were more commonly found in HIV-infected than in non-HIV-infected subjects
Grande et al. 2008	Brazil	50 HIV + patients and 50 HIV-negative patients	Chronic periodontitis	EBV-1 was more frequently recovered in oral sites of HIV-positive patients than in HIV-negative patients

HSV-1 herpes simplex virus type 1; *EBV* Epstein–Barr virus; *HCMVEBV-1* human cytomegalovirus

periods of impaired host defense, resulting from immunosuppression, infection, physical trauma, hormonal changes, etc (Slots 2005). Herpesvirus-activating factors are also known risk factors/ indicators for periodontal disease. Herpesviral activation leads to increased inflammatory mediator responses in macrophages, and probably also in connective tissue cells within the periodontal lesion (Slots 2005). After reaching a critical virus load, activated macrophages and lymphocytes may trigger a cytokine/chemokine "storm" of IL-1β, TNF-α, IL-6, prostaglandins, interferons, and other multifunctional mediators, some of which have the potential to propagate bone resorption. Herpesvirus induced immune impairment may also cause an upgrowth of resident gram-negative anaerobic bacteria, whose lipopolysaccharide together with HCMV, as discussed above, can induce cytokine and chemokine release from various mammalian cells, and may act synergistically in stimulating IL-1 gene transcription (Slots 2005; Botero et al. 2008a). In a vicious circle, the triggering of cytokine responses may activate latent herpesviruses, and in so doing, may further aggravate periodontal disease. Similarly, medical infections by HCMV can lead to increased susceptibility to bacterial and fungal infections and enhance the severity of existing microbial infections (Slots 2005). It is conceivable that herpesviruses rely on coinfection with periodontal bacteria to produce periodontitis and, conversely, periodontopathic bacteria may depend on viral presence for the initiation and progression of some types of periodontitis (Slots 2005). It was also showed that herpesvirus-infected periodontitis lesions tend to harbor elevated levels of classic periodontopathic bacteria, including *Porphyromonas gingivalis*, *Dialister pneumosintes*, *Prevotella intermedia, Prevotella nigrescens*, *Campylobacter rectus, Treponema denticola* and *Actinobacillus (Aggregatibacter) actinomycetemcomitans* (Slots 2005, 2007; Hanookai et al. 2000; Sunde et al. 2008; Saygun et al. 2004a; Kamma et al. 2001).

Table 2.5 resumes the occurrence of herpesviruses in periodontitis patients.

2.5 Transmission of Periodontal Pathogens

In periodontitis, as in other infectious diseases, knowledge of the source of pathogens and the route of infection is important for planning prevention strategies. It was suggested, based on the facts that periodontal pathogens cluster in families, that bacteria are transmitted between family members or that family members share susceptibility to colonisation of these bacteria (Asikainen and Chen 1999). Two types of transmission are recognized, vertical, that is, transmission from parent to offspring and horizontal, that is, passage of an organism between individuals out-side the parent-offspring relationship (Haffajee and Socransky 1994) (Table 2.6).

In *A. actinomycetemcomitans*, but not in *P. gingivalis*, special clones associated with localized juvenile periodontitis have been identified. *Vertical transmission* of *A. actinomycetemcomitans* but not of *P. gingivalis* has been established. Most studies have shown that if children harbor *A. actinomycetemcomitans*, usually one or two parents harbor the same genotype. From these observations, it is assumed that the parent is the source of transmission. However, identical genotypes in family members are not 100% proof of transmission, as there is no infinitive number of genotypes, and finding identical genotypes may have occurred by chance. The frequency of vertical transmission of *A. actinomycetemcomitans* is estimated to be between 30 and 60% based on detection of identical genotypes in children and parents (van Winkelhoff and Boutaga 2005).

Horizontal transmission of *A. actinomycetemcomitans* and *P. gingivalis* between spouses has been documented and may range between 14 and 60% for *A. actinomycetemcomitans* and between 30 and 75% for *P. gingivalis*. Transmission of *A. actinomycetemcomitans* between siblings has been suggested, but infection by the same source cannot be ruled out. Frequency of contact, number of organisms, oral health status, the resident microflora and immunological and genetic factors may determine whether a person will be permanently colonized by periodontal pathogens upon challenge. Although there is some limited evidence to show that cohabitation with a periodontitis patient influences the periodontal status of the spouse, substantially more information is needed to prove this hypothesis (van Winkelhoff and Boutaga 2005).

Since there is no evidence that periodontal pathogens would be disseminated in aerosols as, for example, respiratory pathogens, it is likely that the person-to-person transmission occurs via salivary and mucosal contact or an inanimate object. Therefore, suppression of the

Table 2.5 Recent studies evaluating the presence of selected herpes viruses in periodontal disease patients

Author, year	Country	Study population	Periodontal status	Sampling approach	Main findings
Botero et al. 2007b	Colombia	30 periodontitis patients and 22 healthy controls	Aggressive periodontitis Chronic periodontitis	Plaque samples with paperpoints	HCMV detection was more prevalent in periodontally diseased subjects compared to healthy ones. Furthermore, in all groups, PD and CAL were increased in HCMV-positive sites. In the periodontitis groups, higher frequencies and levels of specific periodontopathic bacteria, such as *P. gingivalis, P. intermedia/ P. nigrescens*, and *E. corrodens*, were detected in HCMV-positive sites
Botero et al. 2008b	Colombia	44 periodontitis patients and 24 healthy controls	Aggressive periodontitis Chronic periodontitis	GCF sample with paperpoints	Patients suffering from periodontitis had a higher frequency of HCMV as detected by nested PCR (79.5%) and real-time PCR (47.7%) in comparison to healthy subjects (25% nested PCR, 4.1% real-time PCR)
Chalabi et al. 2008	Iran	61 periodontitis patients and 40 healthy controls	Chronic periodontitis	Plaque samples with curette	Prevalence of EBV- 1, EBV-2 and CMV among opatients with periodontitis were 73.8, 4.9 and 59%; respectively
Contreras and Slots 1996	USA	27 periodontitis patients	Adult periodontitis	GCF sample with paperpoints	89% subjects yielded at least one of the five test viruses from deep periodontal pockets, wheras only 56% showed viruses from shallow periodontal sites. Viral coinfection occurred more frequently in deep than in shallow periodontal sites. HCMV was detected with higher frequency in deep than in shallow periodontal sites
Contreras and Slots 1998	USA	6 periodontitis patients	Adult and juvenile periodontitis	GCF sample with paperpoints	Subgingival HCMV DNA was more present in periodontitis (89%) than in 22% gingivitis sites (22%), suggesting that active HCMV replication can occur in periodontiti sites
Hanookai et al. 2000	USA	19 Trisomy 21 patients	Mild, moderate and advanced periodontitis	Plaque samples with curette	Of 19 Trisomy 21 periodontitis lesions, 32% were positive for EBV-1, 26% were positive for HCMV, 16% were positive for HSV, and 11% showed viral coinfection. Of 19 shallow periodontal sites, only one revealed HCMV. Subgingival debridement did not reduce genomic herpesvirus presence.
Idesawa et al. 2004	Japan	33 periodontitis patients and 20 healthy controls	Chronic periodontitis	Saliva	Salivary levels of EBV was detected in 48.5% periodontitis patients and in 15% healthy subjects. The baseline mean values for BOP in EBV-positive patients were significantly higher than those in EBV-negative patients

Table 2.5 (continued)

Author, year	Country	Study population	Periodontal status	Sampling approach	Main findings
Kamma et al. 2001	Greece	16 periodontitis patients	Aggressive periodonttiis	Plaque samples with paperpoints	HCMV was detected in 59.4% of active and in 12.5% of stable sites, EBV-1 in 43.8% of active and in 12.5% of stable sites, HSV in 34.5% of active and in 9.4% of stable sites, and coinfection with any of the three test herpesviruses in 43.8% of active and in 3.1% of stable sites. All periodontitis sites showing herpesvirus coinfection and all but one site showing *P. gingivalis* and *D.pneumosintes* coinfection revealed bleeding upon probing
Kubar et al. 2004	Turkey	16 periodontitis patients and 15 healthy control	Aggressive periodontitis	Plaque samples with curette	HCMV was detected in 68.8% of aggressive periodontitis lesions but not in any of the periodontally healthy study sites
Kubar et al. 2005	Turkey	20 periodontitis patients	Aggressive periodontitis Chronic periodontitis	Plaque samples with curette	HCMV DNA was detected in 78% of subgingival and 33% of gingival tissue samples from aggressive periodontitis lesions, but only in 46% of subgingival and 9% of gingival tissue samples from chronic periodontitis lesions. In aggressive periodontitis, HCMV subgingival and gingival tissue counts were positively correlated with periodontal probing depth and CAL at sample sites. EBV DNA was identified in 89% of subgingival and 78% of gingival tissue samples from aggressive periodontitis lesions, but only in 46% of both subgingival and gingival tissue samples from chronic periodontitis lesions. In aggressive periodontitis, positive correlations were found for EBV subgingival counts and periodontal probing depth at sample sites and for EBV gingival tissue counts and whole mouth mean gingival index. HCMV–EBV coinfection was revealed in 78% of aggressive periodontitis lesions but only 27% of chronic periodontitis lesions
Ling et al. 2004	USA	20 periodontitis patients	Chronic periodontitis	Plaque samples with paperpoints	The prevalence of HSV or HCMV was significantly higher in the subgroups that had lower plaque index. However, the prevalence of HSV was significantly higher in the subgroup that had higher gingival index, positive BOP, deeper PD or higher PAL. Moreover, the prevalence of EBV-1 was significantly higher in the subgroup that had higher PD. Coinfection of HSV and HCMV was significantly associated with the sites that had higher gingival index or positive BOP. Coinfection of any two herpesviruses was also associated with higher PD or higher PAL

(*continued*)

Table 2.5 (continued)

Author, year	Country	Study population	Periodontal status	Sampling approach	Main findings
Parra and Slots 1996	USA	56 gingivitis and periodontitis patients	Advanced periodontitis and gingivitis	GCF sample with paperpoints	Cytomegalovirus was detected in 60% of the periodontitis patients, EBV in 30%, HSV in 20%, human papillomavirus in 17% and HIV in 7%. Forty percent of the periodontitis patients revealed coinfection by two to five viruses. Only 31% of the gingivitis subjects showed a positive viral identification in crevicular fluid, and infected individuals only revealed human HCMV
Rotola et al. 2008	Italy	24 periodontitis patients, 13 healthy controls	Aggressive periodontitis (11) Chronic periodontitis (13)	gingival biopsies	HHV-7 was detected in 91.7% of periodontitis patients and in 61.5% of healthy subjects, EBV in 50.0% samples of periodontitis patients and 7.7% of H subjects and HCMV only in one sample from H group. EBV was more frequently detected in biopsies from affected sites (50.0%) than from nonaffected sites (16.7%). HHV-7 transcription was detected in 15.4% of affected and 15.4% of nonaffected sites
Saygun et al. 2002	Turkey	30 periodontitis patients, 21 healthy controls	Chronic periodontitis	Plaque samples with paperpoints	HCMV was detected in 44.3% of chronic periodontitis patients and 14.3% of healthy persons; EBV-1 in 16.7% of chronic periodontitis patients and 14.3% of healthy persons; and HSV in 6.7% of chronic periodontitis patients and in no healthy persons. HCMV and EBV-1 detected and undetected sites in patients with periodontitis showed statistically significant differences in sampling clinical depth and sampling CAL
Saygun et al. 2004a	Turkey	18 periodontitis patients, 16 healthy controls	Aggressive advanced periodontitis	Plaque samples with curette	HCMV, EBV-1 and HSV-1 were each detected in 72–78% of the aggressive periodontitis patients. HSV-2 occurred in 17% of the periodontitis patients. EBV-1 was detected in one periodontally healthy subject. HCMV, EBV-1 and HSV-1 were positively associated with *P. gingivalis, P. intermedia, T. forsythia* and *C. rectus*, but not with *A. actinomycetemcomitans*. HSV-2 was not associated with any test bacteria
Saygun et al. 2008	Turkey	15 periodontitis patients, 15 healthy controls	Aggressive periodontitis (9) Chronic periodontitis (6)	Plaque samples with curette	HCMV was detected in eight periodontitis lesions and in one normal periodontal site, EBV was detected in nine periodontitis lesions and in two normal periodontal sites. Correlations were found between counts of HCMV and EBV, between counts of HCMV and *P. gingivalis, T. forsythia* and *C. rectus*, and between counts of EBV and *P. gingivalis* and *T. forsythia*. HCMV and EBV virus counts were also positively associated with the level of PAL, PD and BOP

Table 2.5 (continued)

Author, year	Country	Study population	Periodontal status	Sampling approach	Main findings
Slots et al. 2002	Greece	16 periodontitis patients	Aggressive periodonttiis	Plaque samples with paperpoints	HCMV, EBV-1, HSV, *D. pneumosintes* and *P. gingivalis* were detected more frequently in periodontitis active than in periodontitis stable sites. HCMV was significantly positively associated with *D. pneumosintes*. HCMV was positively associated with PD, average percentage bone loss, disease-active periodontitis and gender. EBV-1 was positively associated with PD, PAL, disease-active periodontitis and total number of teeth. HSV was positively associated PD, PAL and disease-active periodontitis
Slots et al. 2003	Greece	16 periodontitis patients	Aggressive periodontitis	Plaque samples with paperpoints	HCMV and HSV were both significant predictors of the presence of subgingival *P. gingivalis*
Sunde et al. 2008	USA	25 periodontitis patients	"Refractory" marginal periodontitis	GCF sample with paperpoints	40% were positive for EBV and 12% for HCMV of the samples. Significant associations were found between periodontal EBV and the presence of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*
Ting et al. 2000	USA	11 periodontitis patients	Localized juvenile periodontitis	GCF sample with paperpoints	Of 11 deep periodontal samples, 72% showed HCMV, 63% showed Epstein–Barr virus type 1 (EBV-1), 9% showed EBV type 2, 54% showed HSV and 72% showed viral coinfection. Of 11 shallow periodontal samples, 18% showed HCMV, 18% showed EBV-1, 9% showed HSV and 18% showed viral coinfection
Watanabe et al. 2007	Brazil	30 periodontitis patients	Aggressive periodontitis	GCF samples by paperpoints	77% of patients were positive for EBV-1, while only 6% were positive for HCMV. A positive association between EBV-1 and periodontal lesions was revealed. 57% of periodontitis sites were positive for EBV-1, whereas 30% of gingivitis sites were positive
Wu et al. 2007	Chinese	143 periodontitis patients, 65 gingivitis patients and 76 healthy controls	Chronic periodontitis Gingivitis	Plaque samples with paperpoints	HCMV was detected in 79.0% of chronic periodontitis patients, 78.5% gingivitis patients, and 76.3% periodontally healthy individuals, while EBV was found in 63.6, 32.3, and 30.3% of the three groups of subjects, respectively. HCMV gB-II infection and HCMV gB-II coinfection with EBV-1 are closely associated with periodontal tissue inflammation and destruction

(*continued*)

Table 2.5 (continued)

Author, year	Country	Study population	Periodontal status	Sampling approach	Main findings
Yapar et al. 2003	Turkey	17 periodontitis patients and 16 healthy controls	Aggressive periodontitis	Plaque samples with curette	HCMV was detected in 64.7% of AgP patients but not detected in healthy subjects and EBV-1 in 70.6% of AgP patients and 6.3% of the healthy controls. HCMV and EBV-1 coinfection was detected in 41.7% of AgP patients. There was a statistically significant decrease in HCMV and EBV-1 following surgical and antimicrobial periodontal therapy

HSV-1 herpes simplex virus type 1; *EBV* Epstein–Barr virus; *HCMV* human cytomegalovirus; *CAL* clinical attachment level; *PD* probing depth; *BOP* bleeding on probing

Table 2.6 Similarities of genotypes of A.actinomycetemcomitans and Porphyromonas gingivalis in bacterium-positive families

Author	Bacterium	Transmission	Method of genotyping
Alaluusua et al. 1993	*A.actinomycetemcomitans*	Vertical (parent–child)	Ribotyping
Petit et al. 1993b	*A.actinomycetemcomitans*	Vertical (parent–child)	Restrictive endonuclease analysis
Petit et al. 1993b	*Porphyromonas gingivalis*	Vertical (parent–child)	Restrictive endonuclease analysis
DiRienzo et al. 1994	*A.actinomycetemcomitans*	Vertical (parent–child)	Restriction fragment length polymorphism
Preus et al. 1994	*A.actinomycetemcomitans*	Vertical (parent-child)	Arbitrary primed polymerase chain reaction (AP-PCR)
Saarela et al. 1996	*A.actinomycetemcomitans* *Porphyromonas gingivalis* *P. intermedia/nigrescens*	Vertical (parent–child)	Ribotyping
Asikainen et al. 1996	*A.actinomycetemcomitans*	Vertical (parent–child)	Arbitrary primed polymerase chain reaction (AP-PCR)
Saarela et al. 1993	*A.actinomycetemcomitans* *Porphyromonas gingivalis*	Horizontal	Ribotyping
Van Steenbergen et al. 1993a, b	*Porphyromonas gingivalis*	Horizontal	Restrictive endonuclease analysis
Petit et al. 1993a	*A.actinomycetemcomitans*	Horizontal	Restrictive endonuclease analysis
Van Steenbergen et al. 1993a, b	*Porphyromonas gingivalis*	Horizontal	Restrictive endonuclease analysis
DiRienzo et al. 1994	*A.actinomycetemcomitans*	Horizontal	Restriction fragment length polymorphism
Preus et al. 1994	*A.actinomycetemcomitans*	Horizontal	Arbitrary primed polymerase chain reaction (AP-PCR)
Von Troil-Lindén et al. 1996	*Campylobacter rectus* *Porphyromonas gingivalis* *P. intermedia/nigrescens*	Horizontal	Ribotyping
Asikainen et al. 1996	*Porphyromonas gingivalis* *A.actinomycetemcomitans*	Horizontal	Arbitrary primed polymerase chain reaction (AP-PCR)

organisms in saliva may prevent their spread among individuals (Asikainen and Chen 1999). The effects of prevention of transmission of *A. actinomycetemcomitans* and *P. gingivalis* have not been studied so far. For *A. actinomycetemcomitans*, screening for and prevention of transmission of specific virulent clones may be feasible and effective in preventing some forms of periodontal disease. *P.gingivalis* is usually recovered from diseased adult subjects, and transmission of this pathogen seems largely restricted to adult individuals.

Horizontal transmission of *P. gingivalis* may therefore be controlled by periodontal treatment involving elimination or significant suppression of the pathogen in diseased individuals, and by a high standard of oral hygiene (van Winkelhoff and Boutaga 2005).

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Particular Aspects of Periodontal Disease Pathogenesis

3

Alexandrina L. Dumitrescu and Masashi Tanaka

The immune system is organized in terms of cells and molecules that have specialized roles for defending against infection. Innate (natural) immunity and adaptive (acquired) immunity are two fundamental aspects of the immune system response to invading microbes. Innate immune responses are mediated by the release of inflammatory cytokines and chemokines, and by phagocytic or killer cells. Adaptive immune responses are mediated by the generation of antigen-specific T and B cells. Antigen-primed T cells induce clonal expansion and differentiate into effector T cells that produce various cytokines or elicit cytotoxicity to eliminate target cells. B cells secrete immunoglobulins, which are responsible for eliminating extracellular microorganisms. Innate responses are generated at the periphery of sites of microbial penetration, whereas adaptive immune responses are generated at secondary lymphoid tissues, such as lymph nodes and the spleen (Azuma 2006) (Table 3.1).

3.1 Aspects of Innate Host Response in Periodontitis

Innate immunity can be defined as a host defense reaction directed against pathogens, designed to maintain host integrity. Unlike acquired (or adaptive) immunity, it is found in all multi-cellular organisms (whereas, acquired immunity is found only in vertebrates), is immediately active, not antigen-specific, requires large numbers of cells for pathogen recognition and has no memory requirement. Given the cells and molecules involved, innate immunity is clearly a silent, unperceived, physiological, inflammatory response. However, if the host response is excessive, it may lead to manifest inflammation that may be considered pathological. The damage inflicted on the host by pathogens may thus result from direct effects (because of the colonization and/or toxigenic potential of the pathogen) or indirect effects mediated by an excessive or inappropriate immune response. Improvements in our understanding of host defense mechanisms against pathogens in general, the triggering of anti-infectious responses and the inflammatory reaction of the lung, in particular, are therefore required for the development of new treatment strategies (Si-Tahar et al. 2009) (Fig. 3.1).

The role of innate defense mechanism is to eliminate pathogens. In the respiratory mucosa, this process depends primarily on the very first cells capable of detecting microbes, migrating toward them, taking them up by phagocytosis and destroying them, i.e., the alveolar macrophages. In other tissues, this initial response is mediated by other cells: Kupffer cells (liver), Langerhans cells (skin), osteoclasts (bone), or peritoneal macrophages (peritoneal cavity). The principal functions of these immune cells are, thus, phagocytosis and the elimination of microorganisms. However, they also have a secondary function: the synthesis and release into the body of biologically active molecules. These molecules include cytokines, chemokines and chemotactic lipids, which direct certain circulating cells to migrate to the site of infection and participate in the destruction of pathogens. The cells concerned in this process are principally polymorphonuclear (PMN) neutrophils, as well as monocytes, natural killer cells, and eosinophils. Finally, when the infection concerns mucosa such as those of

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Table 3.1 A comparison of innate and adaptive immunity (Azuma 2006) (with permission from Elsevier Publishing)

Property	Innate immune system	Adaptive immune system
Cells involved	Phagocytes (macrophages, neutrophils, DCs), NK cells	T cells, B cells
Recognition	Fixed in genome	Encoded in gene segments
Receptors	Rearrangement not necessary	Rearrangement necessary
	Limited diversity	High diversity
Ligand/antigens	Conserved molecular patterns(components of microorganisms)	Details of molecular structure (peptides, proteins, carbohydrates)
Development	Nonclonal	Clonal
	Selected over evolutionary time	Selected in individual
Response time	Immediate (0–4 h)	Delayed (~72 h)
Site of response	Local, periphery	Secondary lympoid tissues
Response	Production of inflammatory cytokines (IL-1, IL-6, TNF-α) and chemokines (IL-8); Induction of costimulatory molecules (CD86, CD40)	Clonal expansion or anergy; production of effector cytokines (IFN-γ, IL-4)

DC dendritic cell; *IFN-γ* interferon-γ; *IL* interleukin; *NK* natural killer; *TNF-α* tumor necrosis factor-α

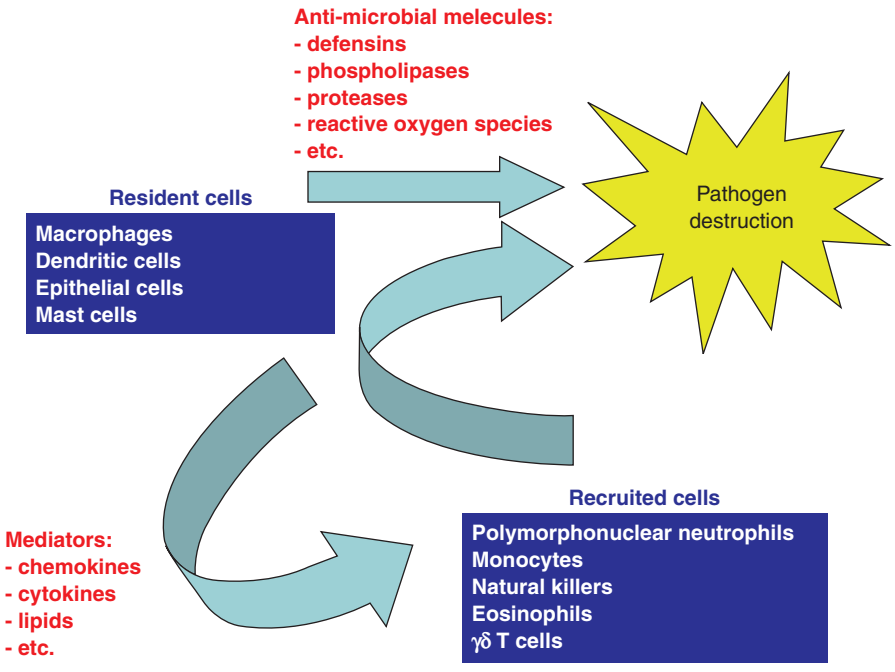

Fig. 3.1 Cells of the innate immune system. Resident cells directly kill pathogens (phagocytosis, release of anti-microbial molecules, etc.) and also synthesize mediators (chemoattractants, etc.) that trigger the recruitment of other cells participating in the pathogen clearance (modified from Si-Tahar et al. 2009) (with permission from Wiley-Blackwell)

the intestine and lungs, resident cells capable of supporting macrophage action include dendritic cells and epithelial cells. Dendritic cells are particularly important because they communicate and present antigens to lymphocytes, linking innate and adaptive immune responses (Si-Tahar et al. 2009).

The innate immune system is an evolutionarily conserved form of host defense found in most multicellular organisms. Induction of innate immunity is triggered by pathogen interaction with germline-encoded pathogen recognition receptors. These receptors recognize conserved pathogen-associated molecular patterns (PAMPs), which represent molecular structures that are produced by microorganisms and not by the host. For example, peptidoglycan and lipopolysaccharide are produced by bacteria, but not by eukaryotic hosts. Recognition of PAMPs by pathogen recognition receptors allows the innate immune system to unerringly discriminate selfmolecules from pathogen-associated nonself structures. PAMPs are invariant and represent conserved molecular patterns that are essential for microbial survival. For example, the lipid A portion of

lipopolysaccharide represents an invariant pattern found in all gram-negative bacteria. Lipopolysaccharide, lipoproteins, peptidoglycan, and lipoteichoic acids are all synthesized by bacteria, and mutation or loss of these molecules is either lethal or reduces adaptation (Azuma 2006).

3.1.1 Main Receptors and Signaling Pathways

Potential host cell receptors involved in recognizing bacterial components and initiating signaling pathways that lead to inflammatory responses include: Toll-like receptors (TLRs), CD14, nucleotide-binding oligomerization domain proteins (Nod), and G-protein-coupled receptors, including formyl-methionyl peptide receptors and protease-activated receptors. Of the above-mentioned bacterial and host molecules, evidence from experimental animal studies implicate TLRs and CD14 in periodontal tissue or alveolar bone destruction (Madianos et al. 2005).

3.1.1.1 TLRs in Periodontitis Lesions

Innate immune recognition relies on a limited number of germline-encoded receptors, which are called pathogen recognition receptors. These receptors recognize conserved molecular patterns PAMPs and produce inflammatory cytokines via intracellular signaling. TLR family is the best characterized class of pathogen recognition receptors and detects multiple PAMPs. TLRs play an essential role in the recognition of microbial components. Dendritic cells within the epithelium express TLRs and play a sentinel role at the front line of defense. Interestingly, different dendritic cell subsets express distinct sets of TLRs, and this leads to them having particular functions in innate responses and the generation of distinct T-cell subsets (Azuma 2006).

Human TLRs are type I transmembrane proteins with an extracellular leucine-rich repeat (LRR) domain and a cytoplasmic carboxy-terminal Toll-interleukin (IL) 1 receptor domain. Based on the chromosomal localization, genomic structure, and amino acid sequences, the human TLRs can be divided into five subfamilies: TLR2, TLR3, TLR4, TLR5, and TLR9. The *TLR2 subfamily* consists of TLR1, TLR2, TLR6, and TLR10, the *TLR9 subfamily* is composed of TLR7, TLR8, and TLR9. TLR3, TLR4, and TLR5 are represented only by one family member, respectively. TLR1 and TLR6 genes are located closely to 4p14, TLR2 maps to 4q32, while TLR3 is located near TLR2, at 4q35. TLR4 resides on 9q33–35, whereas TLR5 is at 1q33.3 (Rock et al. 1998; Takeuchi et al. 1999). TLR7 and TLR8 are located as a tandem in Xp22, TLR9 maps to 3p21.3 (Sandor and Buc 2005). Several studies have attempted to associate the TLR polymorphisms with periodontitis, but controversial results were obtained (Fig. 3.2; Table 3.2).

Various molecules are known to form complexes with TLRs at the host cell membrane. These include CD14, the secreted factor MD-2 and the adaptor protein MyD88, although an MyD88 independent pathway has been described. Additional molecules continue to become known, including Mal and Tollip. In the case of lipopolysaccharide, the ligand is complexed to CD14 and then forms a complex at the cell surface that includes TLR4 and MD-2. Signal transduction includes recruitment of MyD88 and IL-1 receptor associated kinase, and results in nuclear translocation and gene activation of specific inflammatory mediators, including IL-1 and tumor necrosis factor (TNF)-α (Dixon et al. 2004) (Fig. 3.3).

It is clear that periodontal cells actively participate in the innate immune response against dental plaque bacteria. They express different types of TLRs (Fig. 3.4;

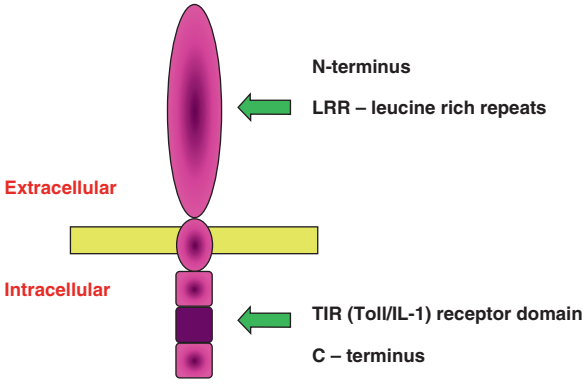

Fig. 3.2 The structure of a human Toll-like receptor (TLR). Human TLRs are type I transmembrane proteins with an extracellular leucine-rich repeat (LRR) domain and a cytoplasmic, referred to as the Toll-interleukin (IL) 1 receptor (TIR) domain, which is homologous to that of the IL-1 receptor (Sandor and Buc 2005) (Reproduced by permission of the Charles University in Prague, first Faculty of Medicine)

Table 3.2 Toll-like receptors (TLRs) in periodontal tissue cells culture

Author (year)	Substrate	TLRs identified
Kusumoto et al. (2004)	Human gingival epithelial cells	LR2, TLR4, TLR5, and TLR9
Mori et al. (2003)	Human gingival epithelial cells	TLR2, TLR4
Beklen et al. (2009)	Human gingival epithelial cells	TLR2, TLR5
Hatakeyama et al. (2003)	Human gingival fibroblasts	TLR2, TLR4, TLR9
Nociti et al. (2004)	Murine cementoblast cell lines	TLR2, TLR4, CD14, and MD-2
Asai et al. (2001)	Human gingival epithelial Cells	TLR2
Asai et al. (2003)	Human osteoblastic cell line	TLR4, MD-2, and nd myeloid differentiation factor 88 but not TLR2
Faure et al. (2000)	Human microvascular endothelial cells	TLR-1, TLR-3, TLR-4, and TLR-5, but express little or no mRNA for TLR2
Faure et al. (2001)	Human endothelial cells	TLR4

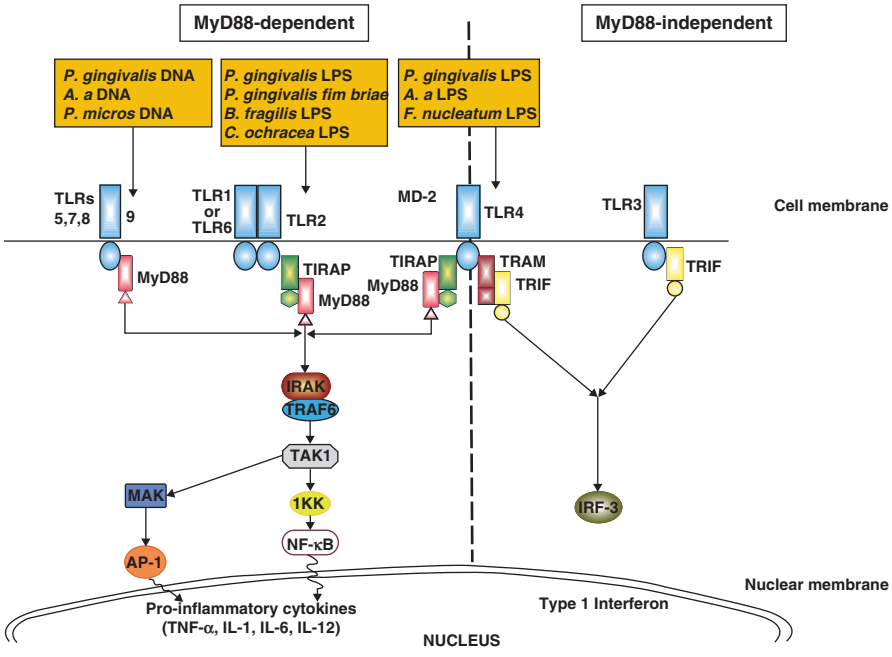

Fig. 3.3 Toll-like receptors (TLRs) signaling pathways (simplified diagram) and TLR ligands derived from bacterial plaque microorganisms. *A.a A. actinomycetemcomitans*; *AP-1* activator protein-1; *IKK* inhibitor of nuclear factor-κB kinase; *IRAK*, interleukin (IL)-1-receptor-associated kinase; *IRF-3* interferon (IFN)-regulatory factor-3; *LPS* lipopolysaccaride; *MAK* mitogen-activated protein kinase; *MyD88* myeloid differentiation primary-response protein 88; *NF-κβ* nuclear factor-κB; *TAK1* transforming growth factor-β-activated kinase 1; *TIRAP* toll-IL-1 receptor domain-containing adaptor protein; *TRAF6* tumor-necrosis-factor-receptor-associated factor 6; *TRAM* TRIF-related adaptor molecules; *TRIF* toll-IL-1 receptor domain-containing adaptor inducing IFN-β (Mahanonda and Pichyangkul 2007) (with permission from Wiley-Blackwell)

Table 3.3). As a result of the specificity of theTLR–ligand interaction, gingival epithelial cells are probably unable to differentiate between commensal and pathogenic bacteria with regard to their responses. It was hypothesized that in the steady-state condition, TLRs expressed on gingival epithelium (especially in the dentogingival region) continually interact with components of oral plaque bacteria that form biofilms attached to the tooth surface. This TLR signaling results in innate immune responses, involving the release of antibacterial peptides (β-defensins, cathelicidin, and calprotectin) and neutrophil-recruiting chemokine (IL-8). TLR signaling, therefore, serves to limit pathogenic infection and to prevent commensal organisms from breaching the epithelial barrier (Mahanonda and Pichyangkul 2007). In a recent study performed by Beklen et al. (2008), the expression and distribution of TLRs (TLR-1–TLR-10) were immunohistochemically detected in

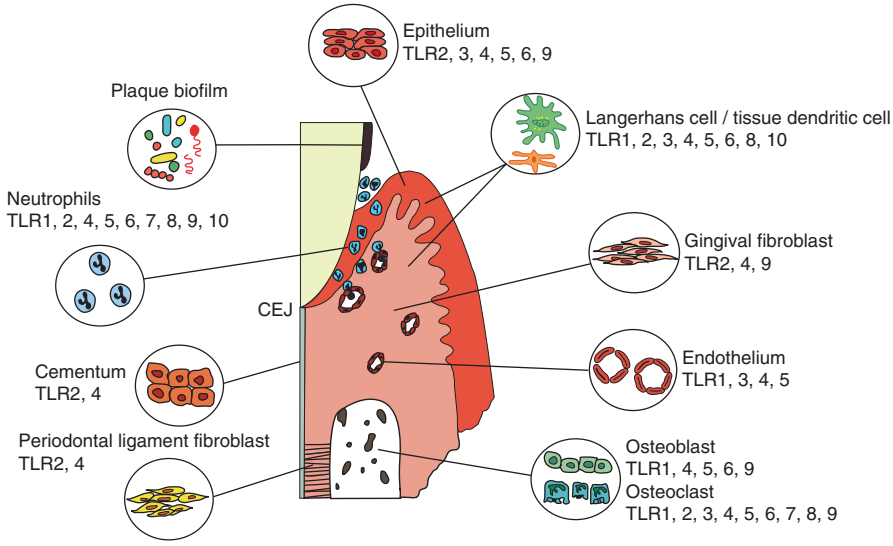

Fig. 3.4 Messenger RNA expression of Toll-like receptors (TLR) on different cell types of periodontium (*CEJ* cementoenamel junction) (Mahanonda and Pichyangkul 2007) (with permission from Wiley-Blackwell)

gingival epithelium and connective tissue. Except for TLR-7 and TLR-8, all the other TLRs showed statistically significant differences between patients with periodontitis and healthy controls, suggesting their involvement in the pathogenesis of periodontitis.

Several studies have investigated the TLR polymorphism in chronic and aggressive periodontitis, as summarized in Table 3.4.

3.1.1.2 Lipopolysaccharide Binding Protein/ CD14

Lipopolysaccharide has been shown to activate a variety of cell types in CD14-dependent manner. Lipopolysaccharide binds to the serum protein, lipopolysaccharide-binding protein, and is transferred to either soluble CD14 (sCD14) or membrane-bound CD14 (mCD14). Some cell types (monocytes, neutrophils) have mCD14 present on their cell surface as a glucosylphosphatidylinositol anchored protein, whereas others (endothelial cells) rely on the presence of soluble protein. The CD14-bound lipopolysaccharide interacts with a receptor complex on the cell surface that includes TLR4 and the accessory protein MD-2, and initiates one or more intracellular pathways leading to expression of inflammatory mediators. Binding of lipopolysaccharide to CD14 has been shown to be the result of electrostatic interactions with numerous charged amino acids present on CD14. It is likely that the nature of this interaction allows CD14 to act as "molecular flypaper," binding not only the structurally diverse lipopolysaccharide but also other bacterial components such as peptidoglycan and lipoteichoic acid (Dixon et al. 2004).

Previous data have shown the strong relevance of the CD14 receptor activity in the clinical manifestation and development of periodontitis (Folwaczny et al. 2004). The concentration of sCD14 receptor in saliva (Isaza-Guzmán et al. 2008) and the systemic level of the soluble form of CD14 (sCD14) is significantly increased in patients with periodontal disease (Hayashi et al. 1999), and showed a severity-dependence with increasing levels of periodontal breakdown (Nicu et al. 2009). It was also revealed that in periodontitis patients, levels of sCD14 correlated positively with C-reactive protein levels, leukocyte numbers and negatively with anti-*A ctinobacillus actinomycetemcomitans* IgG (Nicu et al. 2009). In contrast, the CD14 expression within the periodontal tissue was found to be negatively correlated with the amount of attachment loss (Jin and Darveau 2001; Nicu et al. 2008). Significantly lower levels of the sCD14 protein were observed at sites with advanced attachment loss, indicating a protective effect for CD14. Moreover, a reduced expression of CD14 on monocytes was suggested to be linked with an increasing susceptibility for early-onset periodontitis (Buduneli et al. 2001; Folwaczny et al. 2004).

Several studies have investigated the CD14 polymorphism in chronic and aggressive periodontitis (AP), and controversial results were obtained (Table 3.5) (Holla et al. 2002; Yamazaki et al. 2003; Folwaczny

Table 3.3 Location and expression of Toll-like receptors (TLRs) (Azuma 2006) (with permission from Elsevier) Sandor and Buc 2005) (Reproduced by permission of the Charles University in Prague, first Faculty of Medicine)

TLR	Location	Cells	Ligands
TLR1	Cell membrane	mDCs, monocytes (ubiquitous)	Soluble factors of *Neisseria meningitidis* cell wall Triacylated lipopeptides (G+, G– bacteria), 19 kDa lipoprotein (mycobacteria) Lipoarabinomannan (mycobacterial cell wall) Outer-surface protein – OspA (*Borrelia burgdorferi*)
TLR2	Cell membrane	Monocytes, NK cells, mDCs, mast cells, T cells, epithelial cells	Peptidoglycan, lipoteichoic acids (G+ bacteria) Phenol-soluble modulin (*Staphylococcus epidermidis*) Di- and triacylated lipopeptides, lipoproteins (many pathogens) Outer-membrane porins (*N. gonorrhoeae*, *H. pylori*) OspA (*B. burgdorferi*) Lipoarabinomannan (mycobacterial cell wall glycolipid) Zymosan (yeast) Protozoan cell membrane glycolipids (*Trypanosoma cruzi Neisseria gonorrhoeae, Helicobacter pylori, Leptospira interrogans, Klebsiella pneumoniae*) Wild-type H protein (measles virus) HSV-1, CMV envelope proteins Atypical LSP (*L. interrogans*, *P. gingivalis*) Host HSP70
TLR3	Intracellular	mDCs, NK cells, epithelial cells	Viral and host double-stranded RNA Polyinosinic-polycytidylic acid [poly(I:C)]
TLR4	Cell membrane	Monocytes, mast cells, neutrophils, T cells, epithelial cells, endothelial cells	Lipopolysaccharide-G$^-$ bacteria Fusion protein of respiratory syncytial virus Murine mammary tumor virus Moloney murine leukemia virus Taxol (plant antitumor agent) Extravascular fibrinogen/fibrin (host) Oligosaccharide fragments of hyaluronan (host) Extra domain A of fibronectin (host) Polysaccharide fragments of heparan sulfate (host) Heat-shock protein 60 – HSP60 (host, *Ch. pneumoniae*) HSP70 (host)
TLR5	Cell membrane	Monocytes, NK cells, mDCs, epithelial cells	Flagellin
TLR6	Cell membrane	Myeloid cells, mast cells, B cells, mDCs	Diacylated lipopeptides (*Mycoplasma fermentans*) Zymosan (yeast)
TLR7	Intracellular	pDCs, B cells, eosinophils	Imidazoquinolines (imiquimod, resiquimod, loxoribine, bropirimine)
TLR8	Intracellular	NK cells, T cells, myeloid cells, mDCs	Viral single-stranded RNA (ssRNA) (influenza virus, vesicular stomatitis virus) Guanosine and uridine-rich ssRNA oligonucleotides (HIV-1)
TLR9	Intracellular	pDCs, B cells, NK cells	Unmethylated CpG oligodeoxynucleotides (bacteria) Viral genomic DNA (HSV-2)
TLR10	Cell membrane	B cells, pDCs, mDCs	
TLR11		Uroepithelium (mouse)	Uropathogenic bacteria. *Toxoplasma gondii*

mDCs myeloid dendritic cells; *NK* natural killer; *pDCs* plasmacytoid dendritic cells

et al. 2004; Donati et al. 2005; Tervonen et al. 2007; Laine et al. 2005; James et al. 2007; Schulz et al. 2008; Nicu et al. 2009). However, the local expression of the mCD14 receptor did not vary in gingival biopsies between subjects with different -159 CD14 genotypes (Donati et al. 2008).

3.1.1.3 Nod Proteins

Nod1 and Nod2 belong to a recently discovered family of the nucleotide-binding site and LRR (NBS–LRR) proteins and have been extensively reviwed recently (Shaw et al. 2008; Le Bourhis et al. 2007; Franchi

Table 3.4 TLR2 and TLR4 gene polymorphisms in aggressive and chronic periodontitis

Author	Periodontits	No. of subjects	Ethnicity	Association
D'Aiuto et al. (2004)	Chronic	94 subjects with severe generalized periodontitis	Mixed population	Non-significant
Laine et al. (2005)	Chronic	100 adult patients with severe periodontitis and 99 periodontally healthy controls	Caucasian population	Non-significant
Brett et al. (2005)	Chronic and aggressive	51 aggressive periodontitis (AP) patients, 57 chronic periodontitis (CP) patients, 100 unrelated healthy individuals of unknown periodontal status as controls	Caucasian population	Significant
Schröder et al. (2005)	Chronic and aggressive	197 individuals suffering from generalized periodontitis	Caucasian population	Significant chronic; non-significant aggressive
Holla et al. (2007)	Chronic	171 patients with CP and 218 unrelated controls	Caucasian population	Non-significant
Fukusaki et al. (2007)	Chronic	97 patients with CP and 100 control subjects	Japanese population	Significant
James et al. (2007)	Chronic and aggressive	73 subjects with AP, 95 subjects with CP, 95 healthy controls	Caucasian population	Non-significant chronic; significant aggressive
Berdeli et al. (2007)	Chronic	83 patients with CP and 106 periodontally healthy subjects	Caucasian population	Non-significant
Emingil et al. (2007)	Aggressive	90 patients with generalized aggressive periodontitis and 155 periodontally healthy subjects	Turkish population	Non-significant
Zhu et al. (2008)	Chronic and aggressive	40 patients with generalized AP, 50 patients with chronic periodontitis, and 100 periodontally healthy controls	Chinese population	Non-significant
Noack et al. (2008)	Aggressive	111 patients with AP and 80 periodontally healthy controls	Caucasian population	Non-significant
Schulz et al. (2008)	Chronic and aggressive	133 periodontitis patients (chronic: $n = 60$, aggressive: $n = 73$) and 80 healthy controls	Caucasian population	Non-significant
Imamura et al. (2008)	Chronic	43 periodontitis patients and 49 healthy controls	Japanese population	Non-significant

AP, aggressive periodontitis; *CP*, chronic periodontitis

Table 3.5 CD14 gene polymorphisms in aggressive and chronic periodontitis

Author	Periodontits	Subjects	Ethnicity	Association
Holla et al. (2002)	Chronic	135 patients with chronic periodontitis (CP) and 207 unrelated randomly selected white subjects who did not have a clinical history of periodontal disease	Caucasian population	Significant
Yamazaki et al. (2003)	Chronic	163 subjects with periodontitis and in 104 age- and gendermatched control subjects without periodontitis	Japanese population	Non-significant
Folwaczny et al. (2004)	Chronic	70 patients with periodontal disease and 75 healthy controls	Caucasian population	Significant
Donati et al. (2005)	Chronic	60 patients with severe and generalized CP and 39 periodontally healthy subjects as controls	Caucasian population	Significant
Tervonen et al. (2007)	Chronic	51 subjects with moderate to severe CP and 178 healthy controls	Caucasian population	Significant
Laine et al. (2005)	Chronic	100 patients with severe periodontitis and from 99 periodontally healthy controls	Caucasian population	Significant
James et al. (2007)	Chronic and aggressive	73 subjects with aggressive periodontitis, 95 subjects with chronic periodontitis, 95 healthy controls	Caucasian population	Non-significant
Schulz et al. (2008)	Chronic and aggressive	133 periodontitis patients (chronic: $n = 60$, aggressive: $n = 73$) and 80 healthy controls	Caucasian population	Non-significant
Nicu et al. (2009)	Chronic	115 subjects with untreated periodontitis (59 moderate and 46 severe) and healthy controls (n¼457)	Caucasian population	Non-significant

AP, aggressive periodontitis; *CP*, chronic periodontitis

et al. 2008; Kumagai et al. 2008). In contrast to TLRs, which are mainly integral membrane proteins, Nod proteins are cytosolic and are involved in intracellular recognition of microbes and of their products. Nod1 and Nod2 molecules have a series of LRRs at their C-terminal, which is the domain that senses the microbial ligand. This domain is connected to the NBS, which is important for the oligomerization of the receptor, which is necessary for the signal transduction induced by the N-terminal caspase-activating and recruitment domain (Madianos et al. 2005).

Nod1 and Nod2 are expressed in epithelial cells lining mucosal surfaces, but Nod2 is predominantly expressed in cells of the myeloid lineage (Madianos et al. 2005). Stimulation of NOD1 and NOD2, two prototypic NLRs, results in the activation of MAPK and NF-κB. On the other hand, a different set of NLRs induces caspase-1 activation through the assembly of an inflammasome. The inflammasome is a signaling platform scaffolded by NLR proteins and mediates the activation of caspase-1, which is required for the processing and maturation of the proinflammatory cytokines, IL-1β and IL-18 (Shaw et al. 2008).

A clear mRNA expression of NOD1 and NOD2 was revealed in gingival fibroblasts (Uehara and Takada 2007) and in oral epithelial cells (Sugawara et al. 2006).

3.1.1.4 G-Protein-coupled Receptors (GPCR) and hBDs

GPCR are receptors composed of seven transmembrane domains with loops spanning both the intracellular and extracellular faces of the cell membrane. They have a wide range of ligands, such as sensory stimuli, nucleotides, ions, lipids, hormones, chemokines, complement molecules, etc. Two such receptors that may interact with bacterial virulence factors are the fMLP receptor and the PARs (Madianos et al. 2005).

Activation of the fMLP receptor is initiated after binding with its ligand. This induces a conformational change in the receptor. Heterodimeric G-proteins, consisting of α, β, and γ subunits, interact selectively with the cytoplasmic face of the activated receptor. Hence, the Ga subunit releases guanine di-phosphate (inactive form) and subsequently binds to guanine tri-phosphate, resulting in activation and dissociation of the heterotrimer into α and βγ constituents, which activate downstream effectors. Specifically, the fMLP receptor may activate the MAPK p38 and ERK1/2 signaling pathways, leading to pro-inflammatory cytokine production. Also, these receptors may induce degranulation via activation of inositol triphosphate and diacylglyserol (Madianos et al. 2005).

Only three investigators have evaluated the association between n-Formyl-L-methionyl-L-leucyl-L-phenyl alanine receptor polymorphisms and AP. Gwinn et al. (1999) has reported an association between localized AP with 2 polymorphisms of FRP (Phe110Ser and Cys126Trp). On the contrary, Zhang et al. (2003) could not demonstrate, in African-American patients, that two FPR gene polymorphisms (c.329T > C [Phe110Ser] and + 378C > G[Cys126Trp]) play an etiologic role in AP, but it was suggested that single nucleotide polymorphisms in the second extracellular loop may be etiologically important. In a Japanese population, Gunji et al. (2007) performed an association study with 49 AP patients and 373 controls using 30 variations identified by sequencing the 21.1-kb gene region. Five polymorphisms (−12915C > T, −10056T > C, −8430A > G, 301G > C, and 546C > A) showed significant association with AP. PMN neutrophils from subjects carrying the −12915T allele expressed significantly lower levels of FPR1 transcripts than those homozygous for the −12915C allele. Furthermore, the −12915T allele decreased activity of transcriptional regulation in a luciferase assay. Haplotype association analysis with three SNPs (−12915C > T, 301G > C, and 546C > A) revealed that one haplotype (−12915T−301G−546C) was significantly represented in AP patients.

PARs are GPCRs that mediate cellular responses to extracellular proteinases. Various proteases, such as thrombin and trypsin-like serine proteases, bind to PAR's N-terminal domain and cleave it (after Arg41) to generate a new N-terminus for the receptor. Currently, four PARs have been identified (PAR-1–4). PAR-1 is expressed by platelets, fibroblasts, endothelial cells, and neurons, whereas PAR-2 is expressed by epithelial cells, endothelial cells, smooth muscle cells, T cells, neutrophils, and neurons. PAR-1, -3, -4 are activated by thrombin, and PAR-2 is activated by trypsin and a number of trypsin-like serine proteases. PARs are considered to mediate cellular responses to tissue injury, like expression of IL-8, GM-CSF, intracellular adhesion molecule (ICAM)-1, VCAM-1, platelet activating factor, IL-6, nitric oxide, etc. that may

serve to recruit platelets and leucocytes to sites of injury and promote access of plasma proteins to the extravascular space. PAR-1 may also activate degranulation of mast cells, which may further activate PAR-2 via the released tryptase (Madianos et al. 2005).

As observed by Madianos et al. (2005), only a few studies have been addressed to evaluate the role of PARs in periodontal tissues (Hou et al. 1998; Lourbakos et al. 2001; Chung et al. 2004).

3.1.2 Complement System

The complement system is made up of a large number of distinct plasma proteins that react with one another to opsonize pathogens and induce a series of inflammatory responses. Although these proteins are not receptors of bacterial virulence factors themselves, they can be activated on the surface of a pathogen. Most of these proteins are proteases that are themselves activated by proteolytic cleavage through a triggered-enzyme cascade. In this way, the activation of a small number of complement proteins at the start of the pathway is hugely amplified by each successive enzymatic reaction, resulting in the rapid generation of a disproportionately large complement response. In the early phases of an infection, the complement cascade can be activated through any one, or more, of the three pathways: the classical, the mannan-binding lectin, or the alternative. (Madianos et al. 2005). The classical pathway is usually mediated by binding of the C1 complex (composed of recognition molecule C1q and two proteinases C1s and C1r) to invading pathogens either directly or via immunoglobulins. The lectin pathway is able to recognize, via mannose-binding lectin, polysaccharide molecules normally present only on microbial surfaces. Finally, complement can also be activated through the alternative pathway, which is not so much an activation pathway but as a failure to appropriately regulate the constant low-level, spontaneous activation of C3 (constantly initiated due to inherent instability of this protein). All three pathways lead to opsonisation of the pathogen with C3b (activated form of complement factor C3), which enhances phagocytosis by phagocytes. Furthermore, anaphylatoxins C5a and C3a are released as byproducts to attract phagocytes to the site of infection. Finally, the end result of the complement cascade is formation of the membrane attack complex and bacterial cell lysis. Host cells protect themselves from bystander damage following complement activation through the expression of membrane-bound or recruitment of soluble endogenous complement inhibitors (Potempa et al. 2009).

As the role of the complement system in inflammatory response is very important, several recent studies have evaluated its interactions with periodontal bacteria, such as *Treponema denticola* (McDowell et al. 2009), *Prevotella intermedia* (Potempa et al. 2009), *Porphyromonas gingivalis*, (Hajishengallis et al. 2007, 2008; Wang et al. 2007; Popadiak et al. 2007) and *A. actinomycetemcomitans* (Permpanich et al. 2006).

Polymorphisms and deficiencies in the genes encoding for complement proteins modify inflammatory responses, microbial killing, opsonization, chemotaxis, and scavenging of non-viable cells. Classical pathway complement factor C4 is encoded by closely linked C4A and C4B genes, which form extended haplotypes together with major histocompatibility complex (MHC) class I and II genes. C4A and C4B proteins may be reduced in level or absent in plasma because of null alleles (<2 C4 genes) in either locus. Seppänen et al. (2007) tested recently whether complement levels are systemically altered and C4 deficiencies associated with severe chronic periodontitis (CP). In a case-control study, there were analysed levels of plasma C3, and C4, serum classical pathway haemolytic activity, C4 allotypes and C4 gene numbers in 37 patients with severe CP and in 150 voluntary controls. Plasma levels of C3 were higher, and classical pathway haemolytic activity was lower in patients than in controls. Partial C4 gene deficiencies were more frequent in patients than in controls (odds ratio = 2.4, 95% confidence interval (CI) 1.1–5.5, $P = 0.032$). As was summarized by Potempa et al. (2009), in a patient with AP and severe edema, localized to the free gingival tissues was reported to be deficient in C1-inhibitor (Roberts et al. 2003), and the highest salivary levels of C3 were measured in periodontally healthy subjects while low levels were often found in edentulous and CP patients (Aurer et al. 2005).

The gingival crevice provides a unique environment for its complex microbial flora that has been demonstrated to be an etiologic factor in periodontal disease. Complement proteins are present in gingival fluid at levels as high as 85% of that reported for serum (Schenkein and Genco 1977). Several complement

components have been identified in crevicular fluid. In addition, there is evidence that complement is more active in saliva than in serum (Boackle 1991; Boackle et al. 1978).

3.1.3 Initiation of Inflammation in Periodontal Tissues

Periodontal disease (gingivitis and periodontitis) is an inflammatory process of the gingiva and supporting structures of the teeth induced by a microbial biofilm. Based on in vitro and in vivo studies, as described, and histological assessments of inflamed and healthy gingival tissues (Page and Schroeder 1982), a model of the initial events that may occur when bacteria set up inflammatory responses in the gingival can be proposed. Of course, this model should not be considered as a "new" hypothesis, but as a summary of the existing knowledge (Madianos et al. 2005). It was concluded that the developing lesion in the gingiva during plaque accumulation followed a series of stages, which by clinical and histopathological criteria could be divided into initial, early and established stages in clinically apparent gingivitis, and periodontitis has been designated as the advanced stage. In humans, the *initial lesion* is seen within about 4 days of the beginning of plaque accumulation. The infiltrated area comprises 5–10% the marginal gingival connective tissue, and in this zone much of the collagen is destroyed. The *early lesion* evolves from the initial lesion within about 1 week following the beginning of plaque accumulation. It is characterised by an infiltrate in which small, medium, and large lymphocytes and macrophages predominate, along with small number of plasma cells located around the periphery of infiltrate. Lymphocytes account for approximately 75% of the total inflammatory population. The acute inflammation persists as evidenced by vasculitis and the presence of neutrophils, especially in the junctional epithelium. The infiltrated area may occupy from 5 to 15% of the marginal connective tissue, and collagen loss in the affected area may reach 60–70%. The resident fibroblasts become pathologically altered as evidenced by electron-lucid nuclei, swollen mitochondria, and vacuolisation of the endoplasmic reticulum with rupture of the cell membranes (Table 3.6).

In time, the *established lesion* characterised by a predominance of plasma cells and B lymphocytes evolves. A large number of neutrophils appear in the junctional and pocket epithelium, and macrophages are present in the lamina propria region of the pocket wall. In tissue specimens designated as severe as severe gingivitis, the lymphocytes continue to predominate over the plasma cells, and almost equal numbers of B and T lymphocytes are seen (Page 1986). There is further increase in the proportion of B cells and plasma cells in specimens classified as established lesions (Lindhe 1980). In contrast, the inflammatory infiltrate in the inflamed gingiva of the children was dominated by T lymphocytes (Alcoforado et al. 1990).

Different host mechanisms, such as the regular shedding of epithelial cells, the washing effect of the saliva and the gingival crevicular fluid (GCF), and most importantly the phagocytic action of neutrophils that migrate continuously through the junctional epithelium into the sulcus/pocket, are all able to maintain normal, non-irritating environment for the host bacterial flora. Once this equilibrium is disturbed and more pathogenic bacteria populate the periodontal niche, the host becomes challenged. The first cells to be challenged are the *epithelial cells*. Epithelial cells are the first cells to be challenged by bacteria in the sulcus/pocket. This interaction triggers the first steps of the inflammatory response and leads to cell activation in the connective tissue compartment and the recruitment of neutrophils in the crevice. Bacterial adhesion through fimbriae activates epithelial cells to secrete IL-1β, TNF-α, IL-6, and IL-8. Activation, leading to IL-8 release, can also occur when primed epithelial cells interact with lipopolysaccharide (LPS), PGN, and LTA. At the same time, virulence factors that have diffused in the connective tissue, as well as inflammatory mediators produced by epithelial cells stimulate host cells resident in the area, such as *monocytes/macrophages*, *fibroblasts*, *and mast cells*, to produce and release pro-inflammatory cytokines (IL-1β, TNF-α, IL-6, IL-12), chemotactic molecules (MIP-1a, MIP-2, MCP-1, MCP-5, and IL-8), prostaglandins (PGE_2), histamine, leukotrienes, as well as matrix metalloproteinases (MMPs) that degrade collagen from the connective tissue compartment. In addition, macrophages also express co-stimulatory molecules (B7) and MHC class II molecules, and dendritic cells engulf bacteria and their products and process them for antigen presentation at the local lymph nodes. Hence, while the

Table 3.6 Histopathogenesis of gingivitis and periodontitis Page & Schroeder, 1982 (reprinted with permission S. Karger AG, Basel)

Histopathologic condition	Clinical condition				
	Healthy gingiva	Initial gingival lesion	Early gingivitis	Established gingivitis	Periodontitis (Advanced lesion)
Plaque	Little, primarily Gram +, aerobic	Primarily Gram+, aerobic	Primarily Gram+, aerobic	Gram+ and −, in gingival pocket	Adherent Gram +, nonadherent Gram− (in pocket)
Junctional epithelium/ Pocket epithelium	Normal junctional epithelium without rete pegs	Alteration of the most coronal portion of the junctional epithelium (JE)	Initial alteration and lateral proliferation of the junctional epithelium in coronal region	Lateral proliferation of JE, deepening of sulcus with formation of gingival pocket or pseudopocket	Apical and lateral proliferation of pocket epithelium, with formation of periodontal pockets; ulceration of pocket epithelium
Vessels inflammatory cells, infiltrate excudate	Few polymorpho-nuclears from subepithelial vasculature in junctional epithelium, very minimal exudates from the sulcus	Classic vasculitis of vessels subjacent to the JE; exudation of fluid from the gingival sulcus; Increased migration of leukocytes into the JE and gingival sulcus	Vasculitis, exudation of serum proteins, PMN migration, accumulation of lymphoid cells immediately subjacent to the JE at the site of acute inflamma-tion; very few plasma cells;	Acute inflammatory alterations; predominance of plasma cells; Presence of Immunoglobulins extravascularly in the connective tissue, JE and gingival sulcus; increased sulcus exudate	Acute inflammatory alterations as in gingivitis; predominance of plasma cells; Copious exudates often suppurative; widespread manifestations of inflammatory and immunopatho-logic reactions
Fibroblasts, connective tissue, collagen	Normal	Loss of perivascular collagen	Cytopathic alterations in resident fibroblasts; collagen loss in infiltrated connective tissue areas	Severe fibroblast injury, further loss of collagen, continued infiltration	Further collagen loss in the infiltrated tissues, fibrosis in peripheral gingival regions
Alveolar bone	Normal	Normal	Normal	Normal	Extension of the lesion into alveolar bone with significant bone loss
Course of disease	–	2–4 days after plaque accumulation	4–7 days after plaque accumulation	1–3 weeks after plaque accumulation	Periods of quiescence and exacerbation

inflammatory response is getting organized, the host also prepares for the adaptive immune response (Madianos et al. 2005) (Fig. 3.5).

Mediators, such as, IL-1β, TNF-α, and histamine released from activated host cells, participate along with bacterial factors in the activation of endothelial cells, which express surface molecules such as P- and E-selectins and ICAMs that are important for *leucocyte extravasation*. Leucocytes then migrate through the tissues against a concentration gradient of chemoattractants derived either from the host (IL-8, MCP-1, etc.) or from bacteria (fMLP, fimbriae) toward the focus of infection, where they start phagocytosing bacteria and their virulence factors. TNF-α, PGE_2, and histamine increase vascular permeability, which leads to efflux of plasma proteins and fluid into the connective tissue, and subsequently into the crevice, consisting part of the gingival crevicular fluid. Finally, locally produced cytokines, such as IL-1β, TNF-α, and IL-6 may enter the circulation and activate hepatocytes to synthesize acute-phase proteins such as LBP, sCD14, complement proteins, and C-reactive

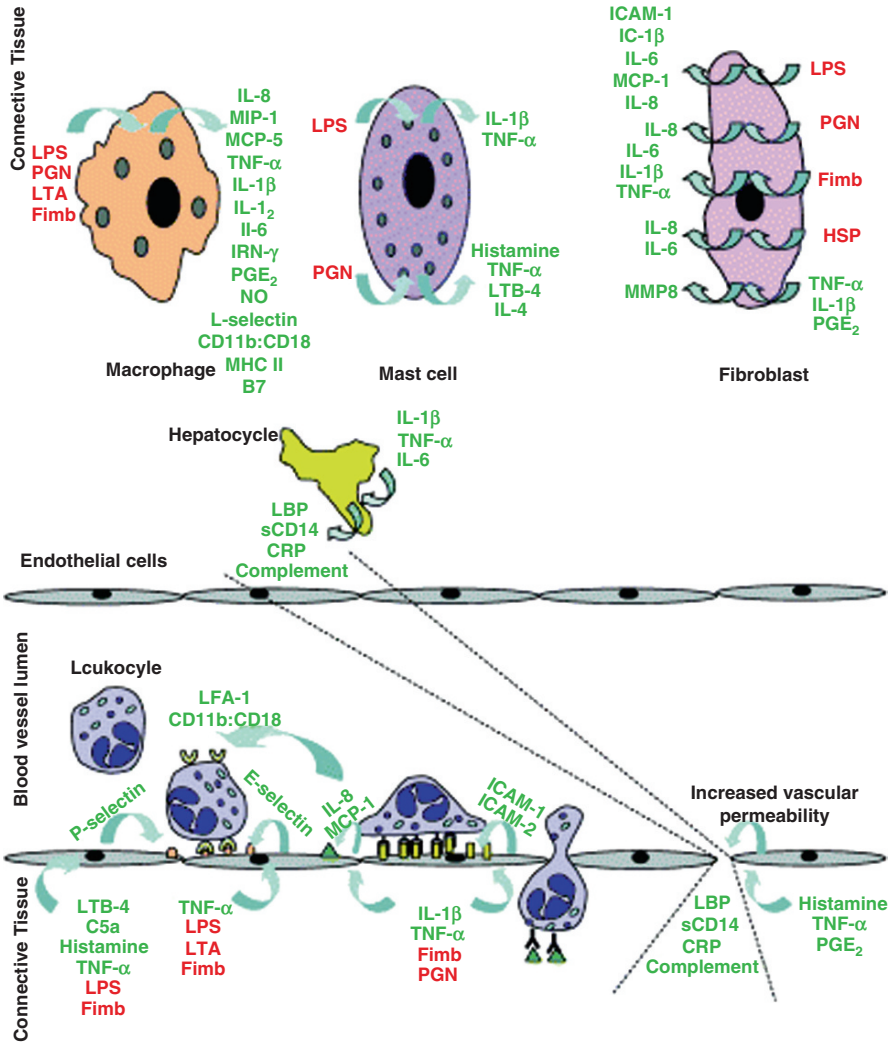

Fig. 3.5 Initiation of inflammatory responses by bacteria: a schematic representation (Madianos et al. 2005) (with permission from Wiley-Blackwell)

protein, which help the host eliminate the infection (Madianos et al. 2005) (Fig. 3.6).

3.1.4 Cells of the Innate Immune Response

3.1.4.1 Neutrophils in Periodontal Lesions

Neutrophils form the first line of defense against infective microbial colonies in plaque biofilms. Neutrophils are attracted to infected periodontal tissues by chemoattractants released from bacteria, host cells, or degraded tissue. As neutrophils themselves cause tissue damage and promote their own chemotaxis, neutrophil accumulation at sites of inflammation is rapidly amplified. Indeed over 90% of leukocytes in GCF are neutrophils. The number of neutrophils increases from ~7 × 10^4 to ~20 × 10^4/mL during the conversion of a healthy sulcus into a diseased gingival pocket. Concurrently, the GCF flow rate adjacent to the tooth surface increases from ~1 to ~5μL/min (Uitto et al. 2003).

Microbicidal Mechanisms of Neutrophils

The killing of microbes is a critical physiological function of phagocytes (Dale et al. 2008). Neutrophils contain morphologically heterogeneous membrane-bound

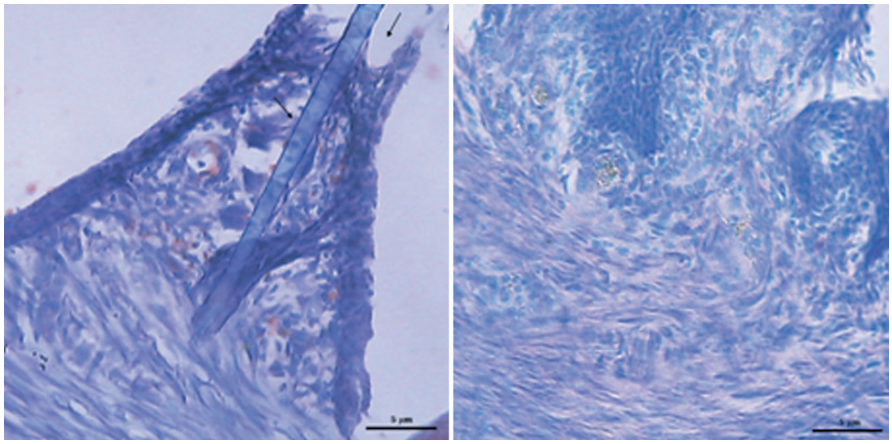

Fig. 3.6 Histological appearance of interdental papilla 3 and 7 days after lipopolysaccharide (LPS) injection in a periodontitis rat model. Giemsa staining reveals inflammatory cell infiltrate in epithelial and subepithelial connective tissues in all LPS-injected animals (University of Bristol)

intracytoplasmic vesicles where a number of molecules used for host defense are stored. Neutrophil granules are generally classified into azurophilic (primary), specific (secondary), and gelatinase (tertiary) types. The azurophilic granules contain hydrolytic neutral enzymes such as elastase, cathepsin G, urokinase, myeloperoxidase, lysozyme, and mannosidase, as well as hydrolases active at acidic pH, including cathepsin B, cathepsin D, and β-glucuronidase. The azurophilic granules also contain four types of defensins (HNP–HNP4), small molecular mass antimicrobial peptides that comprise about 40% of the azurophilic granule contents. The specific (secondary) granules contain lactoferrin, neutrophil collagenase MMP-8), and lysozyme, whereas the main component of the gelatinase granules is the gelatinolytic MMP-9. In addition to these types of granules, a fourth type of granule denoting secretory vesicles is present in mature neutrophils. Amongst other molecules, they contain a serine proteinase, proteinase 3 (Uitto et al. 2003).

Although phagocytes have other microbicidal mechanisms, including antimicrobial peptides (e.g., defensins) and broadly acting proteases, phagocytosis with generation of reactive oxygen species and hypochlorous acid is still regarded as the critical killing mechanism for most invading pathogens (Dale et al. 2008) (Fig. 3.7). In recent years, there has been an important expansion in dental research concerned with free radicals, reactive oxygen species, anti-oxidant defense mechanisms, the possible therapeutic effects of anti-oxidants in treating and/or preventing pathology of inflammatory periodontal disease, with special attention to vitamin E and Co-enzyme Q (Battino et al. 1999). There are also pieces of evidence revealing that *oxidative stress* is possiblly related to periodontitis, as well as obesity, metabolic syndrome, diabetes, coronary heart disease, highlighting the interrelationship between these serious disorders that may co-exist (Boesing et al. 2009; Ohnishi et al. 2009; Southerland et al. 2006).

It was suggested that one cause of the destructive process evident in aggressive, refractory and CP is the "*hyperactivity*" *of neutrophils*, resulting in overproduction of antimicrobial and potentially tissue-damaging oxygen free radicals after exposure to leukotriene B4 and IL-8 due to reduced gene and protein expression of diacylglycerol kinase, inhibition of which is known to amplify the respiratory burst in normal neutrophils (Gustafsson et al. 2006; Fredriksson et al. 1998, 2003 Matthews et al. 2007 a, b).

The specific functions of the PMN include adherence to host substrata, directed migration along chemical gradients, recognition and phagocytosis of microorganisms, followed by bactericidal activity. Alterations in any of these abilities may lead to signs of clinical disease. As previously mentioned, neutrophils enter the gingival crevice by diapedesis through the endothelium of the gingival blood vessels and by migration through the junctional epithelium. PMNs are driven by several chemotactic gradients, including bacterial factors, antibody–antigen complexes, and complement cleavage products, all of which are found in the GCF. Once they enter the dentogingival space, these cells have been activated and possess several mechanisms to counteract microbial colonization and neutralize bacterial products (Table 3.7) (Delima and Van Dyke 2003).

First, PMNs can phagocytose and utilize intracellular killing mechanisms. PMNs can phagocytose bacteria that have been opsonized by complement split products and/or antibodies via specific cell-

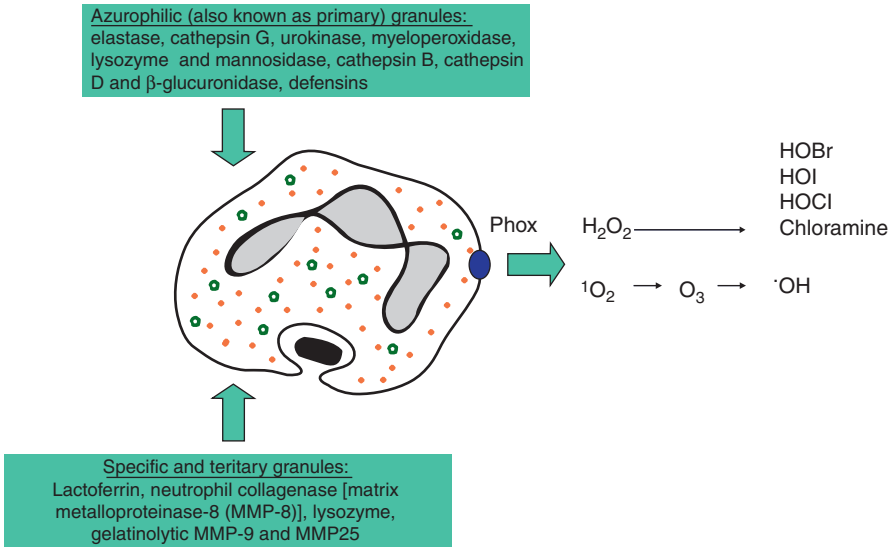

Fig. 3.7 Neutrophils deliver multiple anti-microbial molecules. Microbicidal products arise from most compartments of the neutrophil: azurophilic granules (also known as primary granules), specific granules (also known as secondary granules) and tertiary granules, plasma and phagosomal membranes, the nucleus and the cytosol. *BPI* bactericidal permeability increasing protein; H_2O_2 hydrogen peroxide; *HOBr* hypobromous acid; *HOCl* hypochlorous acid; *HOI* hypoiodous acid; *MMP* matrix metalloproteinase; $^1O_2$ singlet oxygen; $O_{2\cdot}$ superoxide; O_3 ozone; *·OH* hydroxyl radical; *phox* phagocyte oxidase (modified from Nathan 2006) (with permission from Nature Publishing Group)

Table 3.7 Mechanisms of bacterial elimination by neutrophils (Delima and Van Dyke 2003) (with permission from Elsevier Publishing)

Oxygen-independent	Oxygen-dependent
Myeloperoxidase	Myeloperoxidase-mediated
Defensins	Hypochlorous acid
Bactericidal or permeability-inducing protein	Chloramines
Cathepsin G	Myeloperoxidase-independent
Elastase	Hydrogen peroxide
Proteinase 3	Superoxide anion
Azurocidin	Hydroxyl anion
Lysozyme	Hydroxyl radicals
Lactoferrin	Singlet oxygen

surface receptors (for example: Fc receptors, CR1, CR3). The cell sends out cytoplasmic processes that engulf and internalize the pathogen, creating a phagosome. Once the microbes have been engulfed, they can be digested and eliminated. The phagosome subsequently fuses with intracellular lysosomes to create a structure known as a phagolysosome, and consequently digestion and destruction of the pathogen occurs. Although plaque bacteria can be phagocytosed by crevicular neutrophils, this may not be the predominant mechanism of protection in the gingival crevice. A more powerful mechanism of plaque control may be the degranulation of these cells, which results in the extracellular killing of pathogens. This involves the mobilization of the PMNs cytoplasmic granules, following attachment to bacterial surfaces, fusion with the external cell membrane and discharge of the granules into the extracellular environment. The main function of these cells is to deliver antimicrobial substances to microbial targets. This seems to be the principal mechanism of bacterial attenuation used by PMNs in the gingival crevice, especially when confronted by high concentrations of bacterial cells. PMNs are terminally differentiated, and therefore are concerned with growth and survival, thus they are free to use delivery methods that result in their own lysis and death. This "suicidal" action rapidly ejects the cellular contents into the surrounding milieu. Indeed, samples of crevicular fluid contain traces of neutrophil enzymes, particularly in the presence of inflammation (Delima and Van Dyke 2003).

Neutrophil elastase, sometimes referred to as granulocyte elastase, is an abundant proteinase released from the azurophilic granules of neutrophils, and as such is an indicator of neutrophil activity. Neutrophil

elastase is a serine proteinase, active in the degradation of microbiological components in conjunction with, or without, phagocytosis. At the same time, when released extracellularly, this enzyme can degrade host intercellular matrix components, including elastin, fibronectin, and collagen. The elastase activity in gingival crevice fluid may also derive from macrophages. Macrophage elastase, also called MMP-12, may have the same activities as neutrophil elastase, although it is commonly accepted that the majority of elastase activity in gingival crevice fluid originates from the neutrophils. Elastase has been observed in gingival crevice fluid from periodontitis patients at elevated levels, and may be a promising marker for disease progression (Loos and Tjoa 2005).

Alteration of neutrophil/macrophage diapedesis, chemotaxis and migration leading to an absence of the protective inflammatory barrier, or defects within the neutrophil or macrophage themselves render the host susceptible to a wide variety of bacterially induced diseases. These innate cellular abnormalities can occur due to congenital defects or deficiencies (leukocyte adhesion deficiency, Chediak-Higashi syndrome, Papillon-Lefèvre syndrome, and chronic/cyclic neutropenia) of the host or by immunosuppressive agents used to treat other systemic diseases, environmental and behavioral factors (smoking) or a variety of strategies designed by the pathogen itself to avoid the protective mechanisms of the innate system. Regardless of the cause, evidence suggests that during the innate host response phase, diminished or altered function, and/or localization of neutrophils or macrophages is critical for the establishment and severity of chronic inflammatory periodontal disease (Dixon et al. 2004).

Systemic Conditions with Associated Neutrophil Deficits

Papillon-Lefèvre syndrome is a rare autosomal recessive congenital differentiation disorders located at chromosome 11p14-q21 and occurs in children from consanguineous marriages. The prevalence in the general population is 1 to 4 per million, males and females being equally affected with no racial predominance (Hattab and Rawashdeh 1995). The two essential features of Papillon-Lefèvre syndrome are hyperkeratosis of the palms and soles (either diffuse or localized) and generalized rapid destruction of the periodontal attachment apparatus, resulting in premature loss of both primary and permanent teeth (Deas et al. 2003). The external signs are hyperkeratosis of the palms and soles (Kressin et al. 1995). Changes of the skin at electron microscopy revealed the diminution of the tonofibrils, alterations of the keratohialin granules and acanthosis in the stratum spinosum (Kressin et al. 1995). Papillon-Lefèvre syndrome has been associated with decreased neutrophil chemotaxis, reduced random neutrophil migration, impaired neutrophil phagocytosis, reduced myeloperoxidase activity, and increased superoxide radial neutrophil production, associated with a decreased lymphocyte response to pathogens (Velazco et al. 1999; Lundgren et al. 1998).

Intraorally, periodontal symptoms affect primary and permanent dentitions, with an extensive loss of periodontal attachment accompanied by generalized, severe and rapid destruction of the alveolar bone that frequently lead to premature tooth loss (Kressin et al. 1995; Hattab and Rawashdehet al. 1995). Histologically, the gingiva demonstrates epithelial hyperplasia, increased collagen synthesis, parakeratosis, acanthosis and focal aggregates of lymphocytes and plasma cells. In addition, reduced osteoblastic activity and reduced thickness of cementum have been described (Ghaffer et al. 1999; Hattab and Rawashdehet al. 1995). Virulent gram-negative anaerobic microbiota have been considered to be important initiators of the destructive periodontitis observed in these patients. *A. actinomycetemcomitans* has been reported to be the major periodontal pathogen, while *Capnocytophaga gingivalis*, *Eikenella corrodens*, black-pigmented *Bacteroides*, and *Fusobacterium* spp have also been recovered in high numbers in subgingival periodontal lesions in Papillon-Lefèvre syndrome patient (Lundgren et al. 1998; Velazco et al. 1999; Ishikawa et al. 1994; Rudiger and Berglundh 1999).

A particular form of Papillon-Lefèvre syndrome has been named the Haim-Munk syndrome. While also characterized by palmo-plantar keratosis and severe early onset periodontitis, the Haim-Munk syndrome additionally presents with digital abnormalities. These include osteolysis of the distal phalanges, abnormal length and slenderness of the fingers and toes, and a claw-like hypertrophic deformity of the nails (Deas et al. 2003).

Down syndrome is a genetic disorder resulting from a trisomy in the 21st chromosome affecting from 1 in 600 to 1 in 1000 live births, and is characterized by generalized growth and mental deficiencies (Amano et al.

2001). The abnormalities in host response may contribute to the development of periodontal disease, which is more frequently seen among Down syndrome patients compared with healthy controls (Barr-Agholme et al. 1998). There are defects of the chemotaxis and intracellular killing of PMN and other phagocytes, which explains the high incidence of pocketing and marginal bone loss (Steenberghe 1997). Increased amounts of salivary collagenase were found in Down syndrome patients (Halinen et al. 1996). No significant difference could be observed concerning the salivary levels of sIgA, the sum of IgG subclasses, IgM or albumin (Barr-Agholme et al. 1998). Several studies of the microbiota and gingival inflammation in Down syndrome children have implicated *Bacteroides melaninogenicus*, *A. actinomycetemcomitans*, *Fusobacterium nucleatum*, or *P. gingivalis* as possible etiological agents in periodontal disease (Barr-Agholme et al. 1992; Morinushi et al. 1997). In contrast, Amano et al. (2001) did not identify specific bacterial agents, which influenced only early-onset periodontitis in Down syndrome.

Leukocyte adhesion deficiency (LAD) is a rare but well-defined autosomal recessive disease that results in the formation of non-functional ICAM receptor. Healthy periodontal tissue is protected from infection by the continuous transit of neutrophils from the highly vascularized tissue surrounding the tooth root surface into the gingival crevice. Individuals with LAD defect in innate host defense display a severe form of periodontitis that does not require specific periodontal pathogens due to entrapment of neutrophils within the blood vessel (Dixon et al. 2004).

A progressive severe periodontitis, alveolar bone loss, periodontal pocket formation and partial or total premature bone loss of the primary and permanent dentitions were reported in a child with leukocyte adhesion deficiency (Majorana et al. 1999). Root surface exhibited cemental and dentinal erosions or "dentinoclastic activity" that may play an important secondary etiologic role in the rapid attachment loss (Waldrop et al. 1995).

Chédiak-Higashi syndrome is a rare autosomal recessive disease associated with impaired function of cytoplasmic microtubules or microtubule assembly in PMNs (Oh et al. 2002). The susceptibility to infections, although humoral and cellular immunity are normal, leads to early death (often before 5 years of age) (Steenberghe 1997). The disease reveals itself periodontally by severe gingivitis and rapid loss of attachment, leading to exfoliation of the teeth (Steenberghe 1997).

Lazy leukocyte syndrome is an extremely rare disorder that manifests in both quantitative and qualitative neutrophil defects. Lazy leukocyte syndrome is characterized by recurrent infections due to both a deficiency in neutrophil chemotaxis and a systemic neutropenia, while the phagocytic function of the neutrophil remains intact. Impaired random and directional motility leads to a diminished in vivo migration of neutrophils into the tissues and to sites of inflammation (Deas et al. 2003). Oral stomatitis, recurrent ulcerations of the buccal mucosa and tongue, severe gingivitis and periodontitis with advanced alveolar bone loss and tooth loss has been described in persons with these conditions (Manson and Eley 2000; Deas et al. 2003).

Chronic granulomatous disease (CGD) is an extremely rare inherited disease with an incidence of 1/200 000–250 000 births. It has two genetic forms: autosomal recessive and X-linked recessive. The defect in this disease is in the ability of phagocytic cells, both PMN leukocyte and monocytes, to perform killing by the oxidative pathway. Hydrogen peroxide cannot be produced, as there is a defect in the activation of the enzyme NADPH oxidase. As a consequence, superoxide and hydrogen peroxide generation are not performed by the phagocyte, and intracellular killing is defective (Kinane 1999). The majority of CGD patients suffer from severe infections, the most common of which are pneumonia, lymphadenitis, cutaneous and hepatic abscesses, osteomyelitis and septicemia (Buduneli et al. 2001). Although CGD has a place in the list of diseases either known or suggested to cause dysfunction of neutrophils, no specific periodontal condition has been attributed to this disease. A generalized prepubertal periodontitis case associated with CGD was reported by Buduneli et al. (2001) in a 5-year old male patient.

Neutropenia is defined as an absolute neutrophil count <1500 cells/mm^3 and can be graded as mild (1000–1500 cells/mm^3), moderate (500–1000 cells/mm^3), or severe (<500 cells/mm^3).

Neutropenias are classified as (Schwartzberg 2006):

(a) Congenital neutropenia

- Cyclic neutropenia: autosomal dominant genetically transmitted
- Severe congenital neutropenia: sometimes referred to as Kostmann syndrome; autosomal dominant and autosomal recessive genetically transmitted
- Congenital genetic syndromes with neutropenia component: Shwachman syndrome, dyskeratosis

congenita, X-linked Barth syndrome, and Chédiak-Higashi syndrome

(b) *Acquired neutropenia*

- Immune-associated neutropenia: Isoimmune (alloimmune) neonatal neutropenia, most common form; Primary autoimmune neutropenia, rare disorder of early childhood, rare in adults; Secondary autoimmune neutropenia, associated with systemic autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus-associated neutropenia, Felty syndrome), infectious diseases, hematologic disorders (e.g., large granular lymphocyte leukemia, other leukemias, multiple myeloma), solid neoplasms, drug therapy, and transplantation
- Chronic idiopathic neutropenia: associated with low-grade underlying inflammatory illness; not immune mediated
- Infection-related neutropenia: associated with viral, bacterial (including rickettsial), and parasitic agents
- Drug-induced neutropenia: associated with chemotherapeutic agents and many other drugs (e.g., arninopyrine, penicillin, clozapine)
- Nutrition-related neutropenia: associated with nutritional deficiencies (e.g., folate, vitamin B_{12})

Neutropenia can develop as a result of more than one pathologic mechanism, including decreased bone marrow production, the sequestering of neutrophils, and increased destruction of neutrophils in the peripheral blood. The clinical result is increased risk for infection. This risk is directly proportional to the severity and duration of neutropenia. Neutropenia is classified according to the etiology as congenital or acquired, with the latter further defined according to the etiology or pathology (Schwartzberg 2006). Congenital neutropenia is divided primarily into two forms: cyclic neutropenia and severe congenital neutropenia. Periodontal manifestations of cyclic neutropenia include inflamed gingival, gingival ulceration, periodontal attachment and bone loss (Kinane 1999). Unfortunately, even with the best of professional and home care, teeth are often lost due to advancing periodontal disease (Deas et al. 2003). Oral manifestations of chronic benign neutropenia include bright red, hyperplastic edematous gingivitis, which affects the free and attached gingivae, and the gingivae bleed easily. There appears to be premature loss of primary teeth due to bone loss. Some older children show a rapidly progressive periodontitis in the permanent dentition, with generalized bone loss. In most reports, attempts to control the condition with periodontal treatment have been unsuccessful, and early loss of primary and permanent teeth seems difficult to prevent (Manson and Eley 2000). Acquired neutropenia encompasses a broad spectrum of causative processes, and includes primary or secondary immune-associated neutropenia, chronic idiopathic neutropenia, infection-related neutropenia, drug-induced neutropenia, chemotherapy-induced neutropenia, and nutrition-related neutropenia. The periodontal manifestations of Familial neutropenia include fiery red edematous gingivitis, which is often hyperplastic and accompanied by periodontal bone loss (Kinane 1999). The periodontal features of chronic idiopathic neutropenia reported include a severeedematous, hyperplastic gingivitis with early bone loss, and the condition does not respond well to the treatment (Manson and Eley 2000).

3.1.4.2 Macrophages in Periodontal Lesions

A currently accepted paradigm, explaining how the predominantly gram-negative infection associated with periodontitis lesions is translated into destruction of bone and connective tissue, revolves around mononuclear phagocytes. In sites of chronic inflammation, bacteria participate in attracting leukocytes into the periodontal tissues either by directly expressing chemoattractant peptides or by stimulating the production and secretion of chemoattractant cytokines and inflammatory lipids from host cells. This leads to accumulation of leukocytes in the host tissue. Bacterial lipopolysaccharide can subsequently interact with macrophage or dendritic cell receptors, including CD14 and TLRs, to stimulate production of inflammatory cytokines and other mediators. This model of tissue destruction focuses on the production of IL-1 as a key mediator of periodontal tissue destruction due to the association of this cytokine with stimulation of collagenolytic and bone-destructive processes (Schenkein 2006).

Macrophage numbers do not increase and there is little evidence of macrophage activation in advanced periodontitis compared with minimally inflamed tissues. Consistent with this, it has been demonstrated that, in the progression from gingivitis to periodontitis, there is a decrease in the macrophage/B-cell ratio while B cells express an increasingly activated phenotype. This suggests that the major source of IL-1 disease may be lymphocytic rather than macrophages, further

supporting the concept that destructive periodontitis is determined by the nature of the lymphocytic response (Gemmell et al. 2002).

The interaction of lipopolysaccharide with macrophages also stimulates production of prostanoids, in particular prostaglandin E_2, which is notably found at high concentrations in gingival crevice fluid from sites undergoing periodontal breakdown (Schenkein 2006). Prostaglandin E_2 is a bone-resorbing inflammatory lipid, and experimental data indicate that this pathway is likely to be important in human periodontal bone loss (reviewed by Noguchi and Ishikawa 2007). It was also recently revealed by Ho et al. (2008) that the −765G to C polymorphism of the cyclooxygenase-2 (COX-2) gene is associated with a decreased risk for periodontitis in Taiwanese, especially in AP (Fig. 3.8).

The physiological importance of prostanoids is highlighted by the use of COX-inhibiting non-steroidal antiinflammatory drugs in the clinical treatment of disorders (Noguchi and Ishikawa 2007). Non-selective cyclooxygenase-1 (COX-1) inhibitors used in periodontal research include compounds such as aspirin, flurbiprofen, ibuprofen, naproxen and piroxicam. Selective COX-2 inhibitors represent a new group of pharmaceutical products termed "coxibs" that include meloxicam, nimesulide, etodolac, and celecoxib. Evidence from animal experiments and clinical trials documents that selective and non-selective NSAIDs are mainly responsible for the stabilization of periodontal conditions by reducing the rate of alveolar bone resorption. This is achieved through local inhibition of both enzymes (e.g., COX-1 and COX-2) responsible for the synthesis of arachidonic acid metabolites. Evidence shows that the effects of NSAIDs drop off rapidly after drug withdrawal. One of the major advantages of selective COX-2 inhibition is the reduction of adverse systemic effects (Salvi and Lang 2005; Kantarci et al. 2006). The adverse effects of NSAIDs, particularly the conventional non-selective drugs, have been confirmed by numerous experimental and clinical trials in the orthopedic domain, with reports of impaired spinal fusion and delayed fracture healing. In contrast, few investigations have been carried out to assess the possible deleterious effects of NSAIDs on alveolar bone healing (Fracon et al. 2008).

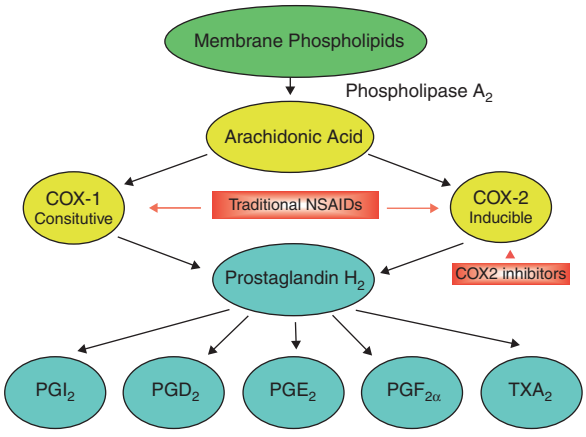

Fig. 3.8 Pathway of prostanoid synthesis. *COX-1* cyclooxygenase-1; *COX-2* cyclooxygenase-2; *PG* prostaglandin; PGD_2 prostaglandin D_2; PGE_2 prostaglandin E_2; $PGF_{2\alpha}$ prostaglandin $PGF_{2\alpha}$; PGH_2 prostaglandin H_2; PGI_2 prostaglandin I_2; TXA_2 thromboxane A_2.(modified from Noguchi and Ishikawa 2007) (with permission from Wiley-Blackwell)

3.1.4.3 Mast cells in Periodontal Lesions

Mast cells are key elements in the innate immune system and have been termed the "antennas" of the immune response for their ability to detect changes in their environment and communicate these to other cells in the vicinity. Mast cells are located throughout the body in close proximity to epithelial surfaces, near blood vessels, nerves and glands, placing them at strategic locations for detecting invading pathogens. In addition, mast cells express a number of receptors that allow them to recognize diverse stimuli. In sensitized individuals, IgE is bound to Fcε receptors expressed on the mast cell surface, and binding of antigen to surface-bound IgE induces mast cell activation. Thus, multiple stimuli (foreign antigens) may trigger the same class of receptor. However, specificity is built into this system as a result of multiple signal transduction pathways that are differentially activated according to antigen size, receptor location, number, and subtype. Human mast cells also express a number of TLRs including TLR-1, TLR-2, TLR-6, and TLR- 4. Expression of TLRs, in combination with other receptors, allows mast cells to recognize many potential pathogens and generate specific responses. Importantly, mast cells are capable of releasing many small molecules that stimulate inflammation and the adaptive immune response and can polarize T cell subpopulations toward Th1 or Th2 subtypes. Mast cell products include preformed mediators that are granule associated (such as histamine), mediators synthesized *de*

Table 3.8 Products of myeloid cells involved in immune regulation and anti-microbial responses (Suzuki et al. 2008) (with permission from Elsevier Publishing)

	Neutrophil	Macrophage	Mast cell	Eosinophil	Dendritic cell
Cytokines	Tumor necrosis factor (TNF)-α, interleukin (IL)-1β, -6	IL-1β, -6, transforming growth factor (TGF)-β, TNF-α	IL-1, -3, -4, -5, -6, -13, -16, VEGF, TNF-α, TGF-β	IL-2, -3, -4, -5, -6, -10, -12, -13, INF-γ, TNF-α, GM-CSF, SCF, TGF-α	IL-1β, TNF-α, IL-5, IL-6, IL-12, IL-18, IFN-γ
Chemokines	IL-8		IL-8, MIP-1α, MCP-1	RANTES, eotaxin, ENA-78/CXL5, GRO-α	MIP-1α, MCP-1, eotaxin
Proteases and anti-proteases	PR3, elastase, cathepsin G, Matrix metalloproteinases (MMPs), TIMPs	MMPs, TIMPs			
Anti-microbial factors	α-Defensin, elafin, SLPI, lactoferin, myelo-peroxidase		LL-37		
Adhesion molecules	CD11(a,b,c,d)/CD18; α4β1	Intracellular adhesion molecule-1			CD11c/CD18, CD86, CD40, major histocompatibility complex/HLA class II
Receptors	fMLP-R, LTB4-R, PAF-R, TLR-1, -2, -4, -6		TLR-1, -2, -4, -6, FcεR		
Others	ROS, RNS	ROS, RNS	LCT4, PAF, prostaglandin D_2		

novo (such as leukotriene C4, platelet activating factor and prostaglandin D2), an array of cytokines, chemokines and growth factors. The strategic location of mast cells in the body and their diversity of receptors and cytokines indicate an important role for mast cells in regulating innate and adaptive immunity (Suzuki et al. 2008) (Table 3.8).

It was revealed in human periodontal disease that there is an increase in the number of mast cells that may be participating either in the destructive events or in the defense mechanism of periodontal disease via secretion of cytokines, including perpetuation of the Th2 response, and cellular migration and healing processes (Batista et al. 2005; Steinsvoll et al. 2004; Gemmell et al. 2004).

3.1.4.4 Dendritic Cells in Periodontitis Lesions

Dendritic cells can be viewed as conductors of the immune response. These cells, resident within tissues, develop in vivo from hematopoietic precursor cells (Suzuki et al. 2008). Dendritic cells exist in two stages of maturation according to their capacity to stimulate T cells, as nonactivated (immature) and as activated (mature) dendritic cells. As immature cells, dendritic cells are scattered throughout the body in nonlymphoid organs, where they are specialized for antigen capture and processing. A number of microbial and inflammatory products, as well as cytokines activate dendritic cells, leading to the increased expression of MHC-II and costimulatory molecules. Activated dendritic cells migrate to draining lymph nodes, where they are able to stimulate T cells. Mature dendritic cells display increased levels of the cell surface costimulatory molecules CD40, CD80, and CD86, as well as HLA-DR. Furthermore, mature dendritic cells express CD83 and secrete increased amounts of various cytokines and chemokines that aid in T-cell activation (Kopitar et al. 2006).

Dendritic cells, including Langerhans cells and dermal dendritic cells, are found in gingival tissue, and mature CD83+ dendritic cells are present in tissues from patients with periodontitis. Furthermore, dermal dendritic cells, which have similarities with monocyte-derived dendritic cells, may be associated with T-cells

in periodontitis, suggesting dendritic-cell-mediated T-cell activation. It was also reported that *P. gingivalis* and *A. actinomycetemcomitans*-stimulated dendritic cells promote a rapid IFN-γ response by stimulating NK cells, as summarized by Kikuchi et al. (2005) (Jotwani et al. 2001; Cirrincione et al. 2002; Jotwani and Cutler 2003; Kikuchi et al. 2004).

In short, a lot of evidence has accumulated that supports the role of dendritic cells in maintenance of oral health and, possibly, in the pathogenesis of oral diseases, especially regarding diseases of the oral mucosa/gingiva and dental pulp, as reviewd by Cutler and Jotwani (2004). In general, there are several pertinent conclusions to be drawn from these studies. CD1a $^{+}$ Langerhans cells appear to be principal leukocytes involved in the response of the oral mucosal epithelium to infectious, atopic or dysplastic diseases, whether it be within keratinized (e.g., gingiva) or non-keratinized (e.g., buccal mucosa) tissue. However, the authors observed that the specific role of Langerhans cells in the diseases studied, including gingivitis, periodontitis, oral lichen planus, oral hairy leukoplakia, oral tumors, contact stomatitis, oral candidiasis, gingival overgrowth/fibrosis is, however unclear at present (Cutler and Jotwani 2004). Cutler and Teng (2007) recently suggested that dendritic cells, macrophages and osteoclasts may share common precursor pool(s), raising the issue of whether the dendritic cell plasticity is restricted to the context of immune responses to environmental factors [i.e., feedback signaling from T cells expressing receptor activator of NF-κβ ligand (RANKL) or other modulating signals] or whether it extends beyond their immune functions.

3.1.4.5 Eosinophils in Periodontitis Lesions

Eosinophils are viewed as effector cells of allergic responses and also function in elimination of parasites. Eosinophils are bone-marrow derived cells that contain four distinct granule cationic proteins: major basic protein, eosinophil peroxidase, eosinophil cationic protein, and eosinophil-derived neurotoxin. During allergic inflammation, eosinophils release granule contents, as well as inflammatory mediators including lipid mediators such as leukotriene C4 and platelet-activating factor, which may cause dysfunction and injury to other cells (Suzuki et al. 2008). Presence of activated eosinophils, high IgE and sCD23 titers in GCF of patients with adult periodontitis was revealed (Suzuki et al. 1995).

3.1.4.6 Natural Killer Cells (NKT Cells) in Periodontitis Lesions

NKT cells represent a unique subset of T cells that express receptors such as CD161 (also called NK1.1 in mouse), typically found on NK cells. A key property of NKT cells is their capacity to rapidly produce a variety of cytokines upon T cell receptors (TCR) engagement. As such, the behavior of NKT cells is more similar to that of innate rather than adaptive immune system cells. Therefore, NKT cells have been classified as innate lymphocytes, which also include B-1 B cells, marginal zone B cells, and γδ T cells (Van Kaer 2004, 2007).

It was demonstrated an elevation of Vα24JαQ NK T cells in the gingival lesion of periodontitis patients and to a lesser extent in that of gingivitis patients as compared with peripheral blood of either periodontitis patients or nondiseased controls (Yamazaki et al. 2001). Although all four subsets of CD1 molecules were expressed in periodontal lesions, CD1d was most abundant. CD1d-expression was more frequent in periodontitis than gingivitis and increased together with increase of invariant NKT cell infiltration, suggesting that CD1d-expressing B cells could activate NKT cells by CD1d-restricted manner and this NKT cell activation may play roles in pathogenesis of periodontal diseases (Amanuma et al. 2006).

3.2 Aspects of Adaptive Host Response in Periodontitis

Mechanisms of host response in the periodontal tissues are complex and involve numerous systems of interactions. While the innate host response is characterized by non-specific reactions, the adaptive response utilizes strategies of recognition, memory and binding to support the effector systems in the elimination of challenging elements (Berglundh and Donati 2005). Parts of the adaptive host response in periodontitis as outlined by Berglundh and Donati (2005) are: (1) the nature of the lymphocyte type (T and B cells), (2) antigen recognition by TCRs, (3) cytokine profiles

of T helper (Th) cells, and (4) autoimmune reactions that may influence the adaptive host response in periodontitis.

3.2.1 Antigen Presenting Cells (APCs) in Periodontitis Lesions

Several cells serve as APCs. Langerhans cells, macrophages, and dendritic cells are professional APCs and contribute to antigen recognition and early response mechanisms in host defense (Berglundh and Donati 2005). As the primary player in humoral immunity, B cells present specific antigens to cognate CD4 T cells with extremely high efficiency so that they obtain help for the production of high-affinity antibodies. Nonspecific antigens derived from endogenous and pinocytosed proteins are also presented by B cells, but the outcome of presentation of nonspecific antigens is T cell tolerance. Therefore, B cells can either activate or inactivate T cells, depending on the nature of the antigen. In the presence of dendritic cells or activated macrophages, the role of B cells in presenting nonspecific antigens is negligible. However, if B cells are the only cells available to present nonspecific antigen, T cell tolerance would be the outcome. Such differences in antigen presentation between antigen-specific and nonspecific B lymphocytes provide assurance that specific helper T cells will be the favorite partners for cognate interactions with specific B cells, a mechanism that is essential not only for the effective induction of specific antibody responses, but also for the prevention of non-specific humoral immunity (Chen and Jensen 2008).

Co-stimulatory molecules associated with APC–TCR interactions in periodontitis were investigated by Gemmell et al. (2001, 2002), Orima et al. (1999), and Mahanonda et al. (2002). Attempts were also made to characterize adults and aggressive periodontitis sites (Liljenberg and Lindhe 1980; Gillett et al. 1986; Joachim et al. 1990; Kleinfelder et al. 2001; Lappin et al. 1999; Berglundh et al. 2001; Celenligil et al. 1993; Hillmann et al. 2001). The reported findings regarding different cell proportions in adult periodontitis are virtually consistent, while larger differences are found in studies on aggressive forms of periodontitis. The overall distribution of inflammatory cells in periodontitis lesions was excellently summarized by Berglundh and Donati (2005). Plasma cells are the most common cell type and represent about 50% of cells, while B cells comprise about 18%. The proportion of B cells is larger than that of all T cells, and Th cells occur in larger numbers than cytotoxic T cells. PMN cells and macrophages are found in fractions of <5% of all cells (Berglundh and Donati 2005).

3.2.1.1 T-cells in Periodontitis Lesions

The primary cells involved in the adaptive immune response are lymphocytes. Lymphocytes are divided into two main groups, B cells and T cells. Both types have the ability to interact with and respond to foreign stimuli. B cells are mainly antibody producers, while T cells are functionally divided into helper T, regulatory T, and cytotoxic T cells. Helper T and regulatory T cells help to regulate the immune response by releasing a variety of regulatory mediators or cytokines. Cytotoxic T cells are able to kill other cells through cell–cell interactions. After activation by antigen-presenting cells, helper T cells begin to secrete a range of cytokines. The helper T cells are classified as T helper type 1 or type 2 cells based on the cytokine profile they secrete. T helper type 1 cells secrete mainly IL-2, interferon-γ (IFN-γ), and lymphotoxin-α, while T helper type 2 cells secrete mainly IL-4, IL-5, IL-10, and IL-13. The T helper type 1 cytokines enhance macrophage activation and support immunoglobulin ((Ig) isotype switching to IgG2a. In contrast, T helper type 2 cytokines up-regulate B-cell activity, support antibody production and promote Ig switching to the IgG1 isotype. In addition, T helper type 1 and type 2 cytokines support clonal expansion of their specific secretor cell while inhibiting the functions of the other type. Therefore, the dominance of either the T helper type 1 or type 2 response determines the outcome of the infection and the fate of the tissues at the inflammatory site (Houri-Haddad et al. 2007; Mathur and Michalowicz 1997; Teng 2003, 2006).

Multiple factors can influence the development of Th subsets, including Ag dose, MHC molecules, the strength of peptide signals, and different levels of co-stimulatory molecule expression. Among these, the most critical factor for the development of Th1 or Th2 phenotype is the cytokines available in the local microenvironment, both in vitro and in vivo (Teng 2003). For instance, IL-4 is absolutely required for committing Th2 development, and IL-12 and IFN-γ

are required for Th1 development. Further, IL-4 and IFN-γ can cross-regulate Th1 and Th2 differentiation while amplifying their own development and growth, respectively. This cross-regulatory mutual antagonism serves to redirect the immune response toward a phenotype or effector function appropriate to resolve the microbial challenges. Thus, Th1/Th2 dichotomy and/or effectors provide a cytokine-based framework for understanding CD4 + Th heterogeneity during development toward more pathogenic (or destructive) vs. beneficial (healing) immune responses (Teng 2003).

However, the results of studies of the contributions of Th1/Th2 or other cytokine profiles to the pathogenesis of periodontal disease have been shown to be conflicting and controversial. It has been postulated that specific Th1 vs. Th2 cytokine profiles may be associated with susceptibility to periodontal infection (Teng 2003; Houri-Haddad et al. 2007). However, many recent studies showed mixed results with respect to the T helper type 1/type 2 dominant response in periodontal disease (Baker et al. 1999; Ebersole and Taubman 1994; Fujihashi et al. 1991; Pilon et al. 1991; Salvi et al 1998; Tokoro et al. 1997). As early observations have revealed that B-cells (humoral immunity) are predominantly enriched in "established" periodontal lesions (Page and Schroeder 1976), while T-cells (cell-mediated immunity) are more predominant at the "early" stage of the disease, directing or influencing Th1 vs. Th2 differentiation at the early stage of the infection could have some impact on the development and/or progression of the periodontal disease (Teng 2003).

T cells can be distinguished from other lymphocyte types, such as B cells and NKT cells by the presence of a special receptor on their cell surface called *TCR*. As was reviewed by Berglundh and Donati (2005), TCR V α/β expression in peripheral blood samples of subjects with periodontitis does not differ from that of healthy individuals. The periodontitis lesion expresses a unique TCR repertoire that is different from that in PBMC. In the lesion, various V β genes seem to be dominating, and the reported variation may be related to differences in the severity of the disease, or in the composition of the subgingival microbiota (Berglundh and Donati 2005).

3.2.1.2 B Cells in Periodontitis Lesions

According to traditional concepts in immunology, B cells serve as a well-controlled part of the adaptive host response and act on systems regulated by T cells. Periodontitis lesions contain large amounts of leukocytes, among which plasma cells appear to be the dominant cell type – together with lymphocytes they represent about 75–80% of all inflammatory cells. It was also observed that lesions in aggressive and chronic forms of periodontitis exhibit similar features with respect to cellular composition. Differences in disease severity, however, may affect plasma cell and B-cell densities in both forms of periodontitis. Thus, the proportions of plasma cells and B cells appear to be larger in lesions obtained from sites of severe periodontitis than in lesions from areas with moderate or mild periodontitis. The fact that plasma cells develop from B cells and that these two groups of cells dominate in periodontitis lesions indicates that specific attention should be given to the role of B cells in periodontitis (Berglundh et al. 2007).

B cells develop from hematopoietic stem cells, initially in the fetal liver before birth, and subsequently in bone marrow. *Among peripheral mature B cells, there are three recognized subsets* as identified in mouse models and to some extent in humans. First, *B-1 cells*, or unconventional B-lineage cells, are divided into B-1a and B-1b. The B-1a cells express the surface marker CD5, while B-1b cells do not. Second, *B-2 cells*, i.e., conventional B cells, are the traditional and representative B cells of the adaptive immune system. The B-2 cells participate in T-dependent germinal center reactions and yield isotype-switched, high-affinity memory cells and long-lived plasma cells. On the other hand, B-1 cells reside in specific anatomical compartments (e.g., peritoneal or pleural cavities) and are responsible for early antibody responses, which may be T-cell-independent, as well as frequently polyreactive and with low affinity. Different subsets of B cells, such as B-1a and B-2 cells are present in periodontitis lesions (Aramaki et al. 1998; Berglundh et al. 2002b; Tabeta et al. 2000). While the conventional B-2 cells represent the traditional B cells of the adaptive host response resulting in long-lived plasma cells and memory cells, the B-1a cells produce natural antibodies of initially IgM types and, following isotype switching, also IgG types. Elevated levels of B-1a cells have been demonstrated in both periodontitis lesions and peripheral blood of subjects with severe forms of periodontitis. Different pathological functions of B-1a cells have been associated with IL-10, and this cytokine serves as an autocrine growth factor for this type of B cells (Berglundh et al. 2007).

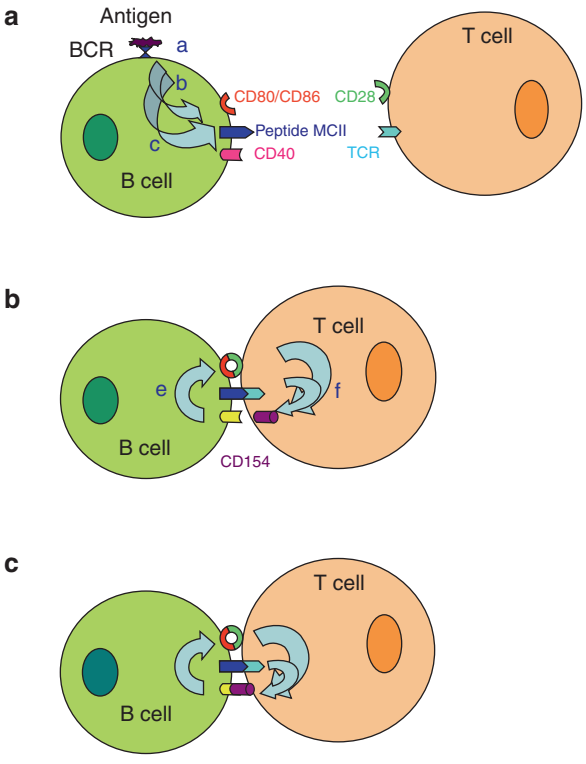

Fig. 3.9 B cell antigen presenting function can be activated without participation of other types of antigen presenting cells. (**a**) Crosslinking of the BCR by its specific antigen (a) leads to the expression of peptide–major histocompatibility complex (MHC) II complexes (b) and low levels of CD86 (c). (**b**) The T cell receptors of a CD4 T cell specific for the same antigen is engaged by peptide–MHCII complexes and CD86 binds to CD28. These signals induce expression of low levels of CD154 (d), which binds CD40 on the B cell, inducing CD80 expression and upregulating CD86 (e). (**c**) The cells continue exchanging signals, which grow progressively and culminate in complete activation of both cells (modified from Rodríguez-Pinto 2005) (with permission from Wiley-Blackwell Publishing) (Berglundh et al. 2007) (with permission from Elsevier)

B lymphocytes contribute to immunity in multiple ways, including production of antibodies, presentation of antigen to T cells, organogenesis of secondary lymphoid organs and secretion of cytokines (Hoelig et al. 2008).

B Cells as Antigen-presenting Cells

The function of B cells in antigen presentation differs to some extent from that of other, so-called professional, antigen-presenting cells, e.g., Langerhans cells, macrophages, and dendritic cells. Thus, other antigen-presenting cells take up antigens through pinocytosis or internalization of receptors for immune complexes, while B cells internalize antigens by an Ig receptor (the B-cell receptor) in the cell membrane. The antigen is degraded into peptides and subsequently attached to class II molecules of the MHC. Finally, the processed antigen is transported to the B-cell membrane for presentation to helper T cells ($CD4^+$). B cells may also, in this process, control and select antigens to be presented to T cells, particularly when the antigen concentration is low. The affinity of the Ig receptor is correlated to the capacity of B cells to present antigens to helper T cells. The density of B-cell clones that express receptors for a particular antigen is low and the expansion of such specific clones and the up-regulation of co-stimulatory molecules require activation. This process involves both the interaction between the Ig receptor and the antigen, as well as the binding of the B-cell–CD40 molecule to the expressed CD154 part on the activated T helper cells (Berglundh et al. 2007). As Berglundh et al. (2007) summarized, the function of B cells as antigen-presenting cells reported above has also been described in studies on periodontitis (Orima et al. 1999; Gemmell et al. 2001, 2002; Mahanonda et al. 2002) (Fig. 3.9).

B Cells and Antibodies

The activation of B cells that takes place during antigen-specific immune responses results in proliferation, maturation, isotype switching, and differentiation into antibody-secreting plasma cells. The production of antibodies involves the response to foreign antigens, as well as to self antigens (Berglundh et al. 2007).

There are four subclasses of IgG, which differ in their distribution in serum, their biological properties, and the types of antigens responsible for their induction. IgG2 is the second most abundant of the IgG subclasses in human serum. Functionally, immune complexes containing IgG2 are relatively poor at activating the classical complement pathway but are the most efficient of the IgG subclasses in activating the alternative pathway. Furthermore, in their interaction with leukocyte Fc receptors, such complexes preferentially bind with greatest affinity to allotypic variants of the receptor FcγRIIa (CD32) (Schenkein et al. 2007).

Leukocyte IgG receptors (*FcγR*) serve as a link between the humoral and cellular branches of the immune system. They confer potent leukocyte effector functions to the specificity of IgG. Binding of the constant region of IgG to FcγR induces a plethora of cell type specific pro- and anti-inflammatory functions. FcγR polymorphisms influence the efficacy of cellular responses, and have been associated with inflammatory disease and disease severity. Leukocyte FcγR belong to the Ig superfamily and are divided in three classes, FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16), encompassing at least 12 isoforms. FcγR classes contain structurally and biochemically distinct molecules, and differ in cell distribution and affinity for IgG subclasses. FcγR induce leukocyte effector functions such as phagocytosis, cytotoxicity, cytokine production, degranulation, antigen presentation, and regulation of antibody production upon crosslinking, e.g., after binding immune complexes (Meisel et al. 2001; Nimmerjahn and Ravetch 2006).

Leukocyte FcγR are encoded by eight genes on the long arm of chromosome 1. In humans, all three FcγR groups present genetically determined polymorphism. The most studied polymorphic forms of FcγR involve changes in the extracellular domains, containing the ligand binding sites, which affect the affinity for immune complexes. The frequency of these polymorphic forms varies among ethnic groups (Ivan and Colovai 2006; Meisel et al. 2001; Rascu et al. 2001; van Sorge et al. 2003). Several invesitigators have evaluated the *Fcγpolymorphism* in chronic, aggressive and refractory periodontal disease in different ethnic population (Table 3.9).

A series of studies on *antibody responses and specificity* in AP patients was performed to address the issue of functionality of antibodies reactive with the periodontal pathogens *A. actinomycetemcomitans* and *P. gingivalis*. These studies revealed that antibody function could be related to clinical status in AP, a fact that was reinforced by studies demonstrating that periodontal therapy resulting in decreased bacterial load could induce production of antibodies to plaque microorganisms in patients with low serum antibody levels and decreased titer, but increased antibody avidity in patients who had high levels of specific antibody (Chen et al. 1991; Schenkein 2006).

To better examine the effects of antibody titer on clinical status, studies have been performed that assess *antibody responses to specific bacterial virulence factors* (Schenkein 2006). As reviewed by Schenkein (2006), several of such studies verified that strong antibody responses against specific bacterial antigens such as the lipopolysaccharide or leukotoxin of *A. actinomycetemcomitans* and the lipopolysaccharide or hemagglutinin of *P. gingivalis*, were associated with less severe disease in patients with aggressive or CP (Califano et al. 1996, 1997, 1999, 2004). These antibodies were identified to be predominantly of the IgG2 subclass (Schenkein 2006). It is crucial to consider the impact of race, genetic predisposition and smoking on antibody and Ig production in periodontitis patients because these factors influence both the design of studies of AP and the interpretation of the results of such studies (Ebersole 2003).

The protective role of local antibody production, as detected by elevated concentrations of *gingival crevice fluid antibodies*, was evaluated in several studies reviewed by Ebersole (2003), Holmberg and Killander (1971), Reinhardt et al. (1989). Certain of these studies have also demonstrated the presence of specific antibody activity to suspected periodontopathogens in the Ig composition of GCF (Berglund 1971; Ebersole et al. 1984, 1985a, b; Smith et al. 1985, Tew et al. 1985). Generally, these results demonstrated localized elevations in specific antibody in GCF (Berglund 1971; Ebersole et al. 1984, 1985a, b; Smith et al. 1985). However, we still have little information on the functional importance of this GCF antibody, somewhat in contrast to a variety of in vitro and in vivo studies describing the capacity of serum antibody to interfere with various virulence properties of oral pathogens. Ebersole (2003) revealed that the presence of all subclasses of IgG have been identified in GCF, with IgG1 and/or IgG4 levels in GCF elevated relative to serum concentrations (Ebersole et al. 1984; Powell et al. 1994; Reinhardt et al. 1989). In particular, IgG4 in active sites was nearly 25 times the serum level (Steubing et al. 1982). Thus, the literature provides rather compelling results supporting a local synthesis of IgG in the periodontium (Ebersole 2003).

Despite the lack of clarity regarding the impact of disease-induced antibody on periodontal clinical status, several investigators have developed *vaccines* based upon immunization with whole bacteria or agents related to specific virulence factors. This approach, which has mainly targeted *P. gingivalis* antigens such as capsular polysaccharide and the gingipain proteases, sheds some light on the pathogenic mechanisms of disease in these models of periodontitis (Schenkein 2006). The current evidence collected from a large series of

Table 3.9 Fc gene polymorphisms in aggressive and chronic periodontitis

Author	Receptor	Study population Cases/Controls	Periodontal diagnosis	Ethnicity	Association
de Souza and Colombo (2006)	FcγRIIa FcγRIIIb	31/49	Generalized aggressive periodontitis (AP)	Brazilian population	Significant
Fu et al. (2002)	FcγRIIa FcγRIIIa	48/67 periodontally-healthy controls	Localized AP	African-American population	Significant
Loos et al. (2003)	FcγRIIa FcγRIIIa FcγRIIIb	12 + 56/61	Aggressive and chronic periodontiti	Caucasian population	Significant
Kobayashi et al. (1997)	FcγRIIa FcγRIIIb	100/105	Adult periodontitis	Japanese population	Significant
Yamamoto et al. (2004)	FcγRIIa	213/209	Chronic periodontitis	Caucasian population	Significant
Yoshihara et al. (2001)	FcγRIIIb	42 + 52/55	Generalized early-onset and adult periodontitis	Japanese population	Significant
Chung et al. (2003)	FcγRIIa FcγRIIIb	50 + 30/74	Chronic and generalized AP	Taiwanese population	Non-significant
Kobayashi et al. (2000a, b)	FcγRIIIa FcγRIIIb	38 + 83/104	Generalized early-onset and adult periodontitis	Japanese population	Significant
Kobayashi et al. (2000a, b)	FcγRIIIb	15/18	Adult periodontits	Japanese population	Significant
Sugita et al. (2001)	FcγRIIIb	46/73	Refractory periodontitis	Japanese population	Significant
Colombo et al. (1998)	FcγRIIa FcγRIIIb	32 + 54/27	Refractory periodontitis	Caucasian population	Non-significant
Kobayashi et al. (2001)	FcγRIIa FcγRIIIa FcγRIIIb	50 + 39/64	Severe and moderate chronic periodontitis	Japanese population	Significant
Meisel et al. (2001)	FcγRIIa FcγRIIIa FcγIIIb	154/0	All stages of periodontal disease	Caucasian population	Significant
Kobayashi et al. (2007a, b)	FcγRIIa FcγRIIIa FcγRIIIb	100/100	Chronic periodontitis	Japanese population	Non-significant
Komatsu et al. (2008)	FcγR FcαR	113/108	Chronic periodontitis	Japanese population	Significant
An et al. (2009)	FcγRIIIa	30 + 131/47	Aggressive and chronic periodontitis	Chinese population	Significant

diverse and independent studies has clearly demonstrated that active immunization using vaccines against *P. gingivalis* will induce a significant humoral response across animal study models. If passive immunization studies are included, such evidence can also be gathered from human observational studies. Further studies are needed to assess the potential of passive immunization by either clinical treatment inducing bacteremia and a host immune response, or from exposure to monoclonal antibodies and vector antigens developed against periodontal bacteria, which can be administered via the mucosal route (Persson 2005). However, successful vaccine development that fully utilizes the current level of understanding has not yet occurred for human use (Sharma et al. 2007).

B Cells and Autoimmune Reactions that May Influence the Adaptive Host Response in Periodontitis

The production of autoantibodies (autoAbs) is a hallmark of systemic autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis. These autoAbs, produced by self antigen-experienced B cells, are involved in pathogenic immune complex

formation and deposit in organs or tissues for disease progression. This also represents a breakdown of autoreactive B cell tolerance. Using different Ig transgenic mouse models, mechanisms by which autoreactive B cells are regulated have been proposed, including clonal deletion, receptor editing (revision), anergy, and ignorance. In addition, autoreactive B cells that escape these mechanisms are subject to regulation by recently identified peripheral checkpoints (Ding and Yan 2007).

As in other chronic infectious diseases, several components related to autoimmune reactions also occur in periodontitis lesions and involve specific cell groups, as well as antibodies to collagen type-1 and other tissue or cell products. However, the role of autoAbs in the regulation of host response in periodontitis as in other chronic infectious diseases needs to be clarified (Berglundh and Donati 2005).

Although *anti-collagen reactions* (Hirsch et al. 1988; Jonsson et al. 1991; Anusaksathien et al. 1992; Sugawara et al. 1992; Rajapakse and Dolby 2004) dominate in studies on autoimmunity and periodontitis, additional targets for analysis were used, such as *auto-antibodies to cellular components* (auto-antibodies to desmosomal proteins, anti-neutrophil cytoplasmatic antibodies, anti-phospholipids antibodies) (Govze and Herzberg 1993; Novo et al. 1999; Schenkein et al. 2003) or *CD51B cells*, also termed *B-1a cells* (Berglundh and Donati 2005). This group of B cells is found in large numbers in the peripheral blood of patients with autoimmune diseases, e.g., RA and Sjögren's syndrome and produces IgM auto-antibodies, as well as antibodies to bacterial antigens, such as LPS. While conventional B (B-2) cells are developed from bone marrow precursors, B-1a cells are developed from peritoneal precursor cells. Further, B-1a cells may develop into plasma cells and produce immunoglobulins of other classes than IgM (Berglundh and Donati 2005).

There is convincing evidence that auto-reactive B cells, i.e., B-1a cells, occur in larger proportions in subjects with chronic and aggressive forms of periodontitis than in healthy controls (Afar et al. 1992; Berglundh et al. 2002a, b; Sugawara et al. 1992; Aramaki et al. 1998; Stein et al. 1997). Results from one study indicate that the elevated numbers of B-1a cells may illustrate a feature of susceptibility rather than the presence of disease (Berglundh et al. 1999). A substantial proportion of B cells in periodontitis lesions are B-1a cells. The enhanced levels of this cell group in periodontitis are associated with increased levels of IL-10, which is considered to be an autocrine growth factor for B-1a cells (Berglundh and Donati 2005).

Other components, such as *heat shock protein 60*, may also contribute to activation of autoimmune reactions in periodontitis (Berglundh and Donati 2005; Tabeta et al. 2000; Yamazaki et al. 2002, 2004a; Honda et al. 2006) and, in this context, may also represent a link between periodontal infections and systemic diseases such as atherosclerosis and coronary heart disease (Yamazaki et al. 2004b; Choi et al. 2004a, b; Hasan et al. 2005).

B Cells and Cytokine Production Involved in Tissue Destruction

Plasma cells also produce cytokines such as TNF-α, IL-6, IL-10, and transforming growth factor (TGF)-β. TNF-α regulates the turnover of extracellular matrix by inducing the expression of MMPs, while TGF-β down-regulates the synthesis and secretion of these MMPs and promotes the production of their inhibitors (tissue inhibitors of MMPs or TIMPs). Plasma cells located in close proximity to blood vessels express the vascular endothelial growth factor, which in turn stimulates angiogenesis and MMP activation (Berglundh et al. 2007).

3.3 Connective Tissue Breakdown in Periodontal Disease

Remodeling of connective tissues occurs in normal growth and development, and many diseases have long been associated with the breakdown of collagenous extracellular matrices. Among host proteases that target the extracellular matrix, matrix metalloproteases play a role in both degradation and remodeling of matrix proteins during different physiologic and pathological processes (Garlet et al. 2004) (Fig. 3.10).

Metalloproteases, which are found in all organisms, are endopeptidases that contain an active site Zn^{2+} (hence, the prefix "metallo") and are divided into subfamilies or classes based on evolutionary relationships and structure of the catalytic domain. The metzincin subfamily of metalloproteases is characterized by 3-histidine zinc-binding motif and a conserved methionine

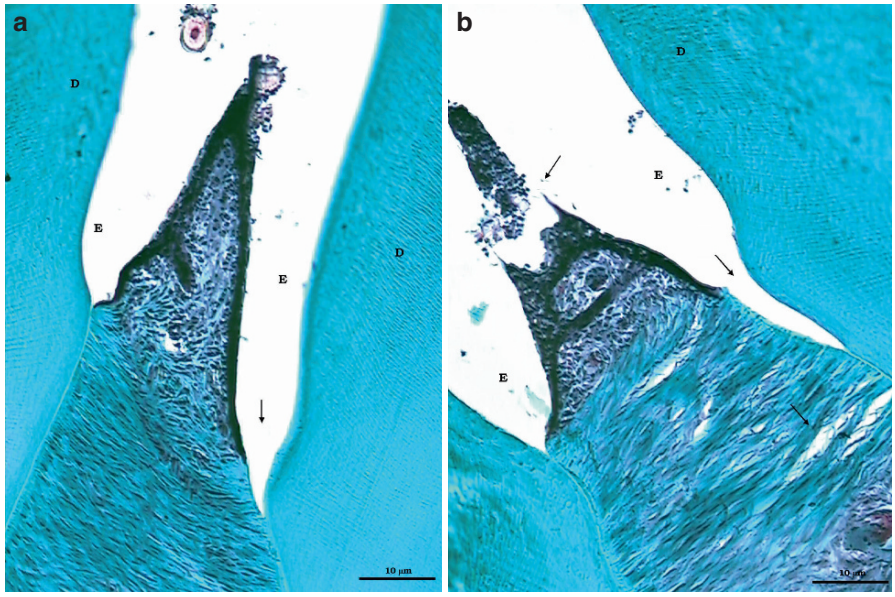

Fig. 3.10 Histological appearance of the interdental space and interdental papilla 3 and 7 days after lipopolysaccharide (LPS) injection in a periodontitis rat model showing crater-like breakdown of the interdental papilla, disruption of transeptal collagen fibres or disruption and apical migration of the junctional epithelium (collagen staining–Masson's trichrome stain) (University of Bristol)

turn following the active site. The members of metzincin family are the reprolysins or ADAMs, (A Disintegrin And Metalloproteinase), serralysins, astacins, and matrixins (aka: MMPs) (Ra and Parks 2007) (Fig. 3.11; Table 3.10).

MMPs comprise a family of currently 25 related, yet distinct vertebrate gene products, of which 24 are found in mammals. MMPs are translated as a zymogen (i.e., an inactive enzyme) and contain a signal sequence peptide for targeting to secretory vesicles. Hence, MMPs are secreted or anchored to the cell surface, thereby confining their catalytic activity to membrane proteins or proteins within the secretory pathway or extracellular space. To be classified as an MMP, a protein must have at least the conserved pro and catalytic domains. The prodomain of a typical MMP is about 80 amino acids and contains the consensus sequence PRCXXPD. The exception to this rule is MMP-23, in which the critical cysteine is found within a distinct run of amino acids. The catalytic domain contains three conserved histidines in the sequence HEXXHXXGXXH, which ligate the active site Zn^{2+}. The glutamate residue within the catalytic motif activates a zinc-bound H_2O molecule, providing the nucleophile that cleaves peptide bonds. The cysteine-thiol and zinc ion interaction keeps proMMPs in a latent state (Ra and Parks 2007).

MMP substrates include peptide growth factors, tyrosine kinase receptors, cell-adhesion molecules, cytokines and chemokines, as well as other MMPs and

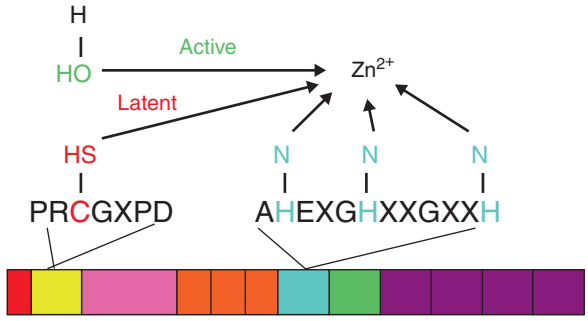

Fig. 3.11 Domain structure of the human matrix metalloproteinase (MMPs). An archetypal MMP contains a signal peptide for secretion, a propeptide, a catalytic domain with a conserved Zn^{2+}-binding motif and a COOH-terminal domain. The hemopexin domain is absent in the smallest MMPs, the matrilysins, whereas the gelatinases incorporate three fibronectin type II repeats for the binding of gelatin, and MMP-9 is the only MMP to possess a Ser/Thr/Pro-rich O-glycosylated domain. Some MMPs are attached to the cell surface through a COOH-terminal transmembrane domain or a GPI anchor. The interaction of a conserved cysteine in the propeptide with the catalytic Zn^{2+} ion seals the catalytic site and results in the latency of the pro-enzyme. MMPs are activated according to the "cysteine switch mechamism" in which removal of the propeptide frees the catalytic Zn^{2+} ion, allowing it to bind a hydrolytic water ion and the substrate. *CA-MMP* cysteine array-MMP; *GPI* glycosyl phosphatidylinositol; *Ig* Immunoglobulin; *RASI-1* rheumatoid arthritis synovial inflammation (adapted from Cauwe et al. 2007) (with permission from Taylor & Francis)

unrelated proteases. MMPs and the related families of proteinases, the ADAMs (a disintegrin and metallo proteases) and ADAM-TSs (ADAMs with thrombo

Table 3.10 Mammalian matrix metalloproteinases (MMPs) (Ra and Parks 2007) (with permission from Elsevier)

Designation[a]	Common name	Other name(s)
MMP-1	Collagenase-1	Fibroblast collagenase, interstitialc ollagenase
	Mcol-A, Mcol-B	
MMP-2	Gelatinase-A	72-kD gelatinase, 72-kD type IV collagenase
MMP-3	Stromelysin-1	Transin-1
MMP-7	Matrilysin	PUMP
MMP-8	Collagenase-2	Neutrophil collagenase
MMP-9	Gelatinase-B92-kD	Gelatinase, 92-kD type IV collagenase
MMP-10	Stromelysin-2	Transin-2
MMP-11	Stromelysin-3	
MMP-12	Metalloelastase	
MMP-13	Collagenase-3	Rat collagenase
MMP-14	MT1-MMP	Membrane-type MMP
MMP-15	MT2-MMP	
MMP-16	MT3-MMP	
MMP-17	MT4-MMP	
MMP-18	RASI-1	
MMP-19	Enamelysin	
MMP-20		
MMP-21		
MMP-22		
MMP-23	CA-MMP	
MMP-24	MT5-MMP	
MMP-25	Leukolysin	MT6-MMP
MMP-26	Endometase	Matrilysin-2
MMP-27		
MMP-28	Epilysin	

[a]MMP-4, -5, and -6 turned out to be either MMP-2 or MMP-3 and, hence, were not unique MMPs. MMP-18 (collagenase-4) has only been cloned from *Xenopus*. A mammalian homologue has not been found

spondin repeats), are important in shedding plasma-membrane bound proteins. ADAMs and ADAM-TSs participate in shedding growth factors that are synthesized as cell-membrane-bound precursor forms, including heparin-binding epidermal growth factor (HB-EGF), neuregulin, amphiregulin and TGFα. Cleavage of other membrane proteins such as E-cadherin and CD44 results in the release of specific, biologically active fragments of their extra cellular domains, and in increased invasive behavior. Cell-surface-adhesion molecules, such as syndecan-1, are also shed by soluble and membrane MMPs. MMP9 and MMP12 contribute to proteolytic shedding of the LPS receptor CD14, and therefore influence innate host defense. TGFβ is another important MMP substrate, the activation of which frequently alters cell migration; for example, MMP9 restrains corneal epithelial migration through the activation of TGFβ34. Both MMP2 and MMP9 can release TGFβ from an inactive extracellular complex that consists of TGFβ, TGFβ latency-associated protein (which is the pro-domain of TGFβ), and latent TGFβ-binding protein (Page-McCaw et al. 2007).

The evidence for the role of MMPs in periodontal destruction is strong and has been supported over many years by a number of findings, including the production of elevated levels of collagenase by diseased gingival tissues in culture and the presence of MMP messenger RNA in cells of the periodontal lesion, such as periodontal ligament and gingival fibroblasts, as well as keratinocytes, endothelial cells, osteoblasts and even osteoclasts. Most of the findings supporting the production of MMPs by these cells have been obtained using cell culture systems. Inflammatory cells, particularly neutrophils, are thought to play a particularly important role in theMMP-mediated periodontal destructive lesion. (Ryan and Golub 2000). Additional evidence for this pathogenic pathway is the presence of elevated MMP protein in periodontal lesions and GCF of patients with different periodontal conditions (Ryan and Golub 2000) (Table 3.11). Similar results were reported in patients with peri-implantitis (Borsani et al. 2005).

Several investigators have evaluated in the recent years the relationship between polymorphisms of genes for MMPs and periodontitis (Astolfi et al. 2006; Cao et al. 2005; Chen et al. 2007; de Souza et al. 2003, 2005; Gürkan et al. 2007, 2008; Holla et al. 2004, 2005, 2006; Itagaki et al. 2004; Pirhan et al. 2008). Due to the limited number of studies carried out to date, it is difficult to relate single nucleotide polymorphisms of MMP genes with periodontitis.

Currently, clinical therapy inhibiting the mediators of connective tissue breakdown is used for the adjunctive treatment of periodontitis. This is accomplished through the non-antimicrobial activities of low-dose tetracycline and tetracycline analogs via the inhibition of MMP-8 and -13 protease mechanisms. The tetracycline analog doxycycline hyclate, available for use specifically in periodontal disease, is the only collagenase inhibitor approved by the United States Food and Drug Administration for any human disease. To clarify, because the low-dose formulations of these drugs have lost their antimicrobial activity, the therapeutic action witnessed is primarily due to the modulation of the host response. This subantimicrobial-dose doxycycline approach has become widely established as an effective adjunctive systemic therapy in the

Table 3.11 Summary of recent studies looking at MMP in gingival biopsies and crevicular fluid of patients with diverse periodontal conditions

Author (Year)	Substrate	MMP	Periodontal diagnosis (n subjects)	Conclusion
Hernandez et al. (2006)	Gingival crevicular fluid (GCF) and gingiva	MMP-13	Destructive periodontitis (periodontally affected sites presenting at least two sites with ≥2 mm CAL loss (21)	MMP-13 activity in GCF samples was significantly increased in active sites from progressive periodontal disease
Hernandez et al. (2007)	GCF and gingiva	MMP-13 and TIMP-1	Active, inactive, and healthy sites from chronic periodontitis (CP) patients (76)	During disease progression, active sites tended to decrease TIMP-1 levels in association with MMP-13 elevation
Kubota et al. (2008)	Gingival tissue	MMP-1, -3, -9, and -13 and TIMP-1, -2, -3, and -4 relative to beta-actin	Generalized CP (16) and healthy gingiva (14)	Levels for MMP-1 and TIMP-4 and the ratios of MMP-1/TIMP-2, MMP-3/TIMP-2, MMP-9/TIMP-2, and MMP-1/TIMP-3 were significantly higher in periodontitis lesions than those in the control tissues
Gonçalves et al. (2008)	Gingival tissue	MMP-1, -2, -9, -13 and TIMP-1, -2	Gingivitis (10), advanced CP (10), generalized aggressive periodontitis (AP) (8) and healthy gingiva (10)	There is a trend towards higher MMP-2 and -9 gelatinase activities in the inflamed samples
Tervahartiala et al. (2000)	GCF and gingiva	MMP-2, -8, -13, -7 and -14	Adult and localized juvenile periodontitis	MMP-8- and -13-positive cells/mm2 were higher in periodontitis gingiva when compared with healthy control tissue
Dahan et al. (2001)	Gingival tissue	MMP-1, MMP-2 and MT1-MMP	Advanced periodontitis (13) and healthy controls (4)	NS
Garlet et al. (2004)	Gingival tissue	MMP-1, MMP-2, MMP-9, TIMP-1, TIMP-2 and TIMP-3	AP(16) and CP patients (20)	The expression of MMPs were similar in AP and CP
Emingil et al. (2006a, b, c)	GCF	MMP-7, TIMP-1	Generalized (20), chronic AP periodontitis (20), gingivitis (20), and healthy subjects (20)	MMP-7 is associated with the innate host defense in periodontal tissues
Emingil et al. (2006a, b, c)	GCF	MMP-25 and MMP-26	Generalized AP (35), CP (29), gingivitis (20), and healthy subjects (21)	Increased levels and activation of MMP-25 and MMP-26 in GCF are associated with an enhanced severity of periodontal inflammation
Ilgenli et al. (2006)	GCF	MMP-13	AP (15), CP (11), gingivitis (17), and healthy subjects (18)	Elevated GCF MMP-13 levels may play an important role in the pathogenesis of CP
Kumar et al. (2006)	Gingival tissue	MMP-8 and -9	Diabetic (DM) CP, non-diabetic CP, and healthy patients	The average concentration of MMP-9 was increased three-fold, and the MMP-8 was increased two-fold in CP patients with DM compared to CP patients without DM
Liu et al. (2006)	Gingival tissue	MMP-8	6 smokers and 6 non-smokers patients	MMP-8 expression were significantly higher in the periodontal tissues of smokers
Maeso et al. (2007)	GCF	MMP-2, MMP-9, TIMP-1	Healthy controls (16), gingivitis (18), and periodontitis (25)	Slightly higher concentrations of MMP-9 were observed in patients with periodontitis but without statistical significance
Söder et al. (2006)	GCF	MMP-8 and -9	CP (33) and healthy controls (31)	Specific periodontal microorganisms appeared to induce host response, with increased release of MMP-8 and MMP-9 in gingival pockets
Smith et al. (2004)	Gingival tissue	MMP-8	Advanced periodontitis, which had at least one periodontal site with 5 mm of probing depth, 3 mm of CAL loss and bleeding upon probing	The study demonstrates the presence of MMP-9 in junctional and pocket epithelium of inflamed gingival tissues
Pozo et al. (2005)	GCF	MMP-8, MMP-9, TIMP-1, TIMP-2	CP (13) and healthy controls (11)	Significant correlations between the severity of the periodontal disease and the actual MMP activity, the active form of MMP-8 and the low level of both TIMP-1 and TIMP-2 were found
Kiili et al. (2002)	GCF and gingiva	MMP-8, MMP-13	CP (12)	The percentages of MMP-8 polymorphonuclear-type enzyme and MMP-13 proenzyme bands correlated significantly with gingival and bleeding indices

management of periodontitis, along with the traditional mechanical therapies of scaling and root planing, as demonstrated by a series of double-blind, placebo-controlled clinical trials (Table 3.12) (Giannobile 2008). A recent meta-analysis done by Reddy et al. (2003) on the studies reporting changes in clinical attachment and periodontal probing depth following administration of subantimicrobial doses of doxycycline in conjunction with scaling and root planing in patients with periodontitis, showed a statistically significant beneficial adjunctive effect.

3.4 Mechanisms of Alveolar Bone Destruction

Periodontitis is associated with a constellation of oral microorganisms that infect the gingival crevice. These polymicrobial infections cause gingival inflammation and resorption of alveolar bone (Figs. 3.12 and 3.13). Host-mediated immune responses (both innate and adaptive) to these microorganisms led to the destruction of periodontal tissues. Recent studies have suggested that the host immune response can contribute to protective and/or destructive effects in periodontal disease (Taubman et al. 2005). In particular, attention has been focused on two molecules belonging to the TNF receptor-ligand superfamily, the osteoprotegerin (OPG) and its ligand OPGL, namely the RANKL also known as TNF-related activation-induced cytokine, which have been identified as critical in the regulation of osteoclast activity, leading to a new paradigm in the bone biology (Giuliani et al. 2004).

RANKL is a polypeptide of 217 amino acids that exerts its biological activity both in a trans-membrane form of about 40–45 kDa and in soluble form of 31 kDa. It has been demonstrated that stromal/osteoblastic cells express RANKL in response to either the systemic factors such as PTH, dexamethasone, and vitamin D_3, or local osteoclastogenic cytokines such as IL-1, TNF, and IL-11. RANKL directly induces osteoclastogenesis together with M-CSF and inhibits osteoclast apoptosis by binding to its specific receptor (RANK) present on osteoclast progenitors and mature osteoclasts. More recently, it has been suggested that activated T lymphocytes, other than stromal/osteoblastic cells, produce RANKL and may maintain bone homeostasis through the cross-talk between RANKL production and IFN-γ secretion. In pathophysiological conditions such as arthritis, activated T cells are capable of regulating bone loss through the expression of RANKL. OPG is a soluble decoy receptor of about 100–110 kDa, produced by stromal/osteoblastic cells, that antagonizes the effects of RANKL on osteoclastic cells inhibiting bone resorption Khosla (2001). It has been shown that OPG binds RANKL and prevents the interaction between RANKL and its receptor RANK, blocking the osteoclast formation (Giuliani et al. 2004). Previous research revealed that *A. actinomycetemcomitans* extracts induce RANKL production from periodontal connective tissue cells, possibly through the cytolethal distending toxin (Belibasakis et al. 2005), and also that *A. actinomycetemcomitans*-responsive B lymphocytes had greater levels of RANKL expression and induced a significantly higher level of osteoclast differentiation in rats (Han et al. 2006; Sakellari et al. 2008) (Fig. 3.14).

The key roles of RANKL and OPG expression in the regulation of bone destruction have been demonstrated in several in vivo disease models, including bacterial arthritis, rheumatoid arthritis, and periodontitis (Bostanci et al. 2007).

Recent clinical studies have confirmed that both RANKL and OPG can be detected in human gingival crevicular fluid, and indicate that RANKL is elevated, whereas OPG is decreased in active sites with periodontitis (Silva et al. 2008; Bostanci et al. 2007; Lu et al. 2006; Vernal et al. 2004), or during orthodontic tooth movement (Kawasaki et al. 2006; Nishijima et al. 2006; Mogi et al. 2004). It has been also shown that RANKL has a membrane-bound (mRANKL) as well as a soluble form (sRANKL). The latter is antagonized by OPG for binding to the receptor RANK, therefore preventing an interaction that leads to osteoclastogenesis. Higher levels of free sRANKL in GCF were reported in periodontitis subjects and correlated significantly with mean counts of *T. denticola* on the subject level and *P. gingivalis, T. denticola* on the site level. No correlations were found between the levels of free sRANKL and investigated parameters in periodontally healthy individuals (Sakellari et al. 2008). It was also suggested that the crevicular fluid level of RANKL and OPG deserves further investigation as a possible marker to evaluate the health status of surrounding tissues of dental implant (Arikan et al. 2008).

Similar results were obtained from basic research studies in which high levels of RANKL and low levels of OPG were observed in gingival tissue from

Table 3.12 Clinical studies of systemic tetracyclines therapy in periodontal disease

Study	No. Patients	Periodontal condition	Study period	Periodontal treatment	Outcome
Caton et al. (2000)	Test: 90 Control: 93	Chronic periodontitis (CP)	9 months	Test: SRP + SDD Control: SRP + placebo	Periostat in conjunction with SRP was shown to significantly improve clinical attachment and reduce probing depth compared with placebo in conjunction with SRP
Novak et al. (2002)	Test: 10 Control: 10	Severe generalized periodontitis	9 months	SRP + SDD (20 mg bid) SRP + placebo	The supplementation of full mouth subgingival and supragingival debridement with a host-modulating agent, SDD, provides clinically and statistically significant benefits in the reduction of deep pockets in patients with severe, generalized periodontitis
Preshaw et al. (2004)	Test: 107 Control: 102	Chronic periodontitis	9 months	SRP + SDD (20 mg bid) SRP + placebo	Adjunctive subantimicrobial dose doxycycline enhances scaling and root planing. It results in statistically significant attachment gains and probing depth reductions over and above those achieved by scaling and root planing with placebo
Choi et al. (2004a, b)	Test: 15 Control: 17	Chronic periodontitis	4 months	SRP + SDD (20 mg bid) SRP + placebo	Significant reduction in PPD and gain of CAL in the SDD-treated group compared with the placebo-treated group. Significant decrease in GCF-MMP-8 level in SDD-treated patients compared with placebo-treated patients
Emingil et al. (2004)	Test: 10 Control: 10	Chronic periodontitis	12 months	SRP + SDD (20 mg bid) SRP + placebo	Significant reduction in PPD and Gingival Index scores in the SDD-treated group compared with the placebo-treated group. Significant decrease in MMP-8 and laminin-5 γ2 chain levels in GCF of SDD-treated patients compared with placebo-treated patients
Lee et al. (2004)	Test: 24 Control: 17	Chronic periodontitis	9 months	SRP + SDD (20 mg bid)	Significant reduction in PPD and gain of CAL in the SDD-treated group compared with the placebo-treated group. Significant decrease in MMP-8 and MMP-13 levels in GCF of SDD-treated patients compared with placebo-treated patients
Gürkan et al. (2005)	Test: 35 Control: 11	Severe, generalized periodontitis	6 months	Supragingival debridment + SDD Supragingival debridment + placebo	Combination of SDD with non-surgical therapy improves clinical parameters of periodontal disease and increases GCF TGF-beta1 levels together with a decrease in prevalence of residual pockets in patients with severe, generalized chronic periodontitis
Mohammad et al. (2005)	Test: 24 Control: 24	Moderate-severe CP	9 months	SRP + SDD SRP + placebo	At all time-points and in both moderate and deep sites, SRP + SDD resulted in significantly greater PD reductions relative to baseline than SRP + placebo. At month 9, in moderate sites, mean PD reductions of 1.57 ± 0.11 mm were reported in the adjunctive SDD group, compared with 0.63 ± 0.11 mm in the adjunctive placebo group. In deep sites at month 9, mean PD reductions of 3.22 ± 0.29 mm were reported in the adjunctive SDD group, compared with 0.98 ± 0.31 mm in the adjunctive placebo group
Emingil et al. (2006a, b, c)	Test: 16 Control: 18	Chronic Periodontitis	12 months	SRP + SDD SRP + placebo	SDD group had lower PPD, CAL and GI scores than placebo group at 6, 9 and 12-months ($P < 0.05$). GCF t-PA levels reduced in both groups over 12-month period ($P < 0.01$). SDD group had lower GCF t-PA levels than placebo group at 6 and 9 months ($P < 0.05$)
Górska and Nedzi-Góra (2006)	Test: 33 Control: 23	Chronic periodontitis	12 months	SRP + SDD SRP + placebo	The application of doxycycline 20 mg resulted in significant improvement in clinical parameters compared with the conventional periodontal treatment. Doxycycline did not produce significant reductions in MMP-8 and MMP-9 levels in saliva observed after the conventional treatment. The study revealed increases in the TIMP-1

(*continued*)

Table 3.12 (*continued*)

Study	No. Patients	Periodontal condition	Study period	Periodontal treatment	Outcome
Needleman et al. (2007)	Test: 16 Control: 18	Chronic periodontitis	6 months	SRP + SDD SRP + placebo	The final improvements were no different between the test and control groups, but multilevel modeling revealed different trajectories for clinical changes. In other words, the endpoint of clinical healing was similar for both experimental groups; however, the rate of improvement was greater for the test group. No differences regarding absolute CAL change or terminal carboxytelopeptide of type 1 collagen in gingival crevicular fluid were evident
Payne et al. (2007)	Test: 64 Control: 64	Post-menopausal osteopenic women with periodontitis	2 years	SRP + SDD SRP + placebo	SDD did not differ overall from placebo on alveolar bone loss
Reinhardt et al. (2007)	Test: 64 Control: 64	Post-menopausal osteopenic women with periodontitis	2 years	SRP + SDD SRP + placebo	DD may be of benefit in reducing progressive attachment loss in post-menopausal females
Tüter et al. (2007)	Test: 18 Control: 18	Patients with both CP and coronary artery disease	6 weeks	SRP + SDD SRP + placebo	Greater improvement was detected for pocket depth and gingival index, and for serum levels of apolipoprotein-A and HDL cholesterol when using SRP + SDD compared with SRP + placebo
Haffajee et al. (2007)	Test: 25 Control: 23	Chronic periodontitis	12 months	SRP alone SRP + azithromycin SRP+ metronidazole SRP+ SDD	The majority of the subjects in each group showed an improvement in both PD and CAL at 12 months. The best response was observed in the Metronidazole and SDD groups where four of 24 (16.6%) and three of 20 (15%) subjects showed mean attachment loss at the end of the study. A larger proportion of subjects in the Azithromycin and SRP groups exhibited attachment loss. Indeed, 39% of subjects exhibited attachment loss at 12 months in the SRP group, more than twice the proportion of subjects seen in the SDD and Metronidazole groups
Guentsch et al. (2008)	Test: 71 Control: 21	Severe chronic periodontitis	12 months	SRP alone (21) SRP+ doxycycline (36) SRP+ moxyfloxacin (35)	All three procedures led to significant reductions in PPD, CAL, and BOP. PPD reduction was significantly greater ($P < 0.05$) in the MOX group (2.46 ± 1.17 mm at 6 months and 2.84 ± 1.53 mm at 12 months) compared to the DOX group (1.85 ± 1.24 and 2.19 ± 1.13 mm at 6 and 12 months, respectively) and the controls (1.77 ± 0.57and 1.86 ± 0.56 mm at 6 and 12 months, respectively)
Gapski et al. (2009)	Test: 35 Control: 35	Chronic severe periodontitis	12 months	Surgery+ doxycycline Surgery+ placebo	Patients treated with SDD and surgery demonstrated stronger reductions in PD in surgically treated sites of ≥7 mm as well as gains in CAL ($P < 0.004$)
Novak et al. (2008)	Test: 83 Control: 88	Chronic periodontitis	6 months	SRP+ SDD+ locally doxycycline hyclate gel SRP+ placebo	In moderate CP (PD of 4–6 mm), combination therapy provided significant benefits over control for PD ($P < 0.01$), CAL ($P < 0.03$), BOP ($P < 0.05$), and GI ($P < 0.03$). In severe CP (PD≥7 mm), combination therapy provided significant benefits over control for PD ($P < 0.01$), CAL ($P < 0.02$), BOP ($P > 0.05$), and GI ($P < 0.01$)

CAL clinical attachment loss; *GCF* gingival crevicular fluid; *MMP* matrix metalloproteinases; *PPD* periodontal pocket depth; *SDD* low-dose doxycycline (20 mg orally administered twice daily); *SRP* scaling and root planning; *TGF-1* transforming growth factor-beta 1; *TIMP* endogenous tissue inhibitors of metalloproteinases

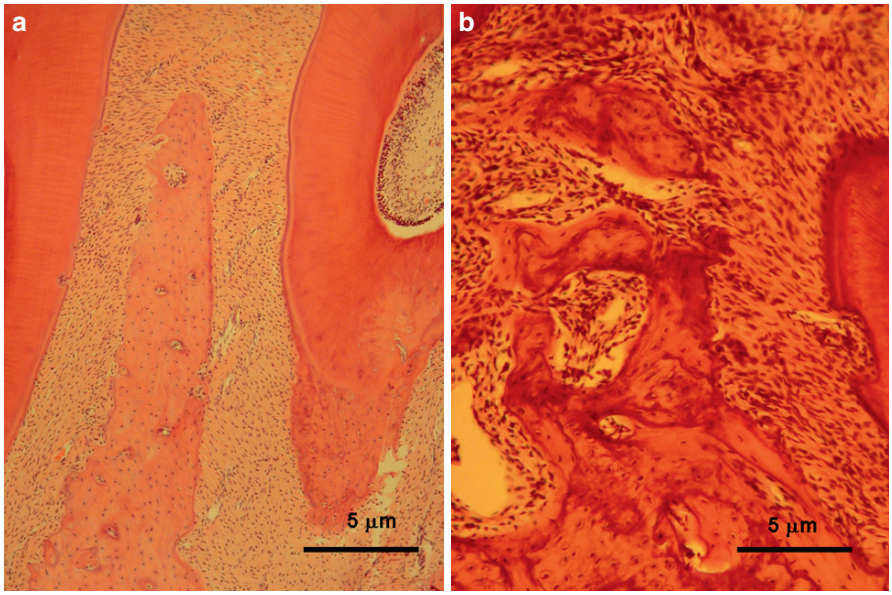

Fig. 3.12 Mesiodistal sections illustrating the interdental and interradicular bony septa in the maxillary molar region of the rat after placing a ligature (2 days observation and 7 days observation) (hematoxylin and eosin staining) (University of Bristol)

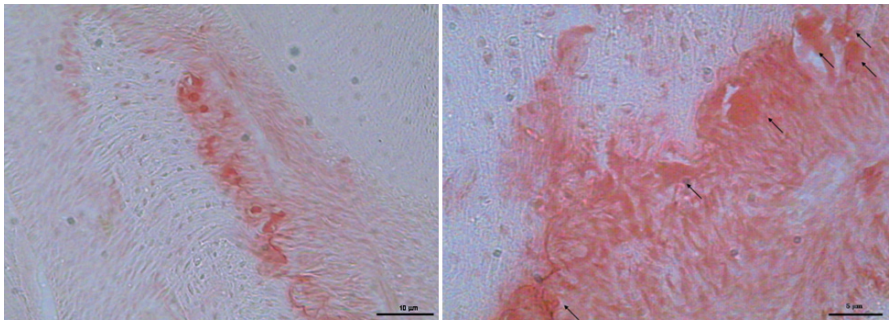

Fig. 3.13 Histological appearance of active osteoclasts (*arrows*) on the mesial surface of the interdental alveolar bone in a rat model of periodontitis stained for tartarate-resistant acid phosphatase (University of Bristol)

periodontitis patients (Crotti et al. 2003; Liu et al. 2003; Garlet et al. 2004; Lu et al. 2006; Kawai et al. 2006).

Cigarette consumption may favor bone resorption through increased ratios of IL-6: IL-10 and RANKL:osteoprotegerin in periodontal tissues (César-Neto et al. 2007) and through suppression of serum OPG production (Lappin et al. 2007).

The association of RANK/RANKL/OPG gene polymorphisms with AP was evaluated by Soedarsono et al. (2006) in a Japanese population. An association analysis with allelotypes showed that single nucleotide polymorphisms (RANK: 27SNPs, RANKL: 7SNPs, OPG: 7SNPs) identified in the RANK/RANKL/OPG genes have no significant association with AP.

Polymorphisms in the OPG gene have a potential impact on the structural and functional properties of the protein and, therefore, may change the OPG/RANKL ratio as well. The following allelic variants were investigated: OPG-Lys3Asn (in exon 1, G > C) and OPG-Met256Val (in exon 4, T > C) in 194 unrelated, nonsmoking Caucasian individuals 35–77 years of age. The homozygous variants coding for Lys3 were present at a higher frequency, whereas Asn3 and Met256 were present at a lower frequency in CP patients/controls (Lys3: 31/25%, Asn3: 23/32%, and Met256: 66/73%). Heterozygosity for Lys3Asn was observed at a higher frequency in CP patients/controls (46/43%) (Wagner et al. 2007). No association between polymorphism OPG-223 (C/T) and chronic periodontal disease in a Brazilian population was observed recently by Baioni et al. (2008), while Park et al. (2008) revealed that the TG haplotype of T950C and G1181C polymorphisms in the OPG gene may be useful genetic markers for the prediction of AP.

The previous-mentioned studies have suggested that excess RANKL shifts the balance of bone metabolism in the direction of catabolism and causes periodontal bone resorption. Therefore, RANKL inhibition offers the therapeutic possibility to treat periodontal bone resorption. This may include reduction of soluble RANKL release or interference with RANKL expression by T/B cells. Interference with these processes should contribute to abrogation of periodontal bone resorption and prevention of periodontal disease progression. It is now clear that multiple novel therapies may be implemented, which more directly address the new periodontal disease pathogenesis concept (Taubman et al. 2007).

3.5 Risk Factors in Periodontal Diseases

A *risk factor* for periodontal disease is an environmental, behavioral, or biological factor confirmed by temporal sequence, usually longitudinal studies. If present, it directly increases the probability of a disease occurring, and if absent, it reduces this probability. Risk factors are part of the causal chain, or expose the host to the causal chain. Once disease occurs, removal of a risk factor may not result in a cure. Some risk factors are modifiable, while others cannot (easily) be modified. Factors that cannot be modified are often called "determinants" or background factors (Timmerman and van der Weijden 2006).

The term *risk indicator* is used to describe plausible correlates of disease identified in cross-sectional studies or case-control studies, while risk factors are best applied to those correlates that are confirmed in longitudinal studies. Risk indicators are not always confirmed as risk factors in longitudinal studies (Timmerman and van der Weijden 2006).

The term *risk marker* is used more in the predictive sense and usually refers to a risk factor. It is associated with an increased probability of disease in the future (Timmerman and van der Weijden 2006).

Numerous studies have shown associations between a myriad of factors (age, race, gender, socioeconomic status, nutrition, psychological factors, excessive alcohol consumption, systemic diseases, genetic factors, etc.) and progression of periodontal disease. In addition, studies of other factors hypothesized to be related to periodontal disease progression have failed to provide compelling evidence. Understanding and evaluation of these risk factors and determinants demand that the evidence for each association be weighed according to the study design, the outcome measures utilized and the strength of the association. With further longitudinal studies evaluating all reported risk factors for periodontal disease progression, it is hoped that we can one day use these risk factors to more accurately predict disease progression as well as long-term outlook for treated teeth (Nunn 2003).

Smoking as a risk factor for periodontal disease

As reviewed by Johnson and Guthmiller (2007), Bergström (2003, 2004, 2006), Kinane and Chestnutt (2000), Barbour et al. (1997), Rivera-Hidalgo (2003) *cigarette smoking is a well-established risk factor for periodontitis* and, second to bacterial plaque, is the strongest of the modifiable risk factors. Smoking is associated with a two- to eight-fold increased risk for periodontal attachment and / or bone loss, depending on the definition of disease severity and smoking dose (Johnson and Guthmiller 2007). It is important to emphasize that the inferior periodontal conditions of smokers cannot be attributed to poorer plaque control, more severe gingivitis or special composition of the subgingival microflora (Papapanou 1996), being suggested that smoking-associated periodontitis should be considered a separate disease entity.

Exposure to tobacco smoking can be measured by interviewing the subjects or by biochemical analysis. The number of cigarettes consumed per day, duration, the number of years of smoking and life-time exposure, the accumulated exposure over time as formed by the product of daily consumption and years of duration ("cigarette-years") are analysed (Bergström et al. 2000). Biochemical assessment by measurement of systemic levels of cotinine, nicotine, thiocyanate or exhaled carbon monoxide is performed (Palmer et al. 1999).

Tomar and Asma (2000) evaluated the relationship between cigarette smoking and periodontitis from the Third National Health and Nutrition Examination Survey, a nationally representative multipurpose health survey conducted in 1988–1994 (n = 12,329). Modeling with multiple logistic regression revealed

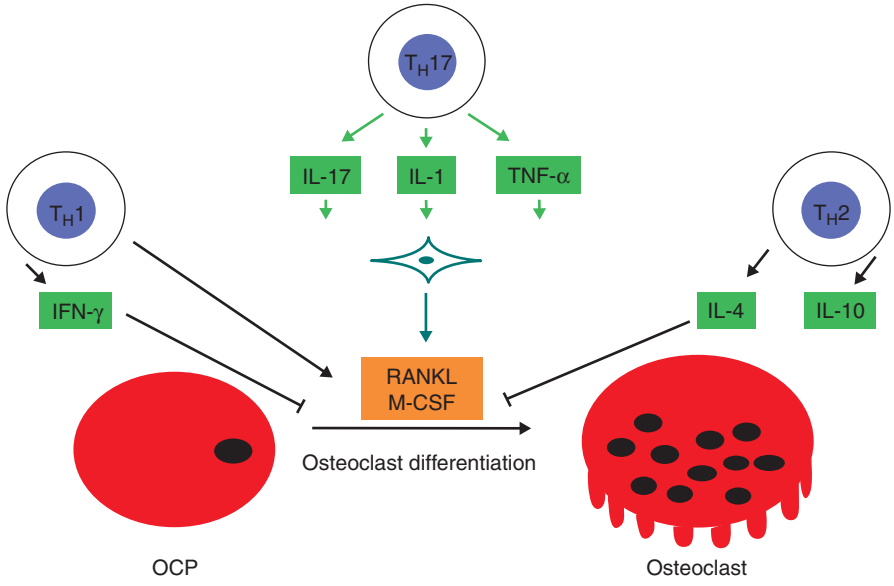

Fig. 3.14 Induction of bone-resorbing osteoclasts. The differentiation of mononuclear osteoclast precursor cells (OCPs) to mature, bone-resorbing osteoclasts is the major step in joint destruction in inflammatory arthritis. This differentiation and maturation process is cytokine-driven. The essential cytokine mediators are macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor-κB ligand (RANKL), which are mainly expressed by fibroblasts and T helper 1 (T_H1) cells. Moreover, cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-17, force and accelerate the process of osteoclast differentiation by upregulating RANKL (modified from Herman et al. 2008) (with permission from Elsevier)

that current smokers were about 4 times as likely to have periodontitis as persons who had never smoked [prevalence odds ratio [OR_p] = 3.97; 95% CI: 3.20–4.93], after adjusting for age, gender, race/ethnicity, education and income:poverty ratio. Former smokers were more likely to have periodontitis than persons who had never smoked (OR_p = 1.68; 95% CI: 1.31–2.17). Among current smokers, there was a dose-response relationship between cigarettes smoked per day and the odds of periodontitis ($P < 0.000001$), ranging from OR_p = 2.79 (95% CI: 1.90–4.10) for ≤9 cigarettes/day to OR_p = 5.88 (95% CI: 4.03–8.58) for ≥31 cigarettes/day. Among former smokers, the odds of periodontitis declined with the number of years since quitting, from OR_p = 3.22 (95% CI: 2.18–4.76) for 0 to 2 years to OR_p = 1.15 (95% CI: 0.83–1.60) for ≥11 years. Calsina et al. (2002) showed that smokers had 2.7 times and former smokers had 2.3 times greater probabilities to have established periodontal disease than nonsmokers, independent of age, sex, and plaque index. Among cases, probing depth, gingival recession and clinical attachment level were greater in smokers than in former smokers or non-smokers, whereas plaque index did not show differences. Bleeding on probing was less evident in smokers than in nonsmokers. There was a dose–response relationship between cigarette consumption and the probability of having advanced periodontal disease.

Furthermore, *smoking has a negative impact on periodontal treatment outcomes*, as excellently reviewed by Labriola et al. (2005) and Heasman et al. (2006) (Table 3.13). Smoking has been also acknowledged as a *pre-disposing factor in implant failure*. Factors that interact with smoking to impact implant outcomes include an IL-1 genotype, implant location (maxillary vs. mandibular) and the presence of periodontal disease (Johnson and Guthmiller 2007).

Smoking in combination with other systemic risk factors further enhances the risk for periodontal destruction (Johnson and Guthmiller 2007). Four lines of evidence suggest that the observed periodontitis–systemic disease associations are, in part, a result of confounding by smoking – the inability to distinguish the effect of smoking on periodontitis from the effect of smoking on systemic diseases. First, no periodontitis–systemic disease associations have been identified among nonsmokers. Second, periodontitis and smoking mimic one another with respect to the types

Table 3.13 Impact of smoking on periodontal therapeutic outcomes

Treatment	Outcomes in smokers compared to nonsmokers	Reference/Year
Scaling and root planing	Less improvement in clinical parameters in smokers (50–75%)	Heasman et al. (2006); Labriola et al. (2005); Jin et al. (2000)
Various forms of surgical periodontal therapy (osseous surgery, modified Widman flap surgery, or flap debridement surgery)	Less improvement in clinical parameters in smokers	Ah et al. (1994); Preber and Bergstrom (1990); Scabbia et al. (2001); Kaldahl et al. (1996)
Periodontal soft- and hard-tissue grafting	Less improvement in clinical parameters in smokers. Smokers exhibit three-quarters of the amount of root coverage shown by nonsmokers	Harris (1994); Harris et al. (2005); Erley et al. (2006); Martins et al. (2004); Silva et al. (2006)
Guided tissue regeneration	Less improvement in clinical parameters in smokers	Tonetti et al. (1995); Trombelli et al. (1997); Stavropoulos et al. (2004); Mayfield et al. (1998); Loos et al. (2002); Cortellini et al. (1996); Bowers et al. (2003); Luepke et al. (1997)
Antimicrobial and host modulatorytherapy	Adjunctive antimicrobial therapy and adjunctive sub-antimicrobial doxycycline therapy brings the smokers' response to that of nonsmokers receiving scaling and root planing alone	Williams et al. (2001); Tomasi and Wennstrom (2004); Paquette et al. (2003); Preshaw et al. (2005)
Implant therapy	Smokers experience approximately twice the failure rate based on a variety of implant designs and surfaces. The survival rate ranged from 80 to 100% in smokers compared to 93 to 98% in nonsmokers	Moy et al. (2005); Schwartz-Arad et al. (2002); Wallace (2000); Bain (2003); Bain and Moy (1993); Chuang et al. (2002); De Bruyn and Collaert (1994); Geurs et al. (2001); Gorman et al. (1994); Jensen et al. (1998); Jones et al. (1999); Kan et al. (1999); Lambert et al. (2000); Levin et al. (2004); Lindquist et al. (1996); McDermott et al. (2003)

of diseases with which they are associated (e.g., lung cancer and Parkinson's disease). Third, only studies with inadequate adjustment for smoking report significant periodontitis–systemic disease associations. Last, elimination of dental infection, unlike smoking cessation, does not reduce coronary heart disease risk (Hujoel et al. 2002).

Also, a *synergistic effect was noted between the IL-1 genetic polymorphism and smoking* (McDevitt et al. 2000; Meisel et al. 2004).

Studies comparing the *subgingival microbiota in smokers and nonsmokers* have yielded conflicting results (Johnson and Guthmiller 2007); this is, in part, the result of variations in sampling, assessment techniques and data presentation. Although several studies reported no difference between smokers and nonsmokers in the prevalence of, bacterial counts of, or percentage of persons infected with selected subgingival bacteria associated with periodontitis (Darby et al. 2000; Preber et al. 1992; Stoltenberg et al. 1993; Bostrom et al. 2001; Mager et al. 2003; van der Velden et al. 2003; Lie et al. 1998), other studies have identified increased prevalence of potential periodontal pathogens in smokers (Zambon et al. 1996; Haffajee and Socransky 2001; Umeda et al. 1998; Eggert et al. 2001; van Winkelhoff et al. 2001; Shiloah et al. 2000; Kamma et al. 1999). The evidence for smoking's deleterious effect on the host response is much stronger; as *smoking affects both the local and systemic innate and adaptive immune responses* (Table 3.14).

Smoking cessation cannot reverse the past effects of smoking; however, the rate of bone and attachment loss slows after patients quit smoking. Periodontal disease severity in former smokers falls between that of current and nonsmokers (Johnson and Guthmiller 2007).

Table 3.14 Potential biological mechanisms underlying the effects of tobacco smoking on periodontitis

Mechanism	Parameters	Reference/Year
Smoking and microflora	Increased prevalence of potential periodontal pathogens	Zambon et al. (1996); Haffajee and Socransky (2001); Umeda et al. (1998); Eggert et al. (2001); van Winkelhoff et al. (2001); Shiloah et al. (2000); Kamma et al. (1999)
Effect on gingival vasculature	Supressive effect in blood flow and vascularity observed through less gingival redness, lower bleeding on probing, fewer vessels visible clinically and histologically	Mirbod et al. (2001); Rezavandi et al. (2002); Bergström and Boström (2001); Dietrich et al. (2004); Bergström et al. (1988); Nair et al. (2003)
Reduced Smoking and the host response	Shift in neutrophil function toward destructive activities: induction of protease release (elastase and matrix metalloproteinase), migration of oral neutrophils, neutrophil priming (hyper-reactivity)	Seow et al. (1994); Seagrave et al. (2004); Ryder et al. (2002); Nowak et al. (1990); Gillespie et al. (1987); Koethe et al. (2000); Matheson et al. (2003); Gustafsson et al. (2000); Ryder et al. (1998a, b); Guntsch et al. (2006); MacFarlane et al. (1992); Pabst et al. (1995); Persson et al. (2001)
	Reduced number and cytolytic activity of circulating Natural Killer cells	Ferson et al. (1979); Tollerud et al. (1989)
	Reduced IgG2 levels	Tangada et al. (1997); Graswinckel et al. (2004)
	Negative effects on cytokine and growth factor production	Bostrom et al. (1998, 1999); Giannopoulou et al. (2003); Johnson and Organ (1997); Ryder et al. (2002); Theiss et al. (2000); Wendell and Stein (2001)
Smoking and fibroblast function	Inhibition of gingival and periodontal ligament fibroblasts growth, attachment and collagen production	Chang et al. (2002); Gamal and Bayomy (2002); Giannopoulou et al. (1999); James et al. 1999; Tanur et al. (2000); Tipton and Dabbous (1995)

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4 Interrelationships Between Periodontal Disease and Mortality, Cardiovascular Disease, Metabolic Syndrome, Diabetes Mellitus

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It is now becoming widely recognized that certain systemic diseases such as osteoporosis, diabetes and immune disorders may increase the risk of periodontal disease. However, until relatively recently, less attention has been devoted to exploring the role that chronic oral diseases may have on systemic health. The hypothesis that oral conditions, such as periodontal infections, may be risk factors or indicators for important medical outcomes represents a paradigm shift in thinking about causality and the directionality of oral and systemic associations (Garcia et al. 2001).

Periodontal medicine defines a rapidly emerging branch of periodontology, focusing on the wealth of new data and establishing a strong relationship between periodontal health or disease and systemic health or disease. This means a two-way relationship in which periodontal disease in an individual may be a powerful influence on an individual's systemic health or disease as well as the most customary, understood role that systemic diseases may have in influencing an individual's periodontal health or disease. Logically included in this definition would be new diagnostic and treatment strategies that recognize the relationship between periodontal disease and systemic disease (Williams and Offenbacher 2000).

To delineate possible causal links between periodontal disease and medical inflammatory diseases, research may examine whether the following criteria can be fulfilled:

1. Prevalence and incidence of the medical disease in question should be significantly higher in periodontitis patients than in periodontally healthy individuals (retrospective data)
2. Onset of the medical disease should follow the onset of periodontitis (prospective data)
3. Removal or reduction of periodontitis should decrease the incidence of the medical disease (effect of treatment)
4. Micro-organism(s) (if recoverable/identifiable) of the medical infection should be the same as (species, biotype, serotype, genotype) the oral microorganism(s) of the patient (specific etiologic agent)
5. Appropriate experimental animals with periodontitis or with inoculated microorganisms should develop more medical diseases than periodontally healthy animals. Human populations with periodontitis in controlled studies should develop the medical disease more frequently than periodontally healthy populations (experimental reproduction/study of medical disease)
6. The postulated association between periodontal disease and medical disease should be biologically feasible (pathogenic mechanism) (Slots 1998)

There is increasing evidence that individuals with periodontal disease may be at increased risk for adverse medical outcomes. A number of studies to date indicate that this increased risk appears to be independent of other known behavioral and medical risk factors and also appear to be related to the severity of periodontal disease (Garcia et al. 2001).

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4.1 Relationship Between Periodontal Disease and Mortality From all Causes

One of the criteria, which has been proposed to be evaluated assessing whether a causal interpretation exists, is the specificity of associations. This means that a causal relationship is less likely between oral health and cardiovascular disease (CVD) if oral health/periodontitis is also related to other diseases. Several studies demonstrate a significant correlation between increased all-cause mortality risk and poor oral health (Table 4.1). The results support a more general association between oral health/marginal periodontitis and systemic health. Further research is needed in the future to be able to interpret the results and clarify whether any biological mechanisms exist between oral infections and systemic responses. The lack of specificity of the associations between oral health and mortality strengthens the hypothesis that the significant correlations could be explained by unidentified confounding factors (Jansson et al. 2001).

DeStefano et al. (1993) analyzed the data from the national examination study I (NHANES I) on 9,760 subjects, aged 25–74 years, who underwent a standard dental examination at baseline and were followed up to 14 years. The mean outcome measures were incidence of mortality or admission to hospital because of coronary heart disease (CHD) as well as total mortality. The severity of periodontal disease increased the risk of total mortality more than the risk of CHD. Compared with subjects with little or no periodontal disease, those with gingivitis were at about 23% higher risk, and those with periodontitis or who had no teeth were at about 50% higher risk of dying during follow up. The strongest association was found between measures of periodontal disease or oral hygiene among men under 50 at baseline. Men with periodontitis had more than a twofold increased risk of dying compared with men who had no periodontal disease at baseline. Young men who had no teeth at baseline had a 2.6-fold increased risk of dying. For the periodontal index, the results reflected the findings with the periodontal classification. The oral hygiene index also showed a stronger association with total mortality. Young men who had a maximum oral hygiene index of six had a risk of dying 3.4 times higher than those who had a hygiene index of 0 (DeStefano et al. 1993).

Garcia et al. (1998) conducted a secondary analysis of data from participants in the VA dental longitudinal study and normative aging study (NAS), a prospective, observational, closed-panel cohort study established in the 1960s to study the determinants of age-related diseases in community-dwelling men. The 804 dentate subjects included in these analyses were, on average, 42 years old at their baseline examination, had 23.8 teeth present, and had a mean whole-mouth radiographic alveolar bone loss (ABL) score (measured with a Schei ruler using full mouth series of periapical films) of approximately 12% (per site per subject). For each 20% increment in mean whole-mouth ABL, the subject's risk of death increased by 51% (RR = 1.51; 95% CI: 1.11–2.04). The increase in risk attributable to periodontal status was found to be similar in magnitude to, and independent of that attributable to cigarette smoking in this cohort. ABL scores (i.e., worst periodontal status) were associated with a 1.85-fold increase in risk (95% CI: 1.25–2.74) using multivariate analysis.

A significant correlation was found between poor oral health (number of lost teeth, number of surfaces with caries, number of teeth with apical lesions and marginal bone loss) and an increased mortality risk even when persons dying from CVDs were excluded from the analyses (Jansson et al. 2002).

Soikkonen et al. (2000) revealed that mortality was increased in subjects having moderate to advanced periodontal attachment loss observed radiographically as infrabony pockets [odds ratio (OR) = 2.2, 95% confidence intervals (CI): 1.0–4.7)]. Mortality tended to be increased in subjects having 5–14 periodontal infrabony pockets, moderate to advanced horizontal bone loss, apical periodontitis lesions, and in association with the pooled sum of all the potentially infectious findings.

A recent longitudinal study in Sweden addresses the issue of periodontal disease as a risk marker for mortality by evaluating the relationship between periodontitis and premature death 16 years after the diagnosis of periodontitis (Söder et al. 2007). Significant differences were present at baseline between survivors and persons who later died, with respect to dental plaque, calculus, gingival inflammation and number of missing molars in subjects with periodontitis. The multiple logistic regression analysis results of the relationship between being dead (dependent variable) and several independent variables identified periodontitis with any missing molars as a principal independent predictor of death.

Table 4.1 Summary of papers relating to the effect of periodontal disease on the mortality of all causes

Author	Length of study	Cases number	Exposure	Outcome	Association
Garcia et al. 1998	25 years	804	Radiographic alveolar bone loss (ABL) Probing depths	Mortality	Significant
DeStefano et al. 1993	14 years	9,760	Oral hygiene index Total dental index (TDI) Periodontal index	Mortality	Significant
Soikkonen et al. 2000	4 years	292	ABL on panoramic radiography supplemented by intraoral radiographs	Mortality	Significant
Jansson et al. 2002	26 years	1,393	Number of caries lesions Number of remaining teeth Number of dental restorations Presence of plaque (Greene and Vermillion 1964) Periodontal health (Russell 1956) Full-mouth intraoral radiographic investigation	Mortality	Significant
Ajwani et al. 2003	10 years	364	Tooth loss Inflammatory conditions of the mouth, including mucosal lesions and denture stomatitis Community periodontal index for treatment needs (CPITN) Salivary microbial (mutans streptococci and yeast) counts	Mortality	Significant
Söder et al. 2007	16 years	3,273	Tooth loss Gingival index (GI) Plaque index (PLI) Calculus index Probing depth	Mortality	Significant

4.2 Interactions Between Periodontal and Cardiovascular Disease

Cardiovascular diseases (CVDs) comprise a variety of heart and vascular conditions including: ischaemia, atherosclerosis, peripheral artery disease, infective endocarditis, and acute myocardial infarction (Persson and Persson 2008).

4.2.1 Periodontal Disease and the Risk of Coronary Heart Disease

Coronary Heart Disease (CHD) is the single most common cause of death in both the United States and the United Kingdom, accounting for approximately one in five deaths. It is estimated that each year, about 700,000 Americans experience symptomatic first-ever myocardial infarction; a further 175,000 have "silent" myocardial infarction (i.e., without the normal indicators such as chest discomfort, shortness of breath, feeling dizzy or lightheaded, or numbness in one or both arms); and a further 500,000 have recurrent myocardial infarction. In the United Kingdom, CHD accounts for more than 208,000 deaths each year. Although the death rate from CHD fell by 33% in the United States from 1994 to 2004 and by 24% in the United Kingdom over the past decade, the condition remains the leading killer worldwide, and its burden is rising rapidly in low- and middle-income countries, particularly in South Asia. Established risk factors for CHD include modifiable factors (such as smoking and high cholesterol and blood pressure levels) and nonmodifiable factors (such as family history, ethnicity, and age) (Sagoo et al. 2008).

Case-control, cross-sectional, and longitudinal studies have found that periodontitis is associated with CHD, even after adjustment for a variety of potential confounders of these associations. However, other studies have found either nonsignificant positive trends or no association after adjustment for variables considered to be confounders (Table 4.2). Concerns about the validity of the periodontitis – CVD associations have been expressed. A review by Danesh (1999) noted that studies were based on clinical measures of periodontal disease and did not have direct measures of the infection, such as bacterial counts or systemic antibody levels to oral pathogens (Beck et al. 2005).

A second criticism focuses on the role of smoking, which is a risk factor for both periodontal disease and heart disease and must be considered as a confounder. Most studies have adjusted for smoking by means of multivariable analyses, an approach open to bias due to residual confounding. As for other morbidities, it has been suggested that statistical adjustment is insufficient to control for smoking and that stratification is needed (Beck et al. 2005).

Several meta-analyses have been performed on this topic (Table 4.3). The meta-analysis of Meurman et al. (2004) is based on nine cohort studies, where the exposure, i.e., periodontal disease, occurred some time before the outcome, i.e., incidence of CVD. It was found that periodontal disease had a stronger effect on fatal CHD and stroke than on nonfatal or fatal and nonfatal CHD combined. They quantified the increased risk of CVD due to periodontal disease by weighted average and found that the increase in risk due to periodontal disease appeared to be approximately 20% (RR = 1.19, 95% CI: 1.10–1.38).

Khader et al. (2004) pooled data from eight cohort studies. Subjects with periodontitis had an overall adjusted risk of CHD that was 1.15 times (95% CI: 1.06–1.25; $P = 0.001$) the risk for healthy subjects. There was no heterogeneity among the studies in the overall relative risk estimate ($P = 0.472$). The relationship between periodontitis and the risk of fatal CHD was explored in three cohort studies. When compared to healthy people, subjects with gingivitis and periodontitis had an overall adjusted relative risk of fatal CHD of 1.52 (95% CI: 0.84–2.75; $P = 0.163$) and 1.20 (95% CI: 0.90–1.60; $P = 0.205$), respectively. None of these relationships was statistically significant.

The meta-analysis conducted by Mustapha et al. (2007) included 12 studies (five cohort and seven cross-sectional studies). Systemic bacterial exposure was measured by periodontal bacterial burden ($N = 1$), periodontitis-specific serology ($N = 12$), or C-reactive protein (CRP) ($N = 1$). It was revealed that periodontitis with elevated markers of systemic bacterial exposure was associated significantly with CHD, with an average OR of 1.75 (95% CI: 1.32–2.34; $P < 0.001$). Among studies of carotid intima-medial thickening, periodontitis increased mean thickness by means of 0.02, 0.03, and 0.05 mm across the three studies, which resulted in significant heterogeneity ($P = 0.0001$). Random-effect models estimated a significant average mean increase of carotid intima-medial thickening of 0.03 mm (95% CI: 0.02–0.04).

The meta-analysis of Bahekar et al. (2007) is based on five prospective cohort studies (follow-up >6 years), five case-control studies, and five cross-sectional studies. Meta-analysis of cross-sectional studies (17,724 patients) indicated that prevalence of CHD is 1.59 times higher (95% CI: 1.329–1.907, $P < 0.001$) in patients with periodontal disease as compared with the people who do not have periodontal disease. Evaluation of five case-control studies (1,423 patients) indicated that there is an even greater risk of developing CHD (OR = 2.22, 95% CI: 1.59–3.117, $P < 0.001$) among patients with periodontal disease. Meta-analysis of the five prospective cohort studies (86,092 patients) indicated that individuals with periodontal disease had a 1.14 times higher risk of developing CHD than the controls (relative risk 1.14, 95% CI 1.074–1.213, $P < 0.001$). When the relationship between number of teeth and incidence of CHD was analyzed, cohort studies showed 1.24 times increased risk (95% CI 1.14–1.36, $P < 0.0001$) of development of CHD in patients with <10 teeth.

Persson and Persson (2008) concluded that meta-analysis of prospective and retrospective follow-up studies have shown that periodontal disease may only slightly increase the risk of CVD. It was observed that studies resulting in higher ORs have commonly used ABL as the definition of periodontitis rather than measure of probing depth (PD) and clinical attachment level (CAL), as Beck et al. (2005) confirmed that clinical signs (bleeding on probing (BPO), PD, CALs) are not representative for the impact of cumulative effects of periodontitis on systemic health. Disparities in prevalence rates of periodontitis in study populations with different age groups, ethnicity and geographic location makes it difficult to assess the likelihood of an association between periodontitis and CVDs (Persson and Persson 2008).

Table 4.2 Summary of papers relating to the effect of periodontal disease on coronary heart disease (CHD)

Author	Type of study	Study population	Periodontal diagnostic	Coronary heart disease assessment	Association
Beck et al. 1996	Cohort study 25 years follow-up	1,147 men	Bone loss. Periodontitis = more than 20% sites with bone loss	New CHD Fatal CHD; stroke	OR = 1.5; 95% CI: 1.01–2.1
DeStefano et al. 1993	Cohort study 14-year follow-up	9,760 men and women (25–74years)	Periodontal classification: no periodontal disease; gingivitis; periodontitis, periodontal index	CHD and mortality	Gingivitis: OR = 1.05 (95% CI: 0.88–1.26) Periodontitis: OR = 1.25 (95% CI: 1.06–1.48) Periodontal Index: OR = 1.04 (95% CI: 1.01–1.08)
Joshipura et al. 1996	Prospective 6 year	44,119 (40–75 years)	Tooth loss due to periodontal disease	Fatal CHD, myocardial infarction, sudden death	RR = 1.67; 95% CI: 1.03–2.71
Loesche et al. 1998	Cross-sectional	165 cases/155 controls	Number of teeth, PD, CAL, R, PI, PBS	CHD	The dentate subjects with CHD had significantly fewer teeth, more % of teeth with pockets >4 mm, CAL loss >4 mm amd R >4 mm than the dentate subjects without CHD
Morrison et al. 1999	Cohort study 21-year follow-up	21,619	Gingivitis Periodontitis Edentulousness	Mortality experience of CHD	RR = 2.15 (95% CI: 1.25–3.72) and 1.90 (95% CI: 1.17–3.10) were observed for severe gingivitis and edentulous status, respectively
Arbes et al. 1999	Cross-sectional study	5,564 people (40 years of age and older)	Percent of periodontal sites per person with attachment loss of 3 mm or greater (categorized as 0, >0–33, >33–67, and >67%)	History of heart attack	Relative to the 0% category, the unadjusted odds of heart attack increased with each higher category of attachment los –2.2 (95% CI: 1.3–3.8), 5.5 (3.4–9.1), and 9.8 (4.5–21.0), respectively
Hujoel et al. 2000	Cohort study 10-year follow-up	8,032 (25–74 years)	Periodontitis, gingivitis	Fatal CHD	Gingivitis was not associated with CHD (hazard ratio = 1.05; 95% CI: 0.88–1.26), while periodontitis was associated with a nonsignificant increased risk for CHD event (hazard ratio = 1.14; 95% CI: 0.96–1.36)
Howell et al. 2001	Cohort study 12.3 years follow-up	2,653 physicians (40–84 years)	Self-reported periodontal disease	Nonfatal myocardial infarction, Cardiova scular death	RR of nonfatal myocardial infarction = 1.12; 95% CI: 0.92–1.36, and cardiovascular death (RR = 1.20; CI: 0.97–1.49)
Wu et al. 2000	Cohort study 10-year follow-up	9,962 (25–74 years)	Gingivitis Periodontitis Edentulousness	CVD	Gingivitis: RR = 1.02 (0.70–1.48) Periodontitis: RR = 1.66 (1.15–2.39) Edentulousness: RR = 1.23 (0.91–1.66)

(*continued*)

Table 4.2 (continued)

Author	Type of study	Study population	Periodontal diagnostic	Coronary heart disease assessment	Association
Buhlin et al. 2002	Cross-sectional study	2,839 (59%) 20–84 years	Self-reported gum bleeding, tooth mobility and periodontal pockets	Self-reported CVD including myocardial infarction and high blood pressure	Significant association between self-reported bleeding gums (OR = 1.60, $P = 0.001$), presence of dentures (OR = 1.57, $P = 0.007$) and known CVD
Geerts et al. 2004	Case-control study	108 CAD patients (mean age 59.2 ± 11 years) and 62 healthy controls (mean age 57.7 ± 9 years)	Moderate periodontits was diagnosticed if at least one pocket >5 mm; severe periodontits if at least one pocket >7 mm	Hospital cases with CHD	The mean number of pockets was 18 ± 17.1 in cardiac patients vs. 7.6 ± 12.7 in controls ($P < 0.0001$), despite the fact that the mean number of missing teeth was significantly greater in cases than in controls (14 ± 7.1 vs. 9 ± 5.2; $P < 0.0001$). Strong association between CAD and periodontitis (OR = 6.5) and a significant dose-response relationship between increasing scores of the periodontal risk of infectiousness and the presence of CAD (adjusted OR = 1.3 per PIRI unit)
Nakib et al. 2004	Cross-sectional study	6,931 participants	Periodontitis defined as CAL >3 mm	Coronary artery calcification	Compared to subjects with no or mild periodontitis (<10% of sites with CAL ≥3 mm), subjects with moderate or severe periodontitis (≥10% of sites with CAL ≥3 mm) were more likely to have CAC ≥100, but this difference was not statistically significant (OR = 1.78; 95%CI: 0.65–4.86)
Shimazaki et al. 2004	Case-control study	957 subjects with ≥10 teeth and without a medical history of CVD	Periodontal status (PD, CAL, PI) of 1,111 residents	ECG abnormalities included left ventricular hypertrophy (Minnesota code 3–1) and ST depression (4–1, 2, 3)	The subjects with deep pockets (mean probing depth (PD) ≥2 mm) had an increased risk for ECG abnormalities (OR = 1.6; 95% CI: 1.01–2.50) compared to the subjects with mean PD <2 mm. Subjects with severe attachment loss (mean CAL ≥2.5 mm) had also significant risk for ECG abnormalities (OR = 1.7; 95% CI: 1.07–2.67) compared to those whose mean CAL was <2.5 mm
Beck et al. 2005	Cross-sectional study	6,793 persons subset of participants in the atherosclerosis risk in communities (ARIC) study	Periodontal status and serum IgG antibody levels against 17 periodontal organisms	Self-reported physician-diagnosed CHD	Clinical signs of periodontal disease were not associated with CHD, whereas systemic antibody response was associated with CHD in ever smokers and never smokers

Table 4.2 (continued)

Author	Type of study	Study population	Periodontal diagnostic	Coronary heart disease assessment	Association
Engebretson et al. 2005	Case-control study	203 stroke-free subjects ages 54–94	Radiographic measurement of ABL; severe periodontitis defined as periodontal bone loss >50%	Carotid plaque thickness evaluated with ultrasound	Severe periodontal bone loss was associated with a nearly fourfold increase in risk for the presence of carotid artery plaque (adjusted OR = 3.64; 95%CI: 1.37–9.65)
Buhlin et al. 2005	Case-control study	143 consecutive women, (43–79 years)	PD, bleeding on probing (BOP), number of remaining teeth, bone loss on panoramic radiographs. Periodontal pockets >4 mm were considered pathogenic	Subjects treated for acute myocardial infarction (percutaneous transluminal coronary angioplasty, coronary artery bypass grafting)	Number of periodontal pockets and CHD: OR = 3.8 (95% CI: 1.68–8.74) Dentures and CHD: OR = 4.6 (95% CI: 0.99–21.28)
Cueto et al. 2005	Case-control study	72 cases (acute myocardial infarction) and 77 controls (trauma patients) (40–75 years)	The degree of periodontitis was defined by the percentage of sites with loss of attachment >3 mm as follows: 0% = absent; 0–32% = mild; 33–66% = moderate; and 67–100% = severe	Acute myocardial infarction	The association between periodontitis (dichotomized) and acute myocardial infarction was high and significant in both the unadjusted (OR = 4.42, $P < 0.001$) and adjusted analyses (OR = 3.31, $P = 0.005$)
Holmlund et al. 2006	Case-control study	3,352 dental patients referred and 902 subjects randomly selected from the general population	Severity of periodontitis was estimated by a combination of the amount of bone loss around each tooth investigated from a full-mouth X-ray, the presence or absence of BOP, and involvement of furcations	Self-reported history of myocardial infarction and hypertension	Severity of periodontitis was significantly associated with myocardial infarction OR: 2.7, 95% CI: 1.1–6.5, but in middle-aged (40–60 years) subjects only
Spahr et al. 2006	Case-control study	263 patients and 526 controls	Community periodontal index of treatment needs (CPITN)	Angiographically confirmed, stable CHD	A statistically significant association between an increase in mean CPITN score by one and the presence of CHD (OR, 1.67; 95% CI: 1.08–2.58; $P = 0.02$) was observed
Geismar et al. 2006	Case-control study	110 individuals with verified CHD and 140 control individuals	ABL was stratified into ABL1 = ABL ≤2 mm; ABL2 = ABL > 2–≤4 mm; and ABL3 = ABL >4 mm	Medically confirmed CHD	For the ABL3 group, there was a significant association with CHD for participants <60 years old (OR = 6.6, 95% CI: 1.69–25.6)
Rech et al. 2007	Case-control study	58 patients and 57 controls	Severity of periodontal disease defined in relation with number of extracted teeth, periodontal pocket depth, BPO, CAL loss and bone loss	History of CHD	Association between periodontal disease and CHD OR = 4.5 (95% CI: 1.3–15.6, $P = 0.019$)

Table 4.3 Meta-analysis on periodontal disease and CHD

Authors	Studies included	Outcomes	Conclusions
Meurman et al. 2004	Nine cohort studies	RR = 1.19 (95% CI: 1.10–1.38)	Periodontal disease may indeed contribute to the pathogenesis of CVD, although the statistical effect size is small
Khader et al. 2004	Eight cohort studies	RR = 1.15 times (95% CI: 1.06–1.25)	No evidence for the existence of strong associations between periodontitis and CHD
Bahekar et al. 2007	Fifteen (five prospective cohort studies, five case-control studies and five cross-sectional studies)	PD and incidence of CHD Cohort studies: OR = 1.14 Case-control studies: OR = 2.22 (95% CI: 1.59–3.12) Cross-sectional studies: OR = 1.59 (95% CI: 1.33–1.91) Number of teeth and incidence of CHD: OR = 1.24 (95% CI: 1.14–1.36)	This meta-analysis indicates that both the prevalence and incidence of CHD are significantly increased in periodontal disease patients. Therefore, periodontal disease may be a risk factor for CHD
Mustapha et al. 2007	12 cohort ($N = 5$) and cross-sectional ($N = 7$) studies	PD and CHD OR = 1.75 (95% CI: 1.32–2.34) PD was associated with mean CIMT (0.03 mm; 95% CI: 0.02–0.04).	Periodontal disease with elevated bacterial exposure is associated with CHD events and early atherogenesis (CIMT)

CAD cardiovascular disease, CAL Clinical attachment level, CHD coronary heart disease, CIMT carotid intima-medial thickening, OR odd ratio, PD Probing depth, PI Plaque Index, PBS papillary bleeding score, PD periodontal disease; RR relative risk (risk ratio)

4.2.1.1 The Relationship Between Effects of Periodontal Therapy on Cardiovascular Disease Outcome (CVD)

Offenbacher et al. (2009) recently revealed that despite the fact that many studies have shown associations that link periodontal disease to CVD, there have been relatively few studies to address the potential effects of periodontal treatment on surrogate markers of cardiovascular risk or cardiovascular outcomes. Recent studies have suggested that periodontal treatments can reduce levels of serum highsensitivity CRP (as determined by hs-CRP assay), lower IL-6, and improve endothelial function as measured by flow-mediated dilation (Offenbacher et al. 2009) (Table 4.4).

This link may, however, be related also to several factors that *transcend interdependency and a putative causal relationship between both diseases* (RCDSO Symposium 2005). It is also possible that the apparent association between these two disease groups is related more to the existence of common risk factors and common underlying physiologies and pathophysiologies.

The biological basis for the hypothetical association of CVD and periodontal disease is presently unclear. According to Paquette et al. (2007) (Fig. 4.1), four specific pathways have been proposed to explain the plausibility of a link between CVD and periodontal infection. These pathways (acting independently or collectively) include:

- Direct bacterial effects on platelets.
- Autoimmune responses.
- Invasion and/or uptake of bacteria in endothelial cells and macrophages.
- Endocrine-like effects of pro-inflammatory mediators.

This link may, however, be related also to several factors that *transcend interdependency and a putative causal relationship between both diseases* (RCDSO Symposium 2005). It is also possible that the apparent association between these two disease groups is related more to the existence of common risk factors and common underlying physiologies and pathophysiologies.

Periodontal pathogens, release of lipopolysaccharide (LPS) and the resultant inflammatory response may systemically trigger several pro-inflammatory cytokines and tissue-destructive mediators, such as C-reactive protein CRP, TNF-α, PGE_2, IL-1β and IL-6. These cytokines can recruit additional monocytes and T-lymphocytes to the lesion sites. The host inflammatory responses have

Table 4.4 Studies on the relationship between effects of periodontal therapy on CVD outcome

Author, year	Length of the study	Study population	Cardiovascular disease outcome	Periodontitis evaluation	Smoking status	Oral intervention	Results
D'Aiuto et al. 2004 UK	6 months	94 subjects (46 ± 9 years)	CRP and IL-6 in serum	PD, marginal ABL, microbiological subgingival periodontal plaque	Smokers and nonsmokers	Oral hygiene instructions and subgingival scaling and root planning (SRP)	Significant reductions in serum IL-6 ($P < 0.001$, median decrease 0.2 ng/L, 95% CI: 0.1–0.4 ng/L) and CRP ($P < 0.0001$, median decrease 0.5 mg/L, 95% CI: 0.4–0.7)
D'Aiuto et al. 2005 UK	2 months	65 subjects	CRP, IL-6, total cholesterol, and low-density lipoprotein (LDL) cholesterol	PD, CAL	Smokers and nonsmokers	Sandard regimen of periodontal therapy (SRPT); intensive course of periodontal treatment (IPT), consisting of SPT with adjunctive local delivery of minocycline-HCl	Significant reductions in CRP in the SPT group 0.5 ± 0.2 mg/L (95% CI: 0–0.9, $P = 0.030$) and IPT group 0 .8 ± 0.2 mg/L (95% CI, 0.3–1.2, $P = 0.001$) compared with the untreated control group. The IPT group showed reduced IL-6 serum concentrations when compared with the untreated controls (mean decrease, 0.5±0.2 ng/L; 95% CI: 0.2–0.9; $P = 0.006$) and with the SPT group (mean decrease, 0.6 ± 0.2 ng/L; 95% CI: 0.2–1.0; $P = 0.002$)
Elter et al. 2006 USA	1 months	22	CRP, IL-6, Serum total and high density lipoprotein (HDL) cholesterol	Mean PD and mean CAL were calculated for each subject as the mean of all measured periodontal sites and as the extent (percentage) of sites with CAL >3 mm and PD >5 mm	Smokers and Nonsmokers	Whole-mouth disinfection completed over one or two visits occurring no less than 2 weeks apart. Periodontitis was treated with SRP, periodontal flap surgery where indicated, and extraction of hopeless teeth	There were no significant changes in clinical periodontal measures, CRP, IL-6, total cholesterol, or high-density lipoprotein cholesterol between the repeated baseline measurements. Periodontal treatment, however, resulted in significant improvements in periodontal pocketing, flow-mediated dilation, and serum IL-6, as well as a trend toward reduction in serum CRP
Mercanoglu et al. 2004	6 weeks	28 patients (45.5 ± 8.6 years)/26 controls (43.7 ± 6.8 years)	Brachial artery responses to reactive hyperemia (EDD) and sublingual nitroglycerin (EID) measured using high-resolution vascular ultrasound	PI, GI, PD, CAL and radiographic bone loss	Smokers and nonsmokers	Full-mouth SRP as nonsurgical periodontal therapy and instructions for proper oral hygiene practices	After nonsurgical periodontal therapy, EDD and EID improved significantly (from 8.4 ± 4.0%–17.7 ± 5.7%, $P < 0.0001$; and from 13.3 ± 6.3%–24.9 ± 7.3%, $P < 0.0001$ for FMD and EID, respectively). The EDD and EID changes in the controls were insignificant

(*continued*)

Table 4.4 (continued)

Author, year	Length of the study	Study population	Cardiovascular disease outcome	Periodontitis evaluation	Smoking status	Oral intervention	Results
Seinost et al. 2005	12 weeks	30 cases with severe periodontitis (40.2 ± 4.7 years.) and 31 control subjects (41.2 ± 7.4 years)	Endothelial function tested with FMD of the brachial artery	PD, R	Smokers and nonsmokers	Oral hygiene instruction with several supragingival cleaning sessions + SRP + Chlorhexidine gluconate (0.1%) mouth washes for 14 days + Systemic antimicrobial therapy for 7 days	Successful periodontal treatment resulted in a significant improvement in FMD (9.8 ± 5.7%; $P = 0.003$ compared to baseline) accompanied by a significant decrease in CRP concentrations (1.1 ± 0.9 vs. 0.8 ± 0.8 at baseline, $P = 0.026$)
Tonetti et al. 2007	180 days	120 patients with severe periodontitis randomly assigned to community-based periodontal care (59 patients) or intensive periodontal treatment (61)	Endothelial function, as assessed by measurement of the diameter of the brachial artery during flow (FMD), inflammatory biomarkers and markers of coagulation and endothelial activation	PI, BOP, PD, R	Smokers and nonsmokers	Control-treatment group: Basic oral hygiene instructions + supragingival mechanical scaling and polishing, Intensive-treatment group: also full-mouth intensive removal of subgingival dental plaque biofilms with the use of SRP with extraxtion of hopeless teeth and local delivery of microspheres of minocycline (Arestin, OraPharma) into the periodontal pockets	Twenty-four hours after treatment, flow-mediated dilatation was significantly lower in the intensive-treatment group than in the control-treatment group (absolute difference, 1.4%; 95% CI: 0.5–2.3; $P = 0.002$)
Offenbacher et al. 2009	6 months	151 subjects assigned to protocol therapy and 152 assigned to community care (PAVE study)	Serum hs-CRP concentrations	PI, GI, CI, PD, CAL, BOP, GCF measurements of IL-1β	Smokers and nonsmokers	Community care group, consisting of oral hygiene instruction plus a letter of referral to seek periodontal care, or a protocol "intensive treatment" group consisting of oral-hygiene instruction plus SRP under anesthesia using a piezoelectric ultrasonic scaler	No significant effect on serum hs-CRP levels

PD probing depths; *R* recession; *CAL* clinical attachment level; *PI* plaque index; *GI* gingival index; *BOP* bleeding on probing; *CI* calculus index; *GCF* gingival crevicular fluid; *SRP* scaling and root planing; *EDD* endothelium-dependent dilatation; *EID* endothelium-independent dilatation; *FMD* flow mediated dilation; *CRP* C-reactive protein; *IL-6* interleukin-6

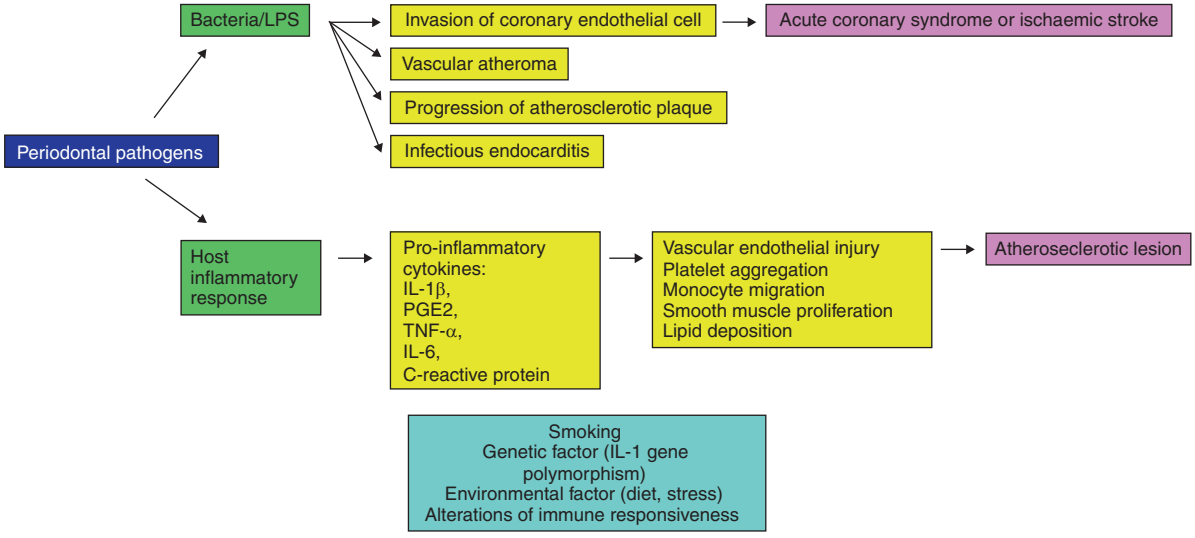

Fig. 4.1 Relationships between periodontitis and cardiovascular disease (CVD) (*LPS* lipopolysaccharide; *IL* interleukin; *TNF* tumor necrosis factor; *PGE* prostaglandin E) (Kuo et al. 2008)

both local and systemic manifestations. Transient bacteraemia and endotoxaemia secondary to periodontal infection may also affect atherogenesis indirectly by stimulating the normal host responses (Kuo et al. 2008).

Several studies assessing the presence of bacteria associated with periodontitis in specimens collected from the aorta or other blood vessels have identified bacteria associated with periodontitis in samples from aorta and heart valves. A recent systematic review performed by Persson and Persson (2008) has presented a summary of studies on serum antibody titers to bacteria associated with periodontitis and CVD. High antibody titers to *Aggregatibacter* (formerly *Actinobacillus) actinomycetemcomitans* specifically have been associated with CHD, while serum immunoglobulin A (IgA) and IgG antibody /titers to *A. actinomycetemcomitans* have also been linked to future stroke event (Persson and Persson 2008).

In addition to studies that link periodontal disease to cardiovascular outcomes, there is literature that begins to indicate that periodontitis is also associated with changes in blood cellular and biochemical composition that are secondary markers of cardiovascular risk, including rheological indicators of hypercoagulation (Beck and Offenbacher 2001).

Inflammatory processes have become an integral part of the pathophysiology of atherosclerosis and are presumed to be involved from the initiation to the progression and final stages of infarction. Normal endothelium does not allow for the attachment of leukocytes. When initial damage of the endothelium occurs, either by infection or by an atherogenic diet, the endothelial cells express adhesion molecules that allow leukocytes to bind to them. These adhesion molecules are called "vascular cell adhesion molecules" (VCAM) and "intercellular adhesion molecules" (ICAM). Selectins and integrins also support leukocyte attachment. Once this attachment is established, the atheroma accumulates more lipids and promotes the production of various chemokines and growth factors that stimulate the recruitment of monocytes and macrophages. These chemokines also promote the migration of smooth-muscle cells. These muscle cells respond to the inflammatory stimuli by secreting specific enzymes (metalloproteinases) that are able to degrade elastin and collagen. Further, these metalloproteinases may disintegrate the fibrous capsule holding the cholesterol plaque together, and cause plaque rupture. Plaque rupture greatly increases the risk of myocardial infarction and stroke (Meurman et al. 2004).

It was also showed that periodontitis is associated with increased levels of low-density lipoprotein (LDL), total cholesterol and triglycerides, as well as with decreased levels and antiatherogenic potency of high density lipoprotein (HDL) (Monteiro et al. 2009).

Other pro-atherogenic markers have also been associated with periodontitis. It was suggested that periodontitis increased the levels of circulating cytokines and inflammatory mediators, such as, C-reactive protein,

which can damage the vascular endothelium and can result in atherosclerosis (Monteiro et al. 2009).

There is evidence to suggest that specific IL-1 genotypes (pattern 1) are associated with severe periodontitis and not atherosclerosis, whereas a different IL-1 genotype (pattern two) is associated with atherosclerosis but not periodontitis. It was speculated that both IL-1 genotypes alter the immunoinflammatory responses in both diseases in a similar manner, and thus provides further evidence of a biological link between susceptibility to periodontal disease and CAD (Seymour et al. 2002). It was recently revealed that allele one of IL-1RN VNTR may be associated with the coexistence of CHD and periodontitis in a multiple regression model (Geismar et al. 2008).

It can be concluded that at present, there is a lack of strong evidence to establish the causal relationship between periodontal diseases and CVD. Confounding factors for both diseases, such as genetic factors (IL-1 gene polymorphism), environmental factors (smoking, diabetes, stress, etc.) and alterations of immune response can mislead interpretations of the study results. Differences can also be observed in the way in which dental data have been recorded. For some studies, mean PD are reported, for others, a composite index has been used, and for others still, self-reported disease is assessed. This makes it difficult to compare studies and to draw common conclusions about which aspect of dental disease might be the reason for a relationship between periodontal disease and coronary artery disease. Periodontal diseases are highly prevalent in middle-age populations, which also commonly suffer from CVD, and prevention and management of periodontal diseases can have a significant impact on improvement of cardiovascular function at a public health level (Kuo et al. 2008).

Properly powered longitudinal case-control and intervention trials are needed to identify how periodontitis and periodontal interventions may have an impact on CVDs.

4.2.2 Periodontal Disease and Stroke

Stroke causes 9% of all deaths around the world and is the second most common cause of death after ischaemic heart disease. The proportion of deaths caused by stroke is 10–12% in western countries, and 12% of these deaths are in people less than 65 years of age. Risk factors for stroke can be broadly classified as modifiable or fixed. Some modifiable risk factors (such as hypertension, diabetes, and smoking) are common and affect health in several ways, providing opportunities to modify risk in large number of people. Other risk factors, such as atrial fibrillation and transient ischemic attacks, are less prevalent and more specific than the common risk factors for stroke. Risk factors that have been identified explain only about 60% of the attributable risk, whereas more than 90% of ischemic heart disease is explained by identifiable risk factors. Strokes are either ischemic or hemorrhagic. Because the management of these subtypes is so different, the clinical distinction between the subtypes is one of the most important and urgent steps in stroke management (Donnan et al. 2008).

Periodontitis and stroke have several common risk factors, such as age, smoking, diabetes, and hypertension. Periodontitis is also associated with elevated markers of inflammation that are themselves indicators of stroke risk. One cross-sectional study, five case-control studies and five cohort studies have investigated the associations between periodontal inflammation and stroke in the United States, Canada, Germany, and Finland. Of these studies, most showed positive results, whereas two others did not (Sim et al. 2008) (Tables 4.5 and 4.6). One cross-sectional study performed by Elter et al. (2003) on a sample of 415 dentate and 1,491 edentulous adults (Dental ARIC Study) revealed that stroke was prevalent in 13.5% of periodontal examinees, 15.6% of dentate nonexaminees, and 22.5% of edentulous persons. The highest quartile of extent (%) of attachment level >3 mm (OR = 1.3, 95% CI: 1.02–1.7) and edentulism (OR = 1.4, 95% CI: 1.5–2.0) were associated with Stroke/TIA.

The meta-analysis performed by Khader et al. (2004) was based on six studies (Beck et al. 1996; Morrison et al. 1999; Wu et al. 2000; Howell et al. 2001; Grau et al. 1997; Buhlin et al. 2002). All of the risk ratios were grater than one, but only three of them were statistically significant. As compared to healthy people, subjects with gingivitis and periodontitis had an overall adjusted relative risk of cerebrovascular disease of 1.37 (95% CI: 1.10–1.73; $P = 0.006$) and 1.13 (95% CI: 1.01–1.27; $P = 0.032$), respectively. When the analysis was restricted to prospective studies only, the relative risk of cerebrovascular disease for subjects with periodontitis was reduced to 1.11 (95% CI: 0.98–1.25; $P = 0.106$); this was not significant. As compared to healthy subjects, edentulous subjects had an overall adjusted relative risk of CVD of

Table 4.5 Summary of case-control studies investigating the association between periodontal disease and stroke

Author, year	Study population	Periodontal disease evaluation	Results	Adjustment factors	Significance
Syrjänen et al. 1989 Finland	40/40	Two indices measuring the severity of infections of teeth and periodontium, or by the presence of subgingival calculus or the presence of suppuration in the gingival pockets	An association between bacterial infection and ischaemic cerebrovascular disease in patients under 50 years of age was suggested. Severe chronic dental infection seems to be an important type of infection associated with cerebral infarction in males	Not known	Significant
Grau et al. 1997 Germany	166/166	TDI that reflects caries, periapical lesions, periodontitis, and other dental lesions and by an orthopantomography index (OPGI)	Patients tended to have a worse dental status (TDI: $P = 0.07$; OPGI: $P = 0.06$) and had more severe periodontitis ($P = 0.047$) and periapical lesions ($P = 0.027$) than control subjects. In age-adjusted multiple logistic regression analysis with social status and established vascular risk factors, poor dental status (TDI) was independently associated with cerebrovascular ischemia (OR: 2.6; 95% CI: 1.18–5.7)	Age	Significant
Grau et al. 2004 Germany	303/468	PI, GI, CAL, radiographic bone loss on panoramic radiographs	Subjects with severe periodontitis (mean CAL >6 mm) had a 4.3-times-higher (95% CI: 1.85–10.2) risk of cerebral ischemia than subjects with mild or without periodontitis (<3 mm)	Age, sex, number of teeth, vascular risk factors and diseases, childhood and adult socioeconomic conditions and lifestyle factors	Significant
Dorfer et al. 2004 Germany	303/300	CAL, GI, PI	A mean CAL >6 mm had a 7.4 times (95% CI: 1.55–15.3), GI >1.2 a 18.3 times (5.84–57.26) and radiographic bone loss a 3.6 times (1.58–8.28) higher risk of cerebral ischemia than subjects without periodontitis or gingivitis, respectively	Age, gender, number of teeth, vascular risk factors and diseases, childhood and adult socioeconomic conditions and lifestyle factors	Significant
Sim et al. 2008 North-Koreea	265/214	CAL	Stroke was strongly associated with periodontitis (presence of CAL ≥6 mm): the odds ratio was 4.0 (95% CI: 2.3–7.0) after controlling for all possible confounders. The association with periodontitis (tertiary percentage of CAL ≥5 mm) had a dose-response effect. The association between periodontitis and stroke was higher among adults younger than age 60 (6.0 vs. 2.6) and normotensives (4.8 vs. 3.2)	Age, gender, income, education, smoking, drinking, history of systemic disease, body mass index (BMI), familial cardiovascular risk factors and oral health behaviors	Significant

PI plaque index; *BOP* bleeding on probing; *PD* probing depth; *CAL* clinical attachment level; *GI* gingival index; *RR* relative risk; *CI* confidence intervals

Table 4.6 Summary of cohort studies investigating the association between periodontal disease and stroke

Author, Year	Study population	Length of the study	Periodontal disease evaluation	Results	Adjustment factors	Significance
Beck et al. 1996 USA	1,147 men during 1968–1971 (normative aging study (NAS) and the dental longitudinal study)	25 years	PD, bone loss	Incidence odds ratio was 2.8 for bone loss and stroke	Not known	Significant
Morrison et al. 1999 Canada	11,251 participants 1970–1972 nutrition Canada survey (NCS)	20 years	Not known	Nonstatistically significantly increased RRs of 1.81 and 1.63 were observed for severe gingivitis and edentulous status for CVD	Age, sex, diabetes status, serum total cholesterol, smoking, hypertensive status and province	Non-significant
Wu et al. 2000 USA	9962 adults (first national health and nutrition examination survey (NHANES))	Dental examination conducted between 1971 and 1974. Follow-up surveys conducted from 1982 through 1984, 1986, 1987 and 1992	Subjects classified with no periodontal disease, mild gingivitis, gingivitis, gingivitis with pocket formation and advanced destruction with loss of masticatory function	Periodontitis is a significant risk factor for total CVA and, in particular, nonhemorrhagic stroke (*ICD-9*, 433–434 and 436–438). Compared with no periodontal disease, the relative risks (95% CI) for incident nonhemorrhagic stroke were 1.24 (95% CI: 0.74–2.08) for gingivitis, 2.11 (95% CI: 1.30–3.42) for periodontitis, and 1.41 (95% CI: 0.96–2.06) for edentulousness. For total CVA, the results were 1.02 (95% CI: 0.70–1.48) for gingivitis, 1.66 (95% CI: 1.15–2.39) for periodontitis, and 1.23 (95% CI: 0.91–1.66) for edentulousness	Age, race, sex, years of schooling, family income level, smoking status, diabetes status, hypertension, alcohol use, serum total cholesterol levels and BMI	Significant
Howell et al. 2001 USA	2,653 physicians (physicians' health study I)	12.3 years of follow-up	Self-reported periodontal disease	Physicians who reported periodontal disease at baseline had slightly elevated, but statistically nonsignificant, relative risks (RR) of nonfatal stroke (RR = 1.10; 95% CI: 0.88–1.37)	Age and treatment assignment	Non-significant
Joshipura et al. 2003USA	41 380 men	12 years of follow-up	Tooth loss, Self-reported periodontal disease was assessed by validated questions	A modest association was seen between baseline periodontal disease history and ischemic stroke (Hazard Ratio = 1.33; 95% CI: 1.03–1.70)	Age, amount smoked, obesity, alcohol, exercise, family history of CVD, multivitamin use, vitamin E use, profession, baseline reported hypertension and hypercholesterolemia	Significant

PI plaque index; *BOP* bleeding on probing; *PD* probing depth; *CAL* clinical attachment level; *GI* Gingival Index; *RR* relative risk; *CI* confidence intervals

1.46 (95% CI: 0.80–2.66; $P = 0.222$); however, this was not significant (Khader et al. 2004).

It is possible that periodontitis increases the risk of cerebrovascular disease through bacteria that induce production of pro-inflammatory cytokines, cause inflammatory cell proliferation into large arteries, stimulate hepatic synthesis of clotting factors, and thus contribute to atherogenesis and thromboembolic events. In addition, several periodontal pathogens can induce platelet aggregation and thus may be thrombogenic when entering the systemic circulation as in periodontitis (Khader et al. 2004).

Additional studies are needed to clarify the association between periodontal inflammation and stroke, properly controling for confounding factors and using adequate measures of periodontal disease status (Sim et al. 2008).

4.2.3 Periodontal Disease and Peripheral Vascular Disease

Peripheral vascular disease (PVD) is generally but not invariably a consequence of an atherosclerotic process and presents as one of its three distinct clinical syndromes that are associated with progressive obstruction. It commonly affects the ileofemoral circulation and encompasses a wide clinicopathological spectrum, ranging from asymptomatic arterial tapering and narrowing of the peripheral arterial bed, to almost complete loss of pulsatile blood flow. Although symptomatic peripheral arterial disease (PAD) patients present initially with intermittent claudication (IC), symptoms may progress to rest pain and critical limb ischemia, and finally to ulceration, gangrene and amputation. PAD afflicts approximately 15% of the U.S. population older than 55 years of age. At least half of the patients with coronary artery disease have some degree of PAD. Although PAD shares the classic risk factors of atherosclerosis, certain risk factors such as smoking and diabetes appear to be more important in its pathogenesis. Even asymptomatic PAD is associated with an increased incidence of myocardial infarction, stroke, and overall cardiovascular morbidity (Al-Mheid and Quyyumi 2008) (Table 4.7).

Very few studies (one cross-sectional study, two cohort studies and one case-control study) have evaluated the relationship between periodontal disease and PAD. The first study by Mendez and colleagues (1998) was conducted as part of the Veterans Affairs Dental Longitudinal Study among 1,110 male veterans. The loss of tooth supporting alveolar bone was estimated from full mouth intraoral periapical radiographs. Mean bone loss across all readable interproximal sites was calculated for each subject. Significant periodontal disease was considered present if the mean whole mouth ABL was >20%. During a 25-year follow-up period, 80 men developed PVD. It was showed that after adjusting only for age, subjects with significant baseline periodontal disease had 2.6-fold greater risk of developing PVD compared with those without significant periodontal disease (95% CI: 1.6–4.2; $P = 0.0001$).

Hung et al. (2003) conducted a prospective cohort study of 45,136 eligible male health professionals (The Health Professionals Follow-up Study) and found that updated history of periodontal disease and PAD were associated with a relative risk of 1.41 (95% CI: 1.12–1.77) during a 12-year follow-up.

In a recent cross-sectional study (national health and nutrition examination survey (NHANES) during 1999–2002.) of 3,585 participants, 172 (4.8%) were recognized as PVD cases. Participants with 33% sites of periodontal attachment loss ≥3 mm had a greater odds ratio over reference participants (OR = 2.25, 95% CI: 1.20–4.22, $P < 0.05$) (Lu et al. 2008).

There are several explanations for the associations between periodontitis and PAD. Locally produced pro-inflammatory mediators, such as IL-6, TNFα and IL-1β, may spill into the circulation and exert systemic or distant effects, including the recruitment of monocytes, up-regulate endothelial adhesion molecules, and increase macrophage uptake of lipids (Chen et al. (2008)). Preliminary observations in a study performed by Chen et al. (2008) suggest that periodontitis might have contributed to the increased IL-6 and TNF-α levels in PAD patients. Periodontopathic bacteria also may directly enhance atherogenesis. The detection frequency of *Porphyromonas gingivalis, Treponema denticola, A. actinomycetemcomitans* and *Prevotella intermedia* in atherosclerostic specimens was 32 (8/25), 32 (8/25), 4 (1/25) and 20% (5/25), respectively (Chen et al. 2008). Lu et al. (2008) also suggested that systemic markers of inflammation (CRP, white cell count, fibrinogen) were also predictors of PVD and were significantly associated with periodontal attachment loss.

Table 4.7 Summary of studies investigating the association between periodontal disease and peripheral arterial disease (PAD)

Author, year	Type of the study	Study population	PVD evaluation	Periodontal disease evaluation	Results	Adjustment factors	Significance
Mendez et al. 1998 USA	Longitudinal study 25–30 years of follow-up	2,280 men (21–80 years old)	Peripheral vascular disease (PVD) was defined as one or more of the following: (1) intermittent claudication (IC); (2) extracranial cerebrovascular disease (ECD); (3) atherosclerosis (including aortic, renal, and mesenteric disease); and (4) arterial embolism and thrombosis	ABL estimated from full-mouth intraoral periapical radiographs	Subjects with clinically significant periodontal disease at baseline had a 2.27 increment in the risk of developing PVD (95% CI: 1.32–3.9, $P = 0.003$)	Age	Significant
Hung et al. 2003 USA	Longitudinal study 12 years of follow-up	45, 136 eligible male health professionals (40–75 years old)	PVD diagnosis on the basis of these criteria: (1) ankle-brachial systolic pressure index <0.80, (2) medical or surgical treatment for PAD, (3) angiogram or Doppler ultrasonic reports of >50% narrowing of the femoral or popliteal arteries, or (4) reported symptoms and positive diagnosis made by physicians	Self-reported periodontal disease measure and remaining number of teeth	The relative risk for history of periodontal disease was 1.41 (95% CI: 1.12–1.77) and for any tooth loss during the follow-up period was 1.39 (95% CI: 1.07–1.82)	Age, smoking, alcohol consumption, BMI, physical activity, family history of myocardial infarction, multivitamin supplement use, vitamin E use, history of hypertension, diabetes, hypercholesterolemia and profession	Significant
Lu et al. 2008 USA	Cross-sectional	3,585 participants who were 40 years or older	PVD assessed by ABI, which is the ratio of systolic blood pressure in the both ankles (posterior tibial arteries) to that in the right arm (brachial artery)	CAL	CAL loss was significantly associated with PVD (OR = 2.25, 95% CI: 1.20–4.22, $P < 0.05$)	Demographic variables, smoking status, alcohol use, physical activity, total cholesterol/HDL ratio, aspirin use, hypertension, renal insufficiency, a history of diabetes and a history of congestive heart failure	Significant
Chen et al. 2008	Case-control	25 cases/32 controls	PAD was diagnosed based on clinical symptoms, ankle brachial pressure index (ABI) and angiographic findings	Number of remaing teeth, pocket depth, CAL	Periodontitis increased fivefold the risk of having PAD (OR = 5.45, 95% CI: 1.57–18.89, $P = 0.007$)	Age, gender, diabetes and smoking status	Significant

4.3 Association Between Periodontal Disease and Metabolic Syndrome

The global obesity epidemic has been described by the World Health Organization (2000) as one of the most blatantly visible, yet most neglected, public health problems that threatens to overwhelm both more- and less-developed countries (Linden et al. 2007). Obesity is a growing problem in Europe, currently affecting between 10 and 20% of the population (Buttriss and Nugent 2005). Understanding the various factors that have contributed to this trend and understanding why obesity is so difficult to treat has become an increasingly important health issue. The metabolic syndrome (also known as syndrome X, insulin resistance syndrome, dysmetabolic syndrome, deadly quartet, and plurimetabolic syndrome) is a cluster of factors associated with increased risk of developing CHD and/or type 2 diabetes. Metabolic syndrome comprises insulin resistance (fasting blood sugar >10 mg/L), dyslipidemia (triacylglycerol >15 mg/L, HDL <4 mg/L for men and 5 mg/L for women, LDL >15 mg/L), essential hypertension (blood pressure of >130 mmHg for systolic and >85 mmHg for diastolic) and visceral obesity (waist circumference of >102 cm for men and >89 cm for women) (Stewart-Knox 2005) (Table 4.8).

A recent number of studies focused on the relationship between periodontal disease and metabolic syndrome. Shimazaki et al. (2007) examined the relationship between periodontitis and five components of metabolic syndrome-abdominal obesity, triglyceride level, HDL cholesterol level, blood pressure, and fasting blood sugar level-in 584 Japanese women. Of the five components of metabolic syndrome, large waist circumference, low HDL cholesterol level, and high fasting plasma glucose level were associated with significantly higher odds ratios (ORs) for greater PD values; the adjusted ORs for these components were 1.8 (95% CI: 1.2–2.8), 2.2 (95% CI: 1.4–3.6), and 2.2 (95% CI: 1.3–3.9), respectively. The participants with low HDL cholesterol had a higher odds ratio (OR = 2.8; 95% CI: 1.4–5.6) for a greater clinical attachment loss value after adjustment for age and smoking status. In multivariate analyses, persons exhibiting more components of metabolic syndrome had significantly higher odds ratios for a greater PD and clinical attachment loss than did those with no components; the odds ratios for a greater PD and clinical attachment loss of the persons exhibiting four or five components were 6.6 (95% CI: 2.6–16.4) and 4.2 (95% CI: 1.2–14.8), respectively.

Table 4.8 WHO definition of metabolic syndrome (2004)

Insulin resistance
Type 2 diabetes
Impaired fasting glucose
Impaired glucose tolerance (>110 mg/dL or >6.1 mmol/L)
Plus at least 2 of the following
High blood pressure (>140 mmHg systolic or >90 mmHg diastolic)
Plasma triglycerides >150 mg/dL (>1.7 mmol/L)
HDL cholesterol <35 mg/dL (<0.9 mmol/L) in men or < 39 mg/dL (<1.0 mmol/L) in women
BMI 430 kg/m^2 and/or waist: hip ratio 40.9 in men or 40.85 in women
Urinary albumin excretion rate >20 mg/min or albumin: creatinine ratio >30 mg/g

HDL high-density lipoprotein; *BMI* body mass index

In a cross-sectional study, Borges et al. (2007) analyzed data from 1,315 Japanese-Brazilians ranging from 30 to 92 years of age, submitted to physical, laboratory, and dental exams. 484 (36.8%) of the 1,315 were edentulous, 215 (16.4%) enjoyed periodontal health, 513 (39%) had gingivitis, 85 (6.5%), showed initial or moderate periodontitis, and 18 (1.4%) suffered from chronic periodontitis. Prevalence of metabolic syndrome was 54.3%, higher among individuals with periodontitis than in the healthy individuals (51.5 vs. 48.8%), but this association was not statistically significant. Individuals with metabolic syndrome showed a worse metabolic and anthropometric profile.

Khader et al. (2008) revealed that patients with metabolic syndrome displayed more severe and extensive periodontal disease, as measured by average PD and average CAL, and the extent of periodontal disease, as measured by the percentage of sites with CAL ≥3 mm and the percentage of sites with PD ≥3 mm, compared to subjects without metabolic syndrome. Similar results were reported by Li et al. (2009), who showed that after adjustment for gender, age, and smoking, the corresponding adjusted odds ratios for metabolic syndrome were 6.9 (95% CI: 1.07–44.77), 9.9 (95% CI: 1.50–65.24), and 15.6 (95% CI: 2.20–110.43) for subjects with attachment loss ≥3 mm in >0–33% of sites, >33–67% of sites, and >67% of sites, respectively.

These results indicate that metabolic syndrome increases risk of periodontitis, and suggest that people exhibiting several components of metabolic syndrome should be encouraged to undergo a periodontal examination (Shimazaki et al. 2007).

4.3.1 Association Between Periodontal Disease and Hypertension

During the past 15 years, mounting evidence for the association between periodontal and CVD has been presented in epidemiological studies. Left ventricular mass is an established independent predictor of CVD. In a cross-sectional study, the association between periodontitis and left ventricular mass was tested in subjects with essential hypertension. One hundred four untreated subjects with essential hypertension underwent clinical examinations, including echocardiographic study, laboratory tests, and assessment of periodontal status according to the community periodontal index of treatment Needs (CPITN) (Angeli et al. 2003). It was observed that with increasing severity of periodontitis, there was a progressive increase in left ventricle mass. Mean values (g/height) were 39.0 ± 2.7) in Code 0 (periodontal health), 40.2 ± 6.4 in Code 1 (gingival bleeding), 42.7 (±6.8) in Code 2 (calculus), 51.4 (±11.7) in Code 3 (pockets 4–5 mm), and 76.7 (±11.3) in Code 4 (pockets ≥6 mm) (overall $F = 51.2$; $P < 0.0001$). Body surface area ($P = 0.04$), systolic ($P < 0.0001$) and diastolic ($P < 0.01$) blood pressure, and left ventricular mass ($P < 0.0001$) were determinants of a composite of Codes 3 and 4. In a multivariate logistic analysis, left ventricular mass was the sole determinant ($P < 0.0001$) of CPITN stages 3 and 4 (Angeli et al. 2003). It was also suggested that periodontitis is associated with endothelial dysfunction in subjects without cardiovascular risk factors, as well as hypertensive patients, through a decrease in nitric oxide bioavailability, and that systemic inflammation may be, at least in part, a cause of endothelial dysfunction, leading to CVDs (Higashi et al. 2008).

Holmlund et al. (2006) investigated how the severity of periodontal disease and number of remaining teeth relates to myocardial infarction and hypertension. Self-reported history of hypertension and myocardial infarction was collected in 3,352 patients referred to the Department of Periodontology, Gävle County Hospital, and in 902 subjects, randomly selected from the general population. Severity of periodontitis was estimated by a combination of the amount of bone loss around each tooth investigated from a full-mouth X-ray, the presence or absence of BPO, and involvement of furcations. The severity of periodontitis was significantly associated with hypertension (prevalence 16%; $P < 0.0005$), even after adjustment for age, gender, number of teeth and smoking in the total sample, and with myocardial infarction (prevalence 1.7%, $P < 0.03$) after above-mentioned adjustments, but in middle-aged (40–60 years) subjects only. The number of diseased periodontal pockets was related to hypertension only ($P < 0.0001$), and this relationship remained after the above-mentioned adjustments. The number of teeth was associated with myocardial infarction ($P < 0.03$) even after correction for age, gender and smoking but was not related to hypertension. These data support the view that oral health is related to CVD in a dose-dependent manner.

More recently, Türkoğlu et al. (2008) investigated whether chronic periodontitis caused the elevated levels of anticardiolipin antibodies and oxidized LDL in subjects with essential hypertension. Seventy-two subjects were categorized as healthy controls, subjects with essential hypertension and periodontal health (healthy-hypertension group), subjects with essential hypertension and gingivitis (gingivitis-hypertension group), or subjects with essential hypertension and chronic periodontitis (periodontitis-hypertension group). The mean IgM anticardiolipin antibodies level and the prevalence of subjects positive for IgM anticardiolipin antibodies were significantly higher in the periodontitis-hypertension group compared to the other groups ($P = 0.001$). The Pearson correlation analysis revealed positive correlations between IgM anticardiolipin antibodies levels and supragingival plaque, BPO and PD scores. It was concluded that chronic periodontitis might play a causal role in the elevated serum levels of anti cardiolipin antibodies in individuals with essential hypertension. These elevated anticardiolipin levels that are due to chronic periodontitis might contribute to an increased risk for atherosclerosis in individuals with essential hypertension.

4.3.2 Association Between Periodontal Disease and Obesity

Various cross-sectional and case-control studies found a strong association between obesity and periodontal disease (Table 4.8). Body mass index (BMI) was calculated as the body weight/height^2 (kg/m^2). The BMI measured was categorized using the World Health Organization (2000) classification: normal weight equated to BMI <25 kg/m^2, overweight >25–<30 kg/m^2 and obese >30 kg/m^2.

4.3.3 Mechanisms Implied in the Association Between Periodontal Disease and Obesity

A few number of reports on the mechanism connecting obesity and periodontal disease have proposed several ways in which *obesity affects periodontal tissue directly*. Obesity affects host immunity (Martí et al. 2001) (Table 4.9). Perlstein and Bissada (1977) evaluated the extent to which obesity and/or hypertension may modify the response of rats' periodontium to chronic gingival irritation. Histopathologic evaluation of the periodontal structure showed both hyperplasia and hypertrophy of the walls of blood vessels supplying the periodontium in the hypertensive and obese-hypertensive animals. The results also indicated that obesity significantly contributed to e severity of periodontal disease. The obese-hypertensive rats showed the most severe periodontal response to local irritation.

The relationship between adipose tissue and immune system is believed to be related to the secretion of numerous adipokines among them leptin of which the amount is correlated to fat mass (Table 4.10). The leptin receptor is largely expressed in hematopoietic and immune cells. Leptin could support T lymphocyte development in thymus, T cell homeostasis, function and proinflammatory immune responses. Furthermore, Th1-promoting effects of leptin have been linked to enhanced susceptibility to experimental autoimmune diseases (Caspar-Bauguil et al. 2006). In addition, leptin has been shown to be involved in bone metabolism. Although reported data appear somewhat conflicting, evidence exists that leptin may decrease bone formation *via* central nervous pathways, and may stimulate bone formation *via* direct peripheral effects on bone cells. The net result on bone formation may depend on various general and bone-specific factors, such as species, age, gender, serum leptin levels, blood-brain barrier permeability, bone tissue, skeletal maturity and signaling pathways. In addition, several studies have suggested that elevated levels of leptin can be found during infection and inflammation (Pischon et al. 2007). It was revealed that human leptin is present within healthy and marginally inflamed gingiva and decreases in concentration as the adjacent PD increases (Johnson and Serio 2001). Recent studies have indicated that adipose tissue, especially visceral adipose tissue, is an important organ that secretes several bioactive substances known as adipocytokines, which include tumor necrosis factor-α that may enhance periodontal degradation (Saito and Shimazaki 2007).

4.3.4 Association Between Periodontal Disease and Hyperlipidemia

An association between hyperlipidemia and periodontal disease in several epidemiological studies was observed (Noack et al. 2000; Losche et al. 2000; Katz et al. 2002). In 302 patients with severe periodontitis and 183 healthy controls, Nibali et al. (2007) revealed that after correcting for differences in age, gender, smoking and ethnicity, periodontitis subjects exhibited a low-grade systemic inflammation (increased white cell counts, $1.10 \pm 1.02 \times 10^9$/l, 95% CI: 1.05–1.15, $P = 0.0001$), dyslipidemia [lower high-density lipoprotein cholesterol, 1.14 ± 1.03 mmol/L, 95% CI: 1.08–1.20, $P < 0.0001$ and higher LDL cholesterol, 1.12 ± 1.03, 95% CI: 1.05–1.19, $P < 0.0001$), and increased nonfasting serum glucose levels (1.04 ± 1.01 mmol/L, 95% CI: 1.02–1.06, $P = 0.01$) when compared with controls. A trend for a dose-dependent effect of the number of periodontal pockets on the tested inflammatory and metabolic markers was observed.

4.4 Conclusion

Recent studies have indicated a close correlation of metabolic syndrome and obesity with periodontal disease and with other chronic inflammatory diseases, including type 2 diabetes and CVD. Whether the relationship between obesity and periodontitis is causal needs to be assessed in future studies. Pro-inflammatory cytokines may be a multidirectional link among periodontitis, obesity and other chronic diseases. The adipose tissue is a large reservoir of biologically active mediators, such as TNF-α and other adipokines. Studies have demonstrated a close involvement of the adipokines – such as leptin, resistin, and adiponectin – in inflammatory processes. However, their role in periodontal inflammation has yet to be defined (Saito and Shimazaki 2007).

These results indicate that metabolic syndrome increases risk of periodontitis, and suggest that people exhibiting several components of metabolic syndrome should be encouraged to undergo a periodontal

Table 4.9 Studies on the relationship between obesity and periodontal disease

Author, year	Subjects, n	Criteria for obesity	Criteria for periodontitis	Association
Saito et al. 1998	241 (172 females, 69 males) 20–59 years	BMI	Probing depth ≥4 mm	Significant
Saito et al. 1999	241 (172 females, 69 males) 20–59 years	BMI	Probing depth ≥4 mm	Significant
Saito et al. 2001	643 (512 females, 131 males) 19–79 years	Waist-hip ratio, body-mass index (BMI), and body fat	Probing depth ≥4 mm	Significant
Wood et al. 2003	NHANES III *N* = 8842	BMI, waist-to-hip ratio (WHR) (visceral fat), log sum of S (subcutaneous fat), and Fat-free mass (FFM)	Percent sites with attachment level ≥3 mm	Significant
Al-Zahrani et al. 2003	NHANES III *N* = 13665 18–90 years	BMI and waist circumference (WC)	Attachment level ≥3 mm and PD ≥4 mm	Significant
Buhlin et al. 2003	50 severe periodontitis and 46 healthy cases. 36–70 years	BMI	Seven or more sites with ≥6 mm of attachment level	Significant
Torrungruang et al. 2005	2,005 individuals, aged 50–73 years	BMI, and waist circumference	Mean attachment level	Non-significant
Alabdulkarim et al. 2005	*N* = 400 200 obese (BMI ≥30) 200 nonobese (BMI <25), ≥18 years	BMI	Radiographic alveolar bone score <60	Significant
Saito et al. 2005	584 Japanese women aged between 40 and 79 years old	BMI, waist-hip ratio, body fat	Upper 20th percentile of mean PD and mean attachment loss	Significant
Nishida et al. 2005	372 Japanese workers	BMI	Upper 20th percentile of percentage PD ≥3.5 mm	Significant
Linden et al. 2007	1,362 men 60–70-year-old	BMI	Percent sites with attachment loss ≥6 mm	Significant
Shimazaki et al. 2007	584 Japanese women 40–79 years	Waist circumference (WC)	Mean pocket depth Mean attachment loss	Significant
Sarlati et al. 2008	80 individuals (40 normal and 40 overweight and obese subjects) 18–34 years	BMI and waist circumference (WC)	PLI, PD and CAL	Significant
Ylöstalo et al. 2008	2841 dentate nondiabetic nonsmoking subjects aged 30–49 years	BMI	Number of teeth with periodontal pockets of 4 mm or deeper and 6 mm or deeper	Significant
Ekuni et al. 2008	618 students (18–24 years)	BMI and body fat	Community periodontal index	Significant
Khader et al. 2009	340 persons (18–70 years)	BMI, weight, height, hip (HC) and waist circumferences (WC)	PLI, GI, PD and CAL	Significant
D'Aiuto et al. 2008	13,994 persons (aged 17 year or older)	Central obesity (waist circumference)	Probing depth (PD), bleeding index	Significant

Table 4.10 Leptin = a pro-inflammatory cytokine (Otero et al. 2006)

Structurally, leptin belongs to the type I cytokine superfamily
Leptin receptor (Ob-R) belongs to the class I cytokine receptor family
Leptin expression is regulated by pro-inflammatory mediators
Circulating leptin levels are increased in both acute and chronic inflammation
Leptin modulates TH1/TH2 balance, regulating cytokines expression pattern
In synergy with other cytokines, leptin induces NOS type II activation in chondrocytes

examination. Further investigations are required to clarify the relationship between metabolic syndrome and periodontal disease, and to determine whether oral health care in individuals exhibiting metabolic syndrome has the potential to reduce the incidence of various systemic diseases (Shimazaki et al. 2007).

4.5 Periodontal Disease and Diabetes Mellitus: A Two-Way Relationship

Diabetes mellitus (DM) is a chronic systemic disease characterized by disorders in insulin production, metabolism of carbohydrate, fat and protein, and the structure and function of blood vessels. The current classification of diabetes is based upon the pathophysiology of each form of the disease. Type 1 diabetes results from cellular mediated autoimmune destruction of pancreatic b-cells, usually leading to total loss of insulin secretion. Markers of autoimmune destruction have been identified and can be used for diagnosis or risk assessment. Type 1 diabetes is usually present in children and adolescents, although some studies demonstrated 15–30% of all cases being diagnosed after 30 years of age. In older type 1 patients, the b-cell destruction occurs more slowly than in children, with a less abrupt onset of symptoms. This demonstrates that the pace and extent of cellular destruction can occur at a different rate from patient to patient. The lack of insulin production in patients with type 1 diabetes makes the use of exogenous insulin necessary to sustain life, hence the former name "insulin-dependent diabetes." In the absence of insulin, these patients develop ketoacidosis, a life-threatening condition (Mealey and Oates 2006).

Type 2 diabetes, previously called noninsulin-dependent diabetes, results from insulin resistance, which alters the use of endogenously produced insulin at the target cells. Type 2 patients have altered insulin production as well; however, autoimmune destruction of b-cells does not occur as it does in type 1, and patients retain the capacity for some insulin production. Because the type 2 patient still produces insulin, the incidence of ketoacidosis is very low compared to type 1; however, ketoacidosis can occur in association with the stress of another illness such as infection. Type 2 patients can be undiagnosed for many years because the hyperglycemia appears gradually and often without symptoms. In many patients, especially early in the disease process, pancreatic insulin production is actually increased to compensate for insulin resistance. As the condition progresses, pancreatic insulin production may diminish over time due to the prolonged increase in secretory demand caused by the insulin resistance. Insulin secretion becomes insufficient to compensate for insulin resistance. Although type 2 patients do not need insulin treatment to survive, insulin is often taken as part of the medical management of type 2 diabetes (Mealey and Oates 2006).

4.5.1 *Effects of Diabetes on the Periodontium*

Several comprehensive reviews have addressed the issue of the possible association between periodontal diseases and DM (Mealey and Oates 2006; Mealey and Ocampo 2007; Preshaw et al. 2007) (Fig. 4.2).

The meta-analysis conducted by Khader et al. (2006) included eight comparative cross-sectional studies, three prospective cohort studies and baseline data of two clinical trials that compared oral hygiene, gingival and periodontal status between diabetics and nondiabetics. This meta-analysis demonstrated that diabetics had poor oral hygiene as measured by the average of plaque index (PLI), higher severity of the gingival disease as measured by the average of gingival index (GI) and BPO score, and higher severity of periodontal disease as measured by the average of PD, clinical attachment loss and proximal bone loss index. On the other hand, this meta-analysis showed that both diabetics and nondiabetics had similar extent of oral hygiene, gingival and periodontal disease. One major limitation of this meta-analysis review was the inclusion of cross-sectional studies with different study populations and sizes, different diagnostic criteria,

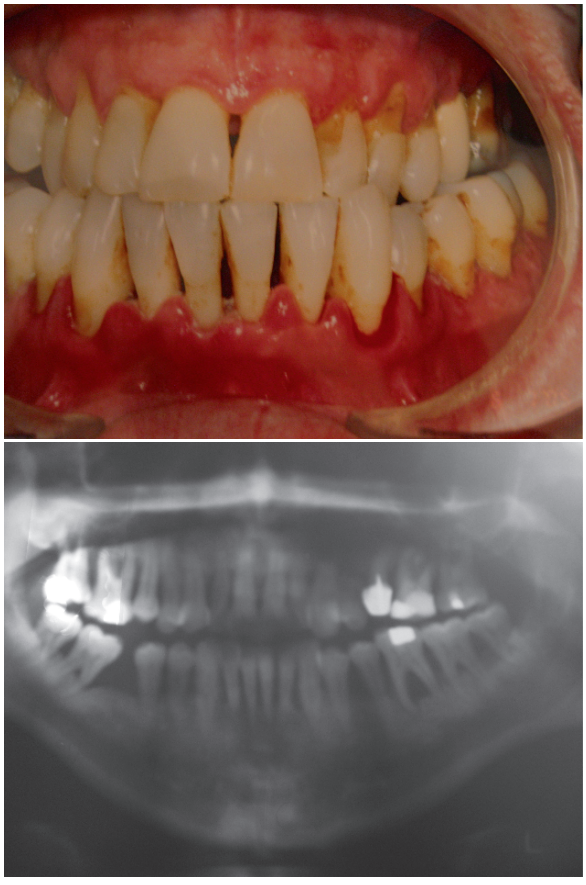

Fig. 4.2 Patient with insulin-dependent diabetes mellitus (DM)

various parameters to assess oral hygiene, gingival and periodontal status, different age groups, and different types of diabetes (Khader et al. 2006).

Specific risk indicators associated with either susceptibility or resistance to severe forms of periodontal disease were evaluated in a cross-section of 1,426 subjects, 25–74 years of age (Grossi et al. 1994). DM was the only systemic disease positively associated with attachment loss with an odds ratio of 2.32 (95% CI: 1.17–4.60).

4.5.1.1 Effects of Type of Diabetes

A number of reports on the relationship between diabetes and periodontal disease have included children and adolescents (Cianciola et al. 1982; Gusberti et al. 1983; Sastrowijoto et al. 1990; de Pommereau et al. 1992; Karjalainen and Knuuttila 1996). The consensus has been that in patients with childhood onset diabetes, periodontitis seems to ensue around puberty and to progress with age (Lalla et al. 2007a). It was suggested that periodontal destruction can start very early in life in diabetes, becomes more prominent as children become adolescents (Lalla et al. 2006a, b), and is related to the level of metabolic control (Lalla et al. 2007b).

The relationship between type 2 diabetes and periodontal disease is complicated by the fact that diabetes onset generally occurs after the age of 40 years, coinciding with the time point when periodontal disease becomes more prevalent (Salvi et al. 2008). The relationship between DM and oral health status was determined in Pima Indians from the Gila River Indian Community in Arizona. This tribe of native Americans has the world's highest reported incidence and prevalence of noninsulin-dependent (type 2) DM. It was reposted that subjects with type 2 diabetes have an increased risk of destructive periodontitis with an odds ratio of 2.81 (95% CI: 1.91–4.13) when attachment loss is used to measure the disease. The odds ratio for diabetic subjects was 3.43 (95% CI: 2.28–5.16) where bone loss was used to measure periodontal destruction (Emrich et al. 1991). Further evidence of type 2 diabetes as a risk factor for more severe periodontal disease has been reported (Tsai et al. 2002; Sandberg et al. 2000; Campus et al. 2005; Jansson et al. 2006; Salvi et al. 2008).

Taylor et al. (1998a) performed a 2-year period longitudinal study of the oral health of residents of the Gila River Indian Community. Change in bone score category was computed as the change in worst bone score reading after 2 years. The cumulative odds ratio for type 2 diabetes at each threshold of ABL of the ordered response was 4.23 (95% CI: 1.80–9.92).

4.5.1.2 Effect of Glycemic Control

As Salvi et al. (2008) reported, while some studies found no relationship between the level of glycaemic control and periodontal status (Hove and Stallard 1970; Barnett et al. 1984; Backley et al. 1988; Hayden and Buckley 1989; Sastrowijoto et al. 1989, 1990; Pinson et al. 1995), other studies indicated that the degree of glycaemic control may influence the severity of periodontal disease (Gislén et al. 1980; Ervasti et al. 1985; Unal et al. 1993; Firatli et al. 1994; Bridges et al. 1996; Taylor et al. 1996, 1998a, b; Tsai et al. 2002; Campus et al. 2005). Evidence comes from several longitudinal studies that revealed that poorly controlled diabetes was associated with

periodontitis (Salvi et al. 2008). Taylor et al. (1998a) analyzed data from a 2-year longitudinal study of the oral health of residents of the Gila River Indian Community. Poorly controlled type 2 diabetes was positively associated with greater risk for a change in bone score (compared to subjects without type 2 DM) when the covariates were included in the model: odds ratio = 11.4 (95% CI: 2.5–53.3). For subjects with better glycemic control, the odds ratio was 2.2 (95% CI: 0.7–6.5), when contrasted to those without type 2 diabetes. Tsai et al. (2002) investigated the association between glycemic control of type 2 DM and severe periodontal disease in 4,343 persons ages 45–90 years from the National Health and Nutrition Examination Study III. It was revealed that individuals with poorly controlled diabetes (glycosylated hemoglobin >9%) had a significantly higher prevalence of severe periodontitis than those without diabetes (OR = 2.90; 95% CI: 1.40–6.03), after controlling for age, education, smoking status and calculus. For the better-controlled diabetes (glycosylated hemoglobin ≤9%), there was only a slight tendency for a higher prevalence of severe periodontitis (OR = 1.56; 95% CI: 0.90–2.68) compared with a nondiabetic status (Table 4.11).

4.5.1.3 Effects of Diabetes Onset and Duration

Controversial results were reported on the effect of diabetes duration on periodontal conditions (Salvi et al. 2008). While outcomes from some studies did not yield any correlation between diabetes duration and periodontal status (Hove and Stallard 1970; Nichols et al. 1978; Barnett et al. 1984; Backley et al. 1988; Rosenthal et al. 1988), other outcomes showed that diabetes duration was of critical importance (Glavind et al. 1968; Cianciola et al. 1982; Hugoson et al. 1989; Löe 1993; Firatli 1997; Firatli et al. 1996; Salvi et al. 2008).

4.5.2 Mechanisms by Which Diabetes May Influence the Periodontium

In subjects with diabetes, chronically elevated blood glucose levels lead to the accelerated formation of advanced glycation end products (AGEs). Endothelial cells and monocytes possess specific receptors for AGEs (i.e., RAGEs) located on their cell surfaces. There is strong indication that the interaction of AGEs with their receptors plays an important role in the development of diabetic complications. The interaction of macrophages with AGEs has been shown to stimulate increased secretions of pro-inflammatory mediators such as tumour necrosis factor a (TNF-α) and interleukin-1 (IL-1). In subjects with type 2 diabetes, deterioration of periodontal status was associated with elevated serum levels of AGEs (Salvi et al. 2008). Also included in the discussion was the possible role of adipokines in periodontal disease and type 2 DM because these mediators may reflect common pathogenic processes relating to imunoregulatory changes (Preshaw et al. 2007; Lalla et al. 2001). Other mechanisms by which diabetes may influence the periodontium includes: vascular abnormalities, nonenzymatic glycosylation, imbalances in lipid metabolism, altered collagen metabolism, neutrofil dysfunction and altered monocytic response (Mealey and Oates 2006; Ryan et al. 2003) (Fig. 4.3).

4.5.3 Effects of Periodontal Status on Diabetic Control

If subjects with diabetes suffer from more severe periodontal disease compared with subjects without diabetes, one would also expect that subjects suffering from periodontal disease would yield a higher prevalence of diabetes compared with periodontally healthy subjects (Salvi et al. 2008). Unfortunately, knowledge on how periodontitis may influence glycaemic control is limited. Outcomes from longitudinal studies showed that severe periodontitis at baseline was associated with poor glycaemic control and diabetes-related complications at follow-up (e.g., overt nephropathy and end-stage renal disease, CVD and associated mortality) (Taylor et al. 1996; Thorstensson et al. 1996; Saremi et al. 2005; Shultis et al. 2007).

Periodontal diseases may induce or perpetuate an elevated systemic chronic inflammatory state. Acute bacterial and viral infections are known to increase insulin resistance in people without diabetes, a condition, which often persists for weeks to months after clinical recovery from the illness. Such illnesses and resultant increases in insulin resistance in people with diabetes

Table 4.11 Summary of studies with information on effects of glycemic control on periodontal status

Author, year	Type of study	Subjects, n	Diabetes type	Periodontitis evaluation	Findings
Baci et al. 1988	Case-control	222 diabetic patients (mean age, 46.9 years) and 189 control subjects (mean age, 43.9 years).	Types 1 and 2	Remaining number of teeth, CPITN index	Diabetic patients demonstrated significantly more missing teeth ($P < 0.001$). The mean number of missing sextants was also significantly higher in diabetics. Pathologic pockets of 6 mm or more were found in 1.3 and 0.3 sextants in the diabetic and control group subjects, respectively ($P < 0.001$). Up to the age of 34, no differences were observed between the diabetic and control group subjects regarding pathologic pockets of 6 mm or more. Above this age, diabetics demonstrated significantly more sextants with deep pockets ($P < 0.001$). Concerning the type of diabetes, no differences related to CPITN score were found between insulin dependent and noninsulin dependent diabetics. Neither were any differences found in the periodontal condition related to the duration and control of diabetes, whereas diabetics with advanced retinopathy demonstrated more sextants with deep pockets
Bridges et al. 1996	Case-control	118 diabetic men and 115 age-matched nondiabetic men	Type 2	Plaque and gingival indices, bleeding scores, PD, loss of attachment, and number of missing teeth	These parameters were significantly higher in diabetic than nondiabetic men: PLI ($P < 0.0001$), GI ($P < 0.0002$), bleeding score ($P < 0.0001$), PD ($P = 0.005$), loss of attachment, $P < 0.0001$; and missing teeth, $P < 0.005$
Campus et al. 2005	Case-control	71 diabetic patients (61.0 ± 11.0 years) and 141 nondiabetic controls in good general health (59.1 ± 9.2 years)	Type 2	Probing depth, attachment level, presence of calculus, BPO, assessment of plaque, *Porphyromonas gingivalis, Prevotella intermedia* and *Tannerella forsythensis* in subgingival plaque samples	Type 2 diabetic patients showed a significantly lower number of teeth present, a significant increase in number of PDs >4 mm, and percent of pocket depths >4 mm, periodontitis, BPO and PLI ($P = 0.01$)
Gislén et al. 1980	Case-control	43 diabetic children and 43 age- and sex-matched healthy controls	Type 1	GI, PLI	The diabetic children with poor metabolic control showed a clear tendency toward higher GI scores than the nondiabetics, while no such tendency was seen between the diabetics with good metabolic control and the nondiabetics
Hayden and Buckley 1989	Cross-sectional	157 insulin dependent DM patients aged 8–78 years	Types 1 and 2	PLI, gingivitis index, periodontal pocket depth and periodontal attachment loss	It was found that none of the diabetic measurements showed any consistent pattern in relation to any of the periodontal measurements

Pinson et al. 1995	Case-control	26 diabetic patients (mean age 13.42 years) and 24 controls	Type 1	PLI, gingival fluid flow, GI, PDs, CALs, recession, and BPO	Analysis of the data demonstrated no statistically significant differences in the overall means for the two groups for average attachment loss, PDs, recession, GI, PLI, gingival fluid flow, or BPO
Sastrowijoto et al. 1989	Case-control	22 type 1 diabetic adults were grouped into patients with *near normal* ($HbAt_{1c}$ ≤7.7%) and *Poor* (HbA_{tc} ≥9.9%) metabolic control	Type 1	Periodontal pocket depth, periodontal attachment loss, bleeding after probing, PLI, *Actinobacillus actinomycetemcomitans*, black-pigmented *Bacteroides* species and *Capnocytophaga* species in subgingival sites	Metabolic control seems to have no direct effect on the periodontium
Sastrowijoto et al. 1990	Longitudinal (4 months)	Six patients	Type 1	Probing depth, probing attachment level, BPO and the PLI	Improved metabolic control in IDDM patients may have no potential impetus for an improved clinical periodontal condition nor on the subgingival bacterial flora
Taylor et al. 1996	longitudinal (2 years)	80 subjects	Type 2	Periodontal attachment loss, radiographic bone loss	Severe periodontitis at baseline was associated with increased risk of poor glycemic control at follow-up
Taylor et al. 1998a	Longitudinal (2 years)	362 subjects (15–57 years)	Type 2	Radiographic bone loss from panoramic radiographs	The study suggests an noninsulin dependent DM-associated increased rate of ABL progression
Tsai et al. 2002	Cross-sectional	4343 persons ages 45–90 years from the national health and nutrition examination study III	Type 2	Probing depth, probing attachment level, the number of teeth (continuous), calculus extent and gingival bleeding	Individuals with poorly controlled diabetes had a significantly higher prevalence of severe periodontitis than those without diabetes (OR = 2.90; 95% CI: 1.40–6.03), after controlling for age, education, smoking status, and calculus

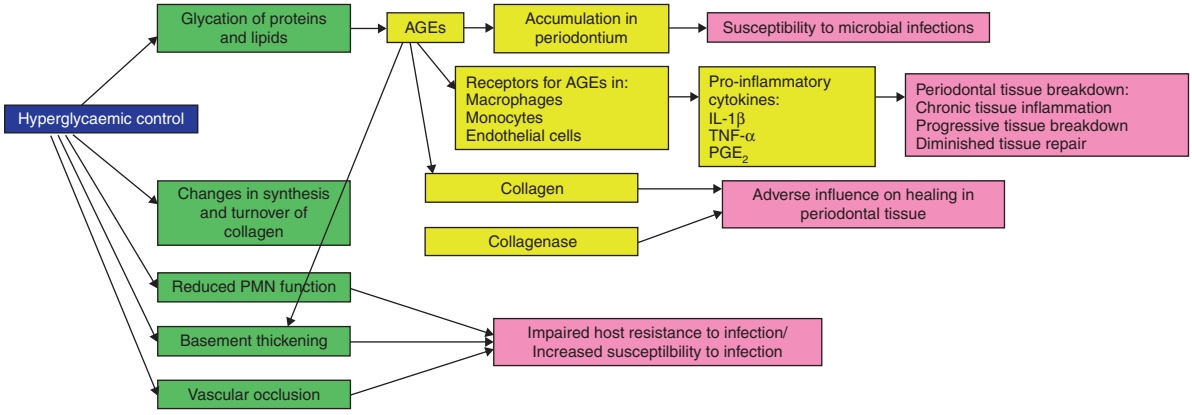

Fig. 4.3 Mechanisms by which diabetes may influence the periodontium. *AGEs* advanced glycation end products; *IL-1* interleukin 1; *TNF* tumour necrosis factor; *PGE*$_2$ prostaglandin E$_2$; *DM* diabetes mellitus (Kuo et al. 2008)

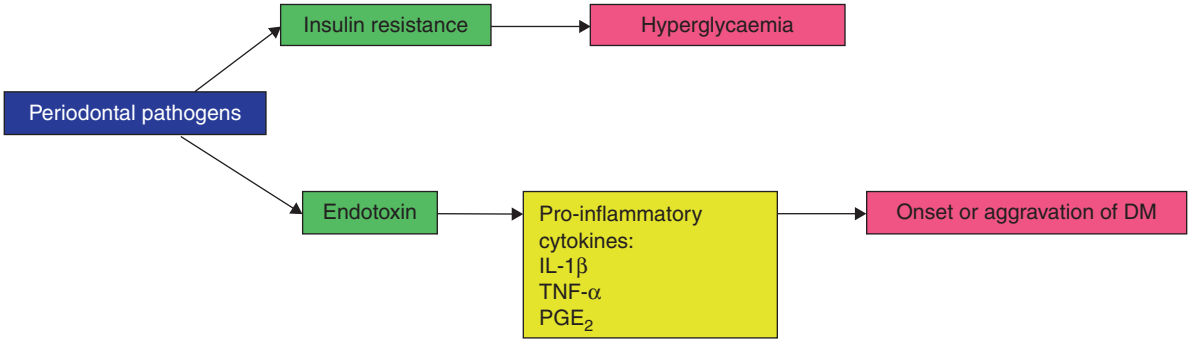

Fig. 4.4 Mechanisms by which periodontitis may influence DM. *IL-1* interleukin 1; *TNF* tumour necrosis factor; *PGE*$_2$ prostaglandin E$_2$; *DM* diabetes mellitus (Kuo et al. 2008)

greatly aggravate glycemic control. Chronic Gram-negative periodontal infections may also result in increased insulin resistance and poor glycemic control (Mealey and Oates 2006). A LPS produced by *P. gingivalis* is a potent inducer of IL-1β, TNF-α and PGE$_2$. TNF-α has been suggested as the mediator of insulin resistance in infection, thus impairing insulin action. (Kuo et al. 2008). Treatment that reduces periodontal inflammation may restore insulin sensitivity, resulting in improved metabolic control (Mealey and Oates 2006) (Fig. 4.4).

4.5.3.1 The Effects of Periodontal Treatment on Diabetes

Several studies have evaluated the effect of periodontal therapy on glycemic control in subjects with diabetes, and the most recent ones (after 2000) are presented in Table 4.12. A meta-analysis was performed by Janket et al. (2005) on a total of 456 patients, with periodontal treatment as predictor and the actual change in hemoglobin A1c level as the outcome. The weighted average decrease in actual HbA1c level was 0.38% for all studies, 0.66% when restricted to type 2 diabetic patients and 0.71% if antibiotics were given to them. However, none was statistically significant. More recently, Darré et al. (2008) performed a review on twenty-five studies, involving 976 subjects altogether. Of these, nine studies, involving a total of 485 patients, were controlled trials and were included in the meta-analysis. The standardized mean difference in HbA_{1c} with the treatment of periodontal disease was 0.46 (95% CI: 0.11–0.82). It was suggested that periodontal treatment could lead to a significant 0.79% (95% CI: 0.19–1.40) reduction in HbA_{1c} level. However, the authors revealed

Table 4.12 Studies on the relationship between effects of periodontal therapy on glycemic control

Author, year	Subjects	Diabetes type	Length of the study	Periodontitis evaluation	Smoking status	Intervention	Improvement of glycemic control in the diabetes patients HbA1C changes
Stewart et al. 2001	72	2	18 months	Not given	Smokers and nonsmokers	SRP + extractions vs. no treatment	Controls: from 9.5 to 7.6 (17.1%) Cases: from 8.5 to 7.7 (6.7%)
Iwamoto et al. 2001	13	2	8 weeks	PD, CPITN, subgingival bacterial sampling, circulating TNFα	Nonsmokers	SRP + local minocycline	Reduced from 7.96 ± 1.9 to 7.12 ± 1.5
Rodrigues et al. 2003	30	2	3 months	Not known	Nonsmokers	SRP + amoxicillin/clavulanic acid vs. SRP	Group 1: change from 9.5 ± 2.4 to 9.2 ± 1.6 Group 2: change from 8.8 ± 1.8 to 7.6 ± 1.4
Al-Mubarak et al. 2002	52	1 and 2	3 months	PI, MGI, PD, R, CAL, BOP, bone loss, mobility	Not known	SRP + subgingival water irrigation	Test: from 8.060 ± 0.29 to 7.7 ± 0.36 Control: from 8.5 ± 0.31 to 8.3 ± 0.36
Rocha et al. 2001	40	2	6 months	PD, R, CAL, BOP, bone loss, mobility	Nonsmokers	SRP + alendronate vs. SRP + placebo	Test: from 11.9 ± 3.2 to 9.4 ± 1.5 Control: from 13.1 ± 2.9 to 10.8 ± 2.4
Skaleric et al. 2004	20	1	6 months	Defined by the presence of four teeth with 5 mm PD, two of which had 6–9 mm PD and BOP	Not known	SRP + minocycline microspheres (Arestin)	Test: reduction of 0.61 ± 0.93 Control: Reduction of 0.96 ± 1.27
Kiran et al. 2005	44	2	3 months	PI, GI, PD, CAL, R, BOP	Smokers and nonsmokers	SRP vs. no treatment	Test: from 7.31 ± 0.74–6.51 ± 0.80 Control: from 7.00 ± 0.72–7.31 ± 2.08
Promsudthi et al. 2005	52	2	3 months	PI, PD, CAL, R, BOP	Nonsmokers	SRP + systemic doxycycline vs. no treatment	Test: changes −0.19 ± 0.74 Control: changes 0.12 ± 1.05
Faria-Almeida et al. 2006	20	2	6 months	PI, PD, CAL, R, BOP	Nonsmokers	SRP	From 7.6 ± 1.5 to 5.8 ± 0.6
Navarro-Sanchez et al. 2007	20 (ten diabetic and ten nondiabetic)	2	6 months	PI, PD, CAL, R, BOP	Smokers and nonsmokers	SRP	Significant decrease in the mean HbA1C level in the diabetic group from 7.2 ± 1.3% at the initial visit to 6.5 ± 1.1% at 3 months and 5.9 ± 0.6% at 6 months

(*continued*)

Table 4.12 (continued)

Author, year	Subjects	Diabetes type	Length of the study	Periodontitis evaluation	Smoking status	Intervention	Improvement of glycemic control in the diabetes patients HbA1C changes
Jones et al. 2007	165	2	4 months	CPITN, GI, R, PD, presence of exudate on palpation	Smokers and nonsmokers	SRP + doxycycline [100 mg by mouth daily for 14 days] + CHX vs. no treatment	Test: −0.63% Control: −0.51%
Gonçalves et al. 2008	40	2	3 months	PI, GI, PD, CAL, BOP and presence of suppuration	Nonsmokers	SRP	−1.71 (−12.18/5.63)
Llambés et al. 2008	60	1	3 months	PI, GI, PD, CAL, BOP	Smokers and nonsmokers	SRP + administration of doxycycline vs. SRP	Changes were 0.07% in group 1 and −0.06% in group 2
Madden et al. 2008	42	2	8 months	PI, GI, PD, CAL, BOP	Nonsmokers	SRP vs. SRP + CHX	Overall mean reduction in HbA1c of 27 subjects with baseline HbA1c >9.0 was 0.6
Da et al. 2009	45	2	3 months	PI, GI, PD, CAL, BOP	Nonsmokers	SRP	Poorly controlled diabetics: from 9.96 ± 1.45 to 9.77 ± 1.15 Well-controlled diabetics: from 6.26 ± 0.72 to 6.05 ± 0.77 Nondiabetic patients: from 5.26 ± 0.40 to 5.24 ± 0.27
Tervonen et al. 2009	65	1	8 weeks	PI, GI, PD, CAL, BOP	Smokers and nonsmokers	SRP	The mean HbA1c level of the whole study group was 8.6% ± 1.5) at the baseline and 8.5% ± 1.5) after treatment

PI plaque index; *GI* gingival index; *PD* probing depth; *CAL* clinical attachment levels; *R* gingival recession; *BOP* bleeding on probing; *CHX* chlorhexidine gluconate rinses; *MGI* modified gingival index

that these results need to be viewed with caution because of a lack of robustness and deficiencies in the design of some of the studies included.

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5 Interrelationships Between Periodontal Disease and Adverse Pregnancy Outcomes, Respiratory Disease, Rheumatoid Arthritis, Renal Disease, Cancer, Inflammatory Bowel Disease, Alzheimer Disease; Assessing Confounding and Effect Modification

Alexandrina L. Dumitrescu and Koji Inagaki

Periodontal medicine defines a rapidly emerging branch of periodontology, focusing on the wealth of new data, and establishing a strong relationship between periodontal health or disease and systemic health or disease. There is increasing evidence that individuals with periodontal disease may be at increased risk for adverse medical outcomes: adverse pregnancy outcomes, respiratory disease, rheumatoid arthritis, renal disease, cancer, inflammatory bowel disease, and Alzheimer disease. The role of confounding and effect modification is discussed in the association between periodontal and systemic diseases.

5.1 Associations Between Periodontal Disease and Respiratory Diseases

Respiratory diseases are responsible for significant morbidity and mortality in human populations. These diseases are widely prevalent and exact an extensive toll on human health and the cost of health care (Scannapieco 1999).

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5.1.1 Studies on the Relationship Between Periodontitis and Bacterial Pneumonia

Pneumonia is one of the commonest serious infections, causing significant morbidity and mortality both in healthy and debilitated subjects (Brown 2007). In pneumonia, the lung alveoli are filled with edema fluid, accompanied by inflammation in the lung parenchyma. Bacteria may enter the lungs by inhalation or through aspiration of oropharyngeal fluid. The latter is thought to be the most common route for bacterial pulmonary infections. Aspiration pneumonia is prevalent in community-acquired pneumonia, as well as in hospital-acquired pneumonia. Pneumonia may be classified as lobar (consolidation of lung tissue limited to one lobe or segment), broncho-pneumonia (consolidation scattered throughout the lung but concentrated mainly at the bases), or atypical (patchy consolidation) (McChlery et al. 2009).

Common clinical features of pneumonia include fevers, rigors, malaise, shortness of breath, cyanosis, and productive cough and lung consolidation. Most cases (about 90%) of pneumonia are acquired in the community, and less commonly in hospitals, although ventilator-associated pneumonia is an important problem. A wide range of microorganisms may be implicated. In otherwise healthy individuals, most pneumonia is caused by *Streptococcus pneumoniae*, viruses or *Mycoplasma pneumoniae*. Patients with underlying disease may develop community-acquired pneumonia from a wider range of organisms, including

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Staphylococcus aureus, *Haemophilus influenzae*, *Klebsiella pneumoniae*, or *Pneumocystis jiroveci*. Hospital-acquired pneumonias are commonly associated with aerobic or facultative Gram-negative bacilli, or with *S. aureus*, and can be transmitted via the contaminated hands of healthcare workers, or through instruments and equipment (McChlery et al. 2009).

Several studies have evaluated the association of oral health with pneumonia (Table 5.1). Overall, the potential risk factors for pneumonia were identified as the presence of cariogenic and periodontal pathogens in saliva and dental plaque [OR (odds ratios) = 4–9.6] and dental decay (OR = 1.2). Higher plaque scores were also shown to be associated with a previous history of respiratory tract infection (RTI) (Azarpazhooh and Leake 2006).

5.1.2 Studies on the Relationship Between Periodontitis and Chronic Obstructive Pulmonary Disease (COPD)

COPD is a major cause of chronic morbidity and mortality throughout the world. COPD is currently the fourth leading cause of death in the world, and further increase in the prevalence and mortality of the disease can be predicted in the coming decades (Pauwels et al. 2001).

COPD is a preventable and treatable disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and is associated with an abnormal inflammatory response of the lungs to noxious particles or gases, primarily caused by cigarette smoking. Although COPD affects the lungs, it also produces significant systemic consequences. The diagnosis of COPD should be considered in any patient who has the following: symptoms of cough; sputum production; dyspnoea; history of exposure to risk factors for the disease (Celli and MacNee 2004).

Because COPD often develops in longtime smokers during middle age, patients often have a variety of other diseases related to either smoking or aging. COPD itself also has significant extrapulmonary (systemic) effects that lead to comorbid conditions. Thus, COPD should be managed with careful attention also paid to comorbidities and their effect on the patient's quality of life. A careful differential diagnosis and comprehensive assessment of severity of comorbid conditions should be performed in every patient with chronic airflow limitation (Rabe et al. 2007).

A recent systematic review by Azarpazhooh and Leake (2006) identified four studies concerning association between oral health and COPD (Hayes et al. 1998; Scannapieco et al. 1998, 2003; Russell et al. 1999). The authors found a weak association [OR/relative risk (RR) < 2.0] between COPD and oral health measures. Recently, Leuckfeld et al. (2008) investigated whether chronic marginal periodontitis is more prevalent in very severe COPD than in other very severe respiratory diseases, and whether periodontitis in COPD is related to risk factors for periodontitis that are often present in COPD subjects. 130 patients with COPD and 50 patients with non-COPD were radiographically evaluated. Chronic marginal periodontitis was defined as a general marginal bone level >4 mm. The prevalence of periodontitis was 44% in the COPD group vs. 7.3% in the non-COPD group. The difference in mean marginal bone level remained statistically significant when adjusting for age, gender, and pack-years smoked. In logistic regression analysis, mean marginal bone level >4 mm was identified as a factor significantly associated with severe COPD (Leuckfeld et al. 2008).

5.1.3 Intervention Studies on the Role of Improved Oral Cleaning in Reducing Pneumonia

Ten studies were retained that examined the evidence that interventions aiming to improve oral health reduced the progression or occurrence of pneumonia (Table 5.2) (Scannapieco et al. 2003; Azarpazhooh and Leake 2006). Recently, Chan et al. (2007) carried out a systematic review and meta-analysis to estimate the effect of oral decontamination using topical antibiotics or antiseptics on ventilator associated pneumonia and mortality in mechanically ventilated adults. Eleven trials totaling 3,242 patients met the inclusion criteria. Among four trials with 1,098 patients, oral application of antibiotics did not significantly reduce the incidence of ventilator-associated pneumonia (RR = 0.69, 95% CI: 0.41–1.18). In seven trials with 2,144 patients,

Table 5.1 Studies on the relationship between pneumonia and periodontal disease

Author, yearear	Subjects, n	Criteria for pneumonia	Oral evaluation	Association
Mojon et al. (1997)	302 frail elders (mean age: 85 years); no controls	History of respiratory tract infection (RTI)	Evaluation of hygiene, quality of prostheses and the prevalence of caries, periodontal disease (CPITN) and mucosal disorders	The incidence of RTI had been greater among dentate subjects (40%) than in edentulous patients (27%), RR = 1.7 (95% CI: 1.1–2.8) The dentate subjects with a history of RTI had higher plaque score (2.9 vs. 2.3; $P = 0.02$) Half (49%) of the subjects had oral disorders that could develop in a dental emergency, and these subjects had had a higher risk of RTI (RR = 1.9, 95% CI: 1.1–3.9) The presence of selected oral disorders associated with low serum albumin increased the RR of having had RTI to 3.2 (1.5–6.7) The association between presence of actual oral health problems and previous experience of RTI was more noticeable in those who had poor general health or were more debilitated
Langmore et al. (1998)	41 subjects with aspiration pneumonia, 148 controls; males aged older than 60 years	Pneumonia diagnosis	Oral/dental status, plaque index; plaque BANA score; periodontal disease score; number of missing teeth; number of sound teeth; number of restored teeth; number of decayed teeth; having full/partial dentures; having full dentures-upper/lower; wearing dentures only when eating; frequency of brushing teeth	The best predictors of aspiration pneumonia were dependent for feeding, dependent for oral care (OR = 2.83, $P = 0.03$), number of decayed teeth, tube feeding, more than one medical diagnosis, number of medications, and smoking
Fourrier et al. (1998)	47 consecutive patients admitted during a 3-months period, mean age 49 ± 18 years	Temperature > 38°C; presence of infiltrates on chest radiographs; leukocytosis (>10 × 10^3/mm^3) or leukopenia (<3 × 10^3/mm^3); positive culture from tracheal aspirate; and positive culture from BAL. Tracheal aspirate cultures were considered positive when ≥10^6 cfu/mL	Decayed-missing-filled teeth index, amount of dental plaque on premolars, quantitative cultures of dental plaque	Dental plaque colonization on days 0 and 5 was significantly associated with the occurrence of nosocomial pneumonia and bacteremia (sensitivity 0.77; specificity 0.96; positive predictive value 0.87; negative predictive value 0.91; RR = 9.6). In six cases of nosocomial infection, the pathogen isolated from dental plaque was the first identified source of nosocomial infection

(*continued*)

Table 5.1 (continued)

Author, yearear	Subjects, n	Criteria for pneumonia	Oral evaluation	Association
Terpenning et al. (2001)	50 subjects with aspiration pneumonia, 308 controls; males aged 55 years and older	Demographic and medical data obtained retrospectively from last 8 years	Comprehensive dental examination; salivary assays including IgA antibodies; and cultures of saliva, throat, and dental plaques	In the dentate subjects, the presence of *P. gingivalis* in dental plaque (OR = 2.7; 95% CI: 1.3–5.3) and of *Streptococcus sobrinus* (OR = 2.3; 95% CI: 0.9–5.9) and *S. aureus* in the saliva (OR = 4.3; 95% CI: 2.0–9.3) was significantly higher in subjects with aspiration pneumonia. Logistic regression model using dentate patients provided estimates for several medical, dental, and bacteriological parameters that were significantly associated with the subsequent diagnosis of aspiration pneumonia: number of decayed teeth (OR 1.2; 95% CI: 1.1–1.4, for each additional decayed tooth) and number of functional (dental) units (OR = 1.2; 95% CI: 1.02–1.4). The presence of *S. aureus* (OR = 7.4; 95% CI: 1.8–30.5) and of *S. sobrinus* (OR = 6.2; 95% CI: 1.4–27.5) in the saliva, and the presence of the periodontopathogen *P. gingivalis* in dental plaque (OR = 4.2; 95% CI: 1.6–11.3). In dentate and edentulous subjects the presence of *S. aureus i*n saliva (OR = 8.3)
El-Solh et al. (2004)	49 critically ill residents of long-term care facilities requiring intensive care treatment: 35 with hospital-acquired pneumonia; (HAP) 14 non-HAP patients; Mean age = 78–80 years	Development of new radiographic infiltrate compatible with pneumonia and the presence of two or more of the following criteria: (1) purulent endotracheal aspirates; (2) temperature of > 38°C or < 35.5°C; or (3) a WBC count of > 12,000 cells/μL, and/or left shift or leukopenia of <3,000 cells/μL. Protected BAL (PBAL) was performed via flexible bronchoscopy in all participants who had a clinical suspicion for pneumonia	Oral examination included the plaque index scores, number of remaining teeth, supragingival plaques culture	28 subjects (57%) had colonization of their DPs with aerobic pathogens *S. aureus* (45%) accounted for the majority of the isolates, followed by enteric Gram-negative bacilli (42%) and *Pseudomonas aeruginosa* (13%) Of the 13 isolates recovered from PBAL fluid, nine respiratory pathogens matched genetically those recovered from the corresponding dental plaques of eight patients

CI confidence intervals; *CPITN* community periodontal index for treatment needs; *OR* odds ratio; *RR* relative risk ICU intensive care unit

Table 5.2 Studies on the relationship between chronic obstructive pulmonary disease and periodontal disease [adapted from Shay et al. (2005)] (with permission from Wiley)

Author, year	Subjects, n	Oral intervention	Conclusion
Pugin et al. (1991)	45 subjects in intensive care unit	Polymyxin B sulfate, neomycin sulfate, and vancomycin hydrochloride	Topical oropharyngeal antibiotic application lowered the rate of ventilator-associated pneumonia by a factor of 5, probably by interrupting the stomach-to-trachea route of infection, and decreased the requirement for intravenous antibiotics
Adachi et al. (2002)	141 elderly persons, mean age was 84 years, and 73.6% of the subjects were women	Professional oral health care (POHC) given by dental hygienists once a week for 24 months	The prevalence of fevers of 37.8°C or more in the subjects receiving POHC was significantly lower than in the non-POHC group. The ratio of fatal aspiration pneumonia in the POHC group during the 24 months was significantly lower than in the non-POHC group. Numbers of *Candida albicans sp*. in samples obtained from the oral cavity after 6 months of POHC were significantly lower than those in the non-POHC group. POHC resulted in the reduction of the presence of *Staphylococcus* but not to a statistically significant extent. The amounts of methylmercaptan exhaled by the POHC group were significantly less than those of the non-POHC group
Houston et al. (2002)	561 patients undergoing aortocoronary bypass or valve surgery requiring cardiopulmonary bypass	0.12% Chlorhexidine gluconate (CHX) oral rinse	Only patients intubated for more than 24 h had pneumonia develop (0/486 vs. 13/75; $P = 0.01$)
Bergmans et al. (2001)	Test group: 87 patients, placebo group: 78 patients, control group: 61 patients	Topical antimicrobial prophylaxis (gentamicin/colistin/vancomycin 2% in Orabase, every 6 h) in the oropharynx	Topical prophylaxis prevented acquired oropharyngeal colonization (10 vs. 59% in placebo and 63% in control ($P < 0.00001$). Incidences of VAP were 10% in study patients, 31% in placebo, and 23% in control patients ($P < 0.001$)
DeRiso et al. (1996)	353 consecutive patients undergoing coronary artery bypass grafting, valve, or other open heart surgical procedures were randomized to an experimental ($n = 173$) or control ($n = 180$) group	0.12% CHX oral rinse	The overall nosocomial infection rate was decreased in the CHX-treated patients by 65% (24/180 vs. 8/173; $P < 0.01$). It was noted a 69% reduction in the incidence of total respiratory tract infections (RTIs) in the CHX-treated group (17/180 vs. 5/173; $P < 0.05$). Gram-negative organisms were involved in significantly less ($P < 0.05$) of the nosocomial infections and total RTIs by 59 and 67%, respectively. The use of nonprophylactic IV antibiotics was lowered by 43% (42/180 vs. 23/173; $P < 0.05$). A reduction in mortality in the CHX-treated group was also noted (1.16 vs. 5.56%)
Fourrier et al. (2000)	60 patients were included; 30 in the treated group and 30 in the control one (mean age: 51 ± 16 years)	Dental plaque decontamination with 0.2% chlorhexidine gel, three times a day (5 days)	Compared with the control group, the nosocomial infection rate and the incidence densities related to risk exposition were significantly lower in the treated group (18 vs. 33% days in the ICU and 10.7 vs. 32.3% days of mechanical ventilation; $P < 0.05$). These results were consistent with a significant preventive effect of the antiseptic decontamination (OR = 0.27; 95% CI: 0.09–0.80) with a 53% RR reduction

(*continued*)

Table 5.2 (continued)

Author, year	Subjects, n	Oral intervention	Conclusion
Genuit et al. (2001)	Surgical ICU patients requiring mechanical ventilation. 10 months study	Chlorhexidine 0.12% oral rinse administered twice daily	The addition of chlorhexidine to the ventilator weaning protocol led to a significant reduction and delay in the occurrence of ventilator-associated pneumonia (37% overall, 75% for late ventilator-associated pneumonia, $P < 0.05$).
Yoneyama et al. (2002)	407 patients randomly assigned to an oral care group or a no oral care group	Nurses or caregivers cleaned the patients' teeth by toothbrush after each meal. Swabbing with povidone iodine was additionally used in some cases. Dentists or dental hygienists provided professional care once a week	During follow-up, pneumonia, febrile days, and death from pneumonia decreased significantly in patients with oral care. Oral care was beneficial in edentate and dentate patients. Activities of daily living and cognitive functions showed a tendency to improve with oral care
Fourrier et al. (2005)	228 nonedentulous patients requiring endotracheal intubation and mechanical ventilation, with an anticipated length of stay ≥5 days	Antiseptic decontamination of gingival and dental plaque with a 0.2% chlorhexidine gel three times a day	The incidence of nosocomial infections was 17.5% (13.2/ 1000 ICU days) in the placebo group and 18.4% (13.3/1000 ICU days) in the plaque antiseptic decontamination group. On day 10, the number of positive dental plaque cultures was significantly lower in the treated group (29 vs. 66%; $P < 0.05$)
Ishikawa et al. (2008)	202 participants from three nursing homes for the dependent elderly, mean age 79 to 83 years old	Professional cleaning of the oral cavity and/or the gargling of a disinfectant liquid solution was performed over a 5-month period	These findings indicate that weekly professional mechanical cleaning of the oral cavity, rather than a daily chemical disinfection of the mouth, can be an important strategy to prevent aspiration pneumonia in the dependent elderly

CI confidence intervals; *OR* odds ratio; *RR* relative risk POHC Professional oral health care

however, oral application of antiseptics significantly reduced the incidence of ventilator-associated pneumonia (0.56, 95% CI: 0.39–0.81). When the results of these 11 trials were pooled, rates of ventilator-associated pneumonia were lower among patients receiving either method of oral decontamination (0.61, 95% CI: 0.45–0.82). Mortality was not influenced by prophylaxis with either antibiotics (0.94, 95% CI: 0.73–1.21) or antiseptics (0.96, 95% CI: 0.69–1.33), nor was the duration of mechanical ventilation or stay in the intensive care unit (Chan et al. 2007). This systematic review suggested that in mechanically ventilated patients, antiseptic oral decontamination prophylaxis reduces the incidence of ventilator-associated pneumonia. However, the authors suggested that more evidence is needed before firm conclusions can be made on the effect of antibiotic oral decontamination. These results should be interpreted in light of the moderate heterogeneity of trial results and possible publication bias. Neither of these two approaches to decontamination seem to affect mortality, duration of mechanical ventilation, or stay in the intensive care unit, although these trials are underpowered for these latter outcomes, and the summary of trials to date does not yet represent the optimum information size. Therefore, more evidence is needed before firm conclusions can be made on the full effect of oral decontamination using antiseptics and, particularly, antibiotics (Chan et al. 2007).

5.1.4 Relationship Between Periodontal Disease and Respiratory Disease

Scannapieco (1999) and Azarpazhooh and Leake (2006) describe four possible mechanisms of the presence of oral bacteria in the pathogenesis of respiratory infections:

1. Aspiration of oral pathogens (such as *Porphyromonas gingivalis*, *Aggregatibacter actynomycetemcomitans*, etc.) into the lung.
2. Second, periodontal disease-associated enzymes may facilitate the adherence of respiratory pathogens to the airways. Among the possibilities of this adherence are the following: (1) mucosal epithelium alteration by elevated levels of proteolytic bacteria of periodontal disease and their specific enzymes, such as mannosidase, fucosidase, hexosaminidase, and sialidase; (2) the loss of surface fibronectin, protein, which coats oral mucosa and masks mucosal surface receptors; (3) the removal of surface fibronectin by hydrolytic enzymes such as salivary fibronectin; and (4) the release of cytokines.
3. Third, hydrolytic enzymes of periodontal disease associated pathogens may destroy protective salivary pellicles such as mucin, resulting in fewer nonspecific host defense mechanisms in high-risk subjects. Travis et al. (1994) pointed out that there are several common parameters to periodontal disease and pulmonary emphysema, since each disease process is characterized by the key role of neutrophils and by proteolysis of connective tissue components in the inflammatory process, leading to tissue breakdown: (a) Both diseases are characterized by the accumulation of neutrophils in the tissues; (b) "Frustrated Phagocytosis" refers to the inability of phagocytic cells to either ingest large, inert materials (e.g., cigarette smoke tars, etc.) or kill and destroy foreign organisms (e.g., resistant bacteria like *P. gingivalis*). In both diseases, the result would be the continued influx and accumulation of inflammatory cells, including neutrophils and macrophages into the region in which the foreign material either has been deposited or is growing. In the case of pulmonary emphysema, there is a continuous influx of neutrophils to remove organic and inorganic materials arising from cigarette smoke inhalation; and as long as this continues, the lungs are always in an inflammatory state. (Travis et al. 1994). (Fig. 5.1)
4. With periodontal disease, the picture is slightly more complex. An extra step is required whereby the pathogenic anaerobes growing in the dentogingival cavity secrete uncontrolled proteinases that activate the complement system and indirectly cause the recruitment of neutrophils to the site of infection. Once again, this process results in the chronic influx of these phagocytes to the dentogingival cavity, further frustrating phagocytosis and releasing the powerful proteinases and oxidases that can not only damage connective tissue of the gingival but also, together with the bacterial proteinases, cause the complete inactivation of regulating inhibitors (Travis et al. 1994).
5. Fourth, in untreated periodontal disease, a large variety of cytokines and other biologically active molecules are continuously released from the periodontium and peripheral mononuclear cells. Upon saliva aspiration in high-risk patients, these cytokines could up regulate the expression of adhesion receptors on the mucosal surface, resulting in respiratory pathogen colonization.

5.2 Relationship Between Periodontal Disease and Adverse Pregnancy Outcome

It is an unfortunate fact that not all pregnancies end with healthy babies and healthy mothers. Families who have experienced an adverse pregnancy outcome require accurate information about the risk of recurrence to plan future childbearing. This article examines the recurrence risk of four complications of pregnancy: (1) gestational diabetes, (2) preterm delivery, (3) stillbirth, and (4) preeclampsia. Combined, these four complications are responsible for approximately 24% of maternal and neonatal morbidity and mortality (Moore 2005). Since Offenbacher et al. (1996) first reported an association between periodontal disease and preterm low birth (infants with birth weights of <2500 g born before 37 weeks) in 1996, adverse pregnancy outcomes that have been linked to periodontal disease include preterm birth, low birth weight, miscarriage or early pregnancy loss, and preeclampsia. Preeclampsia and preterm birth are major causes of maternal and perinatal morbidity and mortality (Xiong et al. 2007).

Preterm delivery is delivery occurring before 37 weeks gestation. Very early preterm delivery (<34 weeks) is associated with significant neonatal morbidity and mortality. The incidence of preterm delivery is estimated between 7 and 12% of all deliveries. One-third of all preterm births occur before 34 weeks. The cause of preterm delivery is multifactorial, and medical and social factors overlap. The largest risk for preterm delivery is a previous preterm delivery. Early preterm delivery (23–27 weeks) is the strongest risk factor for recurrent early preterm delivery. There is a significant

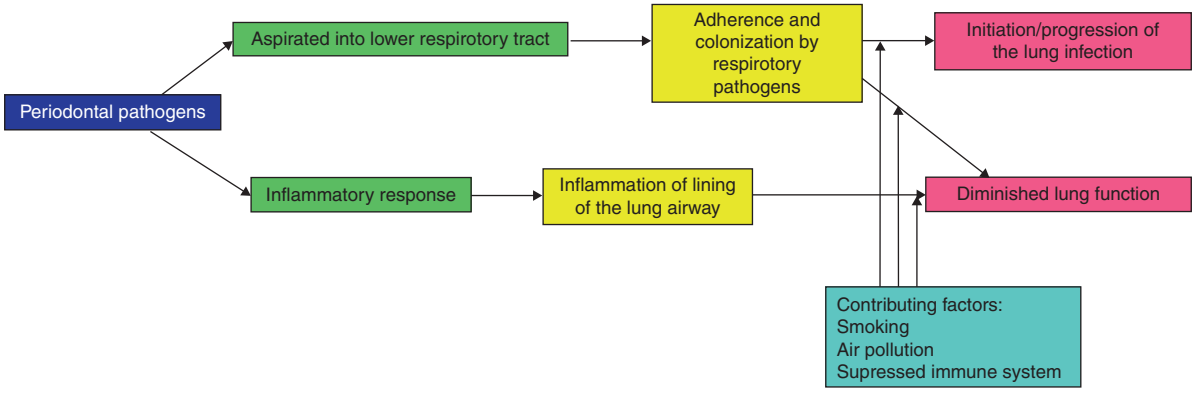

Fig. 5.1 Relationship between periodontal disease and respiratory disease (adapted from Kuo et al. 2008) (with permission from Elsevier)

unexplained racial disparity in recurrent preterm birth. African-American women have a recurrent preterm birth? rate of approximately 26% compared with 20% for white women. Other risk factors include short interpregnancy interval and smoking (Moore 2005).

Stillbirth is defined as the birth of a nonliving fetus after 20 weeks gestation. If gestational age is unknown, a birth weight of 500 g or more constitutes a stillbirth (Moore 2005).

Preeclampsia affects between 5 and 7% of all pregnancies and is responsible for 7–15% of maternal mortality. Neonatal outcomes are directly related to iatrogenic prematurity, although uteroplacental insufficiency may also be associated with the disease. The cause of preeclampsia is unknown. It is unique to human pregnancy and the only known cure is cessation of pregnancy, although temporizing measures are often undertaken. The rate of recurrence is cited between 0 and 60%. Risk factors for recurrent preeclampsia include long interpregnancy interval, primipaternity, chronic hypertension, and a history of preeclampsia (Moore 2005).

5.2.1 Overview on Clinical Trials Evaluating the Relationship Between Periodontal Disease and Adverse Pregnancy Outcome

Several case-control and cohort studies have evaluated the association between periodontal disease and adverse pregnancy outcome as reviewed by Scannapieco et al. (2003), Xiong et al. (2007), Vettore et al. (2008), and recently by Wimmer and Pihlstrom (2008) (Table 5.3). Also, several clinical trials have concluded that periodontal treatment during pregnancy may reduce the rates of preterm birth or the composite outcome of preterm birth and/or low birth weight (Table 5.4).

Three meta-analyses have been performed on this topic (Khader and Ta'ani 2005; Vergnes and Sixou 2007; Xiong et al. 2007) (Table 5.5).

Khader and Ta'ani (2005) evaluated data from two case-control studies and three prospective cohorts. The sample sizes ranged from 80 to 1,313 women, with an age range between 12 and 40 years old. All studies showed that pregnant women with periodontal disease had a significantly higher risk of preterm birth after adjusting for possible confounders. The OR in these studies ranged from 3.5 to 7.5. Pregnant women with periodontal disease had an overall adjusted odds of preterm birth that was 4.28 (95% CI: 2.62 to 6.99; $P < 0.005$) times the odds for healthy subjects.

The meta-analysis performed by Xiong et al. (2007) was based on 44 studies (26 case-control studies, 13 cohort studies, and five controlled trials). It was observed that findings from observational studies yielded inconsistent conclusions on the relationship between periodontal disease and various pregnancy outcomes. Of 39 observational studies, 25 studies (16 case-control and nine cohort) suggested that periodontal disease was associated with increased risk of adverse pregnancy outcomes. Among them, several studies demonstrated a dose–response relationship; that is, the risk of adverse pregnancy outcome increased as the severity of periodontal disease increased, and periodontal disease was associated with

Table 5.3 Case-control studies on the relationship between adverse pregnancy outcome and periodontal disease

Author, year	Subjects, cases/ controls	Adverse pregnancy outcome	Periodontitis evaluation	Smoking status	Findings	Association
Offenbacher et al. (1996)	93/31	Preterm low birth weight (PLBW)	CAL	Smokers and non-smokers	Multivariate logistic regression models demonstrated that periodontal disease is a statistically significant risk factor for PLBW with adjusted OR of 7.9 and 7.5 for all PLBW cases and primiparous PLBW cases, respectively	Significant
Dasanayake (1998)	55/55	LBW	CPITN	Smokers and non-smokers	Mothers with more healthy areas of gingiva (OR = 0.3, 95% CI: 0.12–0.72) had a lower risk of giving birth to an LBW infant	Significant
Dasanayake et al. (2001) USA	17/63	LBW	Porphyromonas gingivalis (P.g)-specific maternal serum IgG levels	Smokers and non-smokers	Women with higher levels of P.g.-specific IgG had higher odds of giving birth to LBW infants (OR = 4.1; 95% CI: 1.3–12.8)	Significant
Davenport et al. (2002)	236/507	PLBW	PD, bleeding index, CPITN	Smokers and non-smokers	No evidence for an association between PLBW and periodontal disease was found	Non-significant
Mokeem et al. (2004)	30/60 Mean age 30.8 ± 6.9 years	PLBW	PD, BOP, CI, CPITN	Smokers and non-smokers	Case mothers with periodontal disease were found to have a 4.21 time higher chance of having PLBW than the mothers with healthy periodontal tissues	Significant
Radnai et al. (2004)	41/44	PTB and/or LTB	PI, CI, BOP, R, PD	Smokers and non-smokers	Having bleeding at >50% of the examined sites and having at least at one site >4 mm probing depth had an OR = 5.46 at the 95% CI: 1.72–17.32	Significant
Goepfert et al. (2004)	95/44	PTB	Attachment loss >5 mm in any one sextant extension and severity index	Smokers and non-smokers	Severe periodontal disease was more common in the spontaneous preterm birth group (49%) than in the indicated preterm (25%, $P = 0.02$) and term control groups (30%, $P = 0.045$). Multivariable analyses supported the association between severe periodontal disease and spontaneous preterm birth (OR = 3.4, 95% CI: 1.5–7.7)	Significant

(*continued*)

Table 5.3 (continued)

Author, year	Subjects, cases/ controls	Adverse pregnancy outcome	Periodontitis evaluation	Smoking status	Findings	Association
Moore et al. (2004)	48/82	PTB	PI, PD, CAL, BOP, interleukin-1(IL-1)β and tumor necrosis factor alpha (TNF- α) polymorphism	Smokers and non-smokers	There was no statistically significant difference in the carriage of the IL-1β $^{+3953}$ allelic variant between cases and controls (29 vs. 18%, $P = 0.112$). However, 23 (48%) of the case subjects and 24 (29%) of controls were heterozygous or homozygous for the variant TNF-α^{-308} gene (OR = 2.2, 95% CI: 1.0–5.0, $P = 0.026$)	Non-significant
Moliterno et al. (2005)	76/75	PLBW	PD, CAL	Smokers and non-smokers	Significant associations with low birth weight babies was periodontitis (OR = 53.48, 95% CI: 1.17–10.36)	Significant
Jarjoura et al. (2005)	83/120	PTB	PI, BOP, PD, CAL, plaque collection and microbiologic analysis, Serum immunoglobulin G (IgG) antibodies to periodontal pathogens	Smokers and non-smokers	Cases showed greater mean attachment loss (1.7 vs. 1.5 mm) and higher prevalence of periodontitis (30.1 vs. 17.5%) ($P < 0.05$). No differences in microbial or serum antibody levels were detected between the groups. Logistic regression revealed that PTB was associated with attachment loss (OR = 2.75, 95% CI: 1.01–7.54)	Significant
Buduneli et al. (2005)	53/128	PLBW	BOP, PD, PI, subgingival plaque samples	Smokers and non-smokers	When subgingival bacteria were evaluated together, *Peptostreptococcus micros (presently Parvimonas micra)* and *Campylobacter rectus* may have a role in increasing the risk for PLBW, although no single bacteria exhibited any relation with the risk of PLBW	Non-significant
Noack et al. (2005)	59/42	PLBW	PI, BOP, PD, CAL, interleukin-1b level in gingival crevicular fluid, periodontal pathogens	Smokers and non-smokers	Periodontitis was not a detectable risk factor for preterm low birth weight in pregnant women	Non-significant
Moore et al. (2005)	61/93	PTB	PI, PD, CAL, BOP	Smokers and non-smokers	No association between periodontal disease and preterm birth was found	Non-significant

Table 5.3 (continued)

Author, year	Subjects, cases/ controls	Adverse pregnancy outcome	Periodontitis evaluation	Smoking status	Findings	Association
Alves and Ribeiro (2006)	19/40	PLBW	The periodontal screening and recording	Smokers and non-smokers	There was a higher rate of periodontal disease in cases (84.21%–16/19) as compared with controls (37.5% –15/40). The data also showed a significant association between periodontal disease and PLBW (OR = 8.9, 95% CI: 2.22–35.65)	Significant
Bosnjak et al. (2006)	17/64	PTB	CAL, PD, R, Papillary bleeding index	Smokers and non-smokers	Multivariate logistic regression model demonstrated that periodontal disease is a significant independent risk factor for PTB, with an adjusted OR of 8.13 for the PTB group (95% CI: 2.73–45.9)	Significant
Radnai et al. (2006)	77/84	PTB	PI, CI, BOP, R, PD	Smokers and non-smokers	A significant association was found between PB and initial chronic localized periodontitis, the criteria being bleeding at >50% of the examined teeth and having at least at one site at >4 mm probing depth ($P < 0.0001$). The adjusted OR for initial chronic localized periodontitis was 3.32, 95% CI: 1.64–6.69	Significant
Skuldbøl et al. (2006)	21/33	PTB	PI, PD, BOP, bone loss on bitewing radiographs, subgingival plaque analyses	Smokers and non-smokers	No association between periodontal disease and preterm birth was found	Non-significant
Wood et al. (2006)	50/101	PTB	Oral hygiene index simplified, PD, CAL, BOP, neutrophil elastase, gingipain and dipeptidylpepti-dase in gingival crevicular fluid	Smokers and non-smokers	There was no difference in the proportion of sites with significant attachment loss (≥3 mm): Cases-3.2%, Controls-2.2%, $P = 0.21$	Non-significant
Gomes-Filho et al. (2006)	44/177	PLBW	PI, PD, R, BOP, CAL	Smokers and non-smokers	There was no statistically significant difference in the periodontal clinical parameters between the groups	Non-significant
Bassani et al. (2007)	304/611	LBW	PD, CAL	Smokers and non-smokers	OR were 0.93 (95% CI: 0.63–1.41) for LBW and 0.92 (95% CI: 0.54–1.57) for preterm LBW in the presence of periodontitis	Non-significant

(*continued*)

Table 5.3 (continued)

Author, year	Subjects, cases/ controls	Adverse pregnancy outcome	Periodontitis evaluation	Smoking status	Findings	Association
Vettore et al. (2008)	150/66 (>30 years old)	PTB, LBW, PTB and/or LBW	PI, CI, BOP, PD, CAL	Smokers and non-smokers	The mean PD was significantly higher in nonpreterm low birth weight controls than in subjects in the preterm low birthweight, preterm and/or low birthweight, and preterm and lowbirthweight groups. CAL measures were not different between all pairs of cases and control groups. Groups did not differ with respect to the mean proportions of different microbial complexes	Non-significant

BOP bleeding upon probing; *CAL* clinical attachment level; *CPITN* community periodontal index for treatment needs; *CI* confidence intervals; *CI* calculus index; *GI* gingival index; *LBW* low birth weight; *OR* odds ratio; *PD* probing depths; *PI* plaque index; PTB: Preterm Birth; *PTLBW* preterm low birth weight

even higher risk of very preterm birth (<32 weeks), birth weight below 1500 g and early pregnancy loss. Fourteen studies (Ten case-control and four cohort) reported no associations. This meta-analysis of the existing controlled trials suggested that treating periodontal disease during pregnancy resulted in reduced risk of preterm low birth weight (PLBW), but did not significantly reduce the rate of preterm birth, low birth weight, or intrauterine growth restriction (IUGR).

The meta-analysis of Vergnes and Sixou (2007) was conducted on 17 observational studies (11 case-controls, four cohorts, and two cross-sectionals). A total of 7,151 individuals were studied, among whom 1,056 were mothers with PLBW infants. The overall OR was 2.83 (95% CI: 1.95–4.10, $P < 0.0001$). The pooled estimate for the risk of having PLBW in mothers with periodontal disease was 2.83 (95% CI: 1.95–4.10, $P < 0.0001$) by using a random effects model. For the outcome "preterm birth" alone, the overall OR was 2.27 (95% CI: 1.06–4.85, $P < 0.05$), whereas for the outcome "low birthweight" alone, we found OR = 4.03 (95% CI: 2.05–7.93, $P < 0.0001$).

As recently reviewed by Wimmer and Pihlstrom (2008), a major reason for the inconsistent findings among many observational studies is likely due to methodological differences, including various definitions of periodontal disease and adverse pregnancy outcomes, lack of control for recognized risk factors and effect modifiers, and statistical heterogeneity. Moreover, many investigations have small sample sizes and do not clearly describe study methods. As a result, authors of systematic reviews and meta-analysis agree that larger and more methodologically rigorous studies using reliable outcome and exposure measures are needed.

Variations in diagnostic criteria can have marked effect on study outcomes. In order to estimate the impact of any disease, it is critical that the disease be well characterized and accurately assessed. Use of well characterized and widely accepted periodontal outcome measures is important for documenting changes in periodontal health over time in intervention studies to investigate the effects of treatment on pregnancy (Wimmer and Pihlstrom 2008). Most of the researchers used their own case definitions (mostly based on disease distribution within the study population) that combined Probing Depth (PD) and Clinical Attachment Level (CAL). Some studies defined periodontal disease in terms of Decayed, Missing, and Filled Teeth (DMFT) and Community Periodontal Index (CPI), the Russell Periodontal Index and similar indexes, all of which have limited sensitivity for disease detection (Xiong et al. 2007).

A second potential bias is confounding effects. For those studies that reported an association, questions remain whether the observed associations represent a

Table 5.4 Studies on the relationship between effect of periodontal therapy in preventing adverse pregnancy outcome

Author, year	Subjects cases/ controls	Adverse pregnancy outcome	Periodontitis evaluation	Smoking status	Oral intervention	Results
López et al. (2002)	200/200	Preterm low birth weight (PLBW)	PI, PD, CAL	Smokers and non-smokers	Plaque control instructions, scaling and root planing (SRP)	Significant
Jeffcoat et al. (2003)	366 women with periodontitis between 21 and 25 weeks' gestation	PTB	PD, CAL	Smokers and non-smokers	(1) dental prophylaxis plus placebo capsule; (2) SRP plus placebo capsule; and (3) SRP plus metronidazole capsule (250 mg t.i.d. for 1 week)	Non-significant
López et al. (2005)	580/290	PLBW	Gingivitis	Smokers and non-smokers	Plaque control, scaling, and daily rinsing with 0.12% clorhexidine	Significant
Sadatmansouri et al. (2006)	30/30	PLBW	PD, CAL, BOP	Smokers and non-smokers	Oral hygiene instructions, use of 0.2% chlorhexidine mouth rinse once a night for 1week period and periodontal evaluation once every 2 weeks	Significant
Offenbacher et al. (2006)	40/34	PTB	PD, R, BOP, GI, PI	Smokers and non-smokers	SRP and the use of a sonic toothbrush	Significant
Michalowicz et al. (2006)	413/410	PTB, LBW	PD, CAL, BOP	Not given	monthly oral hygiene instruction and scaling as needed	Non-significant
Tarannum and Faizuddin (2007)	53/68	PTB, LBW	PD, CAL, Oral hygiene index-simplified, bleeding index	Non-smokers	Plaque control instructions and SRP	Significant

BOP bleeding upon probing; *CAL* clinical attachment level; *CPITN* community periodontal index for treatment needs; *CI* confidence intervals; *CI* calculus index; *GI* gingival index; *LBW* low birth weight; *OR* odds ratio; *PD* probing depths; *PI* plaque index; PTB: Preterm Birth; *PTLBW* preterm low birth weight; PTLBW: Preterm.

causal relationship or are due to the confounding effects of other variables such as low socioeconomic status and smoking. Several important confounding variables such as histories of adverse pregnancy outcomes, infections (e.g., bacterial vaginosis and chorioamnionitis), antibiotic use during pregnancies, excessive body mass index, or maternal disorders (hypertension, diabetes) were not considered. Although some of the studies adjusted for race, smoking, socioeconomic status, and other important confounding variables by using multivariable regression analysis, it is possible that some residual confounding effects remain (Wimmer and Pihlstrom 2008).

Another issue that makes interpretation of adverse pregnancy outcome as related to periodontal disease is the different ways that studies have used to define

Table 5.5 Meta-analysis on periodontal disease and adverse pregnancy outcomes

Authors	Studies included	Outcomes	Conclusions
Khader and Ta'ani (2005)	5 studies (two case-control and three prospective cohorts)	PTB: OR = 4.28 (95% CI: 2.62–6.99; $P < 0.005$) PTLBW: OR = 5.28 (95% CI: 2.21–12.62; $P < 0.005$) Either PTB or LBW: OR = 2.30 (95% CI: 1.21–4.38; $P < 0.005$)	Periodontal diseases in the pregnant mother significantly increase the risk of subsequent preterm birth or low birth weigh
Vergnes and Sixou (2007)	17 observational studies (11 case/controls, four cohorts, and two cross-sectionals)	Preterm low birth weight: OR = 2.83 (95% CI: 1.95–4.10, $P < 0.0001$) LBW: OR = 4.03 (95% CI: 2.05–7.93, $P < 0.0001$)	These findings indicate a likely association, but it needs to be confirmed by large, well-designed, multicenter trials
Xiong et al. (2007)	44 studies (26 case-control studies, 13 cohort studies, and five controlled trials)	Twenty nine suggested an association between periodontal disease and increased risk of adverse pregnancy outcome (ORs ranging from 1.10 to 20.0) and 15 found no evidence of an association (ORs ranging from 0.78 to 2.54) Preterm Low birth weight: RR = 0.53, 95% CI: 0.30–0.95, $P < 0.05$ Preterm birth: RR = 0.79, 95% CI: 0.55–1.11, $P > 0.05$ Low birth weight: RR = 0.86, 95% CI: 0.58%1.29, $P > 0.05$	The published literature is not vigorous to clinically link periodontal disease and/or its treatment to specific adverse pregnancy outcomes

CI confidence intervals; *LBW* low birth weight; *OR* odds ratio; *PTB* preterm birth low birth weight; *PTLBW* preterm low birth weight; *RR* risk ratio

adverse pregnancy outcome. A number of studies do not use the anticipated delivery date as a fundamental reference baseline. This is critical for appraisal of gestational age and birth weight. Moreover, infants can be term born (<37 weeks) and underweight (<2500 g), preterm (<37 weeks) with normal birth weight (>2500 g) or preterm and with low birth weight. Each may have very different genesis. For example, intrauterine growth restriction (IUGR), preterm labor, premature rupture of membranes, preterm birth, and eclampsia may potentially be influenced by many types of inflammation and infection – only one of which may be periodontal disease. It is clear that reports that do not clearly distinguish between the many types of adverse pregnancy outcomes and simply categorize infants as PLBW add little to our understanding of the potential relationship between periodontal disease and adverse pregnancy outcomes (Wimmer and Pihlstrom 2008).

5.2.2 Hypothesis About the Association Between Periodontitis and Adverse Pregnancy Outcomes

The mechanistic aspect of the possible association of periodontal disease with pregnancy complications has been explored in several experimental animal models. In most models, periodontal bacteria (*P. gingivalis* or *Campylobacter rectus*) are injected in a small chamber that previously had been implanted subcutaneously in the pregnant animals (hamsters, mice, rabbits) (Bobetsis et al. 2006; Williams et al. 2000).

The results of these studies reveal that maternal infection with periodontal pathogens has a deleterious effect on fetal growth and viability. Specifically, both *P. gingivalis* and *C. rectus* have the capacity to disseminate from the subcutaneous chamber toward not only maternal organs (liver, uterus), but most importantly toward placental and fetal tissues. This translocation is

accompanied by an increase in inflammatory mediators in the placenta. Moreover, the infection with periodontal pathogens induces a significant alteration in the architecture of the placenta, especially in areas that are critical for the exchange of nutrients between the mother and the fetus. Furthermore, maternal exposure to *P. gingivalis* or *C. rectus* results in a decrease in the size of the fetuses (preterm deliveries do not occur in mice). The reduced size of the fetuses is not the only complication, since the newborns demonstrate a higher risk of experiencing perinatal death, similarly to PLBW human infants. Finally, pups that survive the perinatal period appear to have an increase in inflammatory cytokines (interferon IFN-γ) in the brain tissues along with ultrastructural alterations in the hippocampal region of the brain. Interestingly, these changes in the neonatal brain occur in a manner analogos to the effect of maternal infection on white-matter damage seen in humans. Taken together, these findings suggest that the threat of maternal infection with periodontal pathogens during pregnancy may not be limited to the duration of gestation, but also may affect perinatal neurological growth and development (Bobetsis et al. 2006).

On the basis of the current evidence from both animal and human studies, a hypothetical model of the association between maternal periodontal inflammation and fetal development may be proposed. Periodontal bacteria and their virulence factors, found in the periodontal pockets, induce a local periodontal host-immune response that includes mainly the production of inflammatory cytokines [interleukin-1(IL-1), prostaglandin (PGE_2), tumor necrosis factor alpha (TNF-α) and so forth] and antibodies against the bacteria. If this immune response and the neutrophils are not capable of keeping the infection localized (such as low maternal IgG response to bacteria), then the bacteria and/or their virulence factors and the inflammatory cytokines may gain access systemically via the blood circulation. This would be particularly evidenced clinically by signs of bleeding on probing and increased pocketing during pregnancy. The presence of the bacteria in the blood circulation will trigger the host to elicit a second round of inflammatory response, systemic this time, mainly by the production of more inflammatory cytokines and acute-phase reactants such as C-reactive protein from the liver. Eventually, bacteria and/or their virulence factors and inflammatory cytokines appear to reach the placenta, as about 40% of all pregnancies are associated with some fetal IgM antibody response to organisms of maternal oral origin. This will create another site of bacterial challenge and possibly placental infection, leading to a new inflammatory response, localized at the fetal–placental interface this time, with the production of more inflammatory cytokines. As in periodontal tissues, these cytokines, although produced with the intention to combat the infection, also may cause tissue destruction. Because the structural integrity of the placenta is vital for the normal exchange of nutrients between the mother and the fetus, this placental tissue damage may contribute to impaired fetal growth, which may lead to low birth weight. Also, structural damage in the placenta may disrupt the normal blood flow between the mother and the fetus, affecting the maternal blood pressure and leading to preeclampsia. The increase in the production of inflammatory cytokines such as IL-β and PG E_2 also may contribute to preterm rupture of the membranes and uterine contraction, and lead to miscarriage or preterm delivery. Finally, periodontal bacteria and/or their virulence factors and inflammatory cytokines may cross the placenta and enter the fetal circulation. There, they may trigger a new fetal–host immune response, as evidenced by the observed elevated levels of fetal IgM to periodontal pathogens. If the fetus cannot control the infection, the bacteria and/or their virulence factors may gain access to various tissues and initiate local inflammatory responses and, consequently, structural damage to the fetal tissues and organ systems. Depending on the extent of this damage, the newborn may or may not survive the perinatal period. However, survivors may possess deficiencies that may compromise their quality of life, even throughout adulthood (Bobetsis et al. 2006).

5.3 Interactions Between Periodontal Disease and Chronic Renal Disease

End-stage renal disease is fatal without renal replacement therapy, which can be provided by dialysis, either hemodialysis or peritoneal dialysis (PD), or by renal transplantation. The prevalence of chronic renal disease in industrialized countries is increasing and, when coupled with improved rates of survival for renal replacement therapies, it is evident that patients with chronic renal disease will comprise an enlarging proportion of

the dental patient population in the future. In addition, chronic renal disease and periodontitis can have significant, reciprocal effects. Chronic renal disease and renal replacement therapy can affect oral tissues and can greatly influence the dental management of the renal patient, while recent studies suggest that chronic adult periodontitis can contribute to overall systemic inflammatory burden and may therefore have consequences in the management of the end-stage renal disease patient on hemodialysis maintenance therapy (Craig 2008).

Several studies have evaluated the interactions between periodontitis and chronic renal disease, and revealed an impaired periodontal condition in end-stage renal disease patients on hemodialysis maintenance therapy (Table 5.6).

Several possible reasons have been forwarded to account for the almost universally reported increased levels of plaque, calculus and gingival inflammation in renal hemodialysis populations. Most prominently, end-stage renal disease patients on hemodialysis are in a state of chronic kidney failure resulting in the uremic syndrome, and uremia has been associated with immune dysfunction including defects in lymphocyte and monocyte function. Therefore, if uremia is responsible for the increased gingival inflammation observed in this population, increased dialysis vintage maintenance therapy should be associated with increased gingival inflammation and periodontitis incidence and severity. In addition to uremia, the presence of confounding diseases such as diabetes mellitus could contribute to the increased gingival inflammation reported for renal hemodialysis populations, especially in view of the high incidence of diabetes mellitus in end-stage renal disease populations and the strong association between diabetes mellitus and periodontitis in the general population. Alterations in calcium homeostasis leading to secondary hyperparathyroidism have been suggested as a possible cause of increased gingival inflammation and possible alveolar bone loss in renal hemodialysis populations (Craig et al. 2007).

5.4 Interactions Between Periodontal Disease and Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a common, systemic autoimmune disease affecting 0.5–1% of the population. It is characterized by synovial hyperplasia, inflammatory cell recruitment and intraarticular fibrin deposition that leads to the destruction of the joint architecture and consequent disability (Liao et al. 2009). The cause of RA is not known, although its etiology appears to be multifactorial, and may involve infectious, genetic, endocrine and immune participation. Rheumatoid arthritis is believed to be a T lymphocyte-driven disease in which a sudden influx of T-cells into the affected joint(s) is followed by an increased number of macrophages and fibroblasts, drawn by the release of cytokines, particularly IL-1 and tumor necrosis factor alpha (TNF-α). This cytokines release and subsequent migration of cells is thought to be responsible for the chronic inflammation and characteristic destructive changes in rheumatoid joints. The cause of the initial T-cell influx is not known, but several infectious agents,including both bacteria such as *Streptococci* and mycoplasma, as well as viruses such as parvovirus, Epstein-Barr virus, and retroviruses have been suggested. The general progression of RA leads to an increasing disability and loss of functional capacity; 50 percent of people with RA become unable to work within the decade after the onset of disease. There are extra-articular manifestations of RA as well, including rheumatoid nodules, rheumatoid vasculitis, interstitial lung disease, pericardial disease, episcleritis and scleritis, Felty's syndrome and Sjögren's syndrome. The objective of RA therapies is to restore or at least maintain quality of life by relieving pain, reducing joint inflammation and preventing joint destruction and deformity (Treister and Glick 1999) (Fig. 5.2).

Whether RA is associated with the progression of existing inflammatory conditions, such as periodontitis, is controversial (Al-Katma et al. 2007). Over the last two decades, the interrelationship between RA and periodontitis has become increasingly appreciated and several studies have revealed an increased prevalence of periodontal disease among patients with RA. In patients referred for periodontal treatment, the prevalence of self-reported rheumatoid arthritis was 3.95%, which is significantly higher than that seen in patients not referred for periodontal treatment (0.66%) and that reported in the general population (1%). Of those referred patients with rheumatoid arthritis, 62.5% had advanced forms of periodontal disease (Mercado et al. 2000). In a Romanian adult population, rheumatoid arthritis was found to be more prevalent in periodontitis patients compared with gingivitis affected individuals ($P < 0.05$) (Dumitrescu 2006). Further, patients

Table 5.6 Summary of papers relating to the relationship between periodontal disease and chronic renal disease

Author	Study population	Periodontal disease evaluation	Conclusion	Association
Klassen and Krasko (2002)	147 patients undergoing dialysis (dentate and edentate). No healthy controls	Numbers of remaining natural teeth, mobility, recession, gingivitis	Findings in the dentate group included increased tooth mobility, recession (52%), gingivitis (99%) and a high plaque index (77%).	N/A
Frankenthal et al. (2002)	35 patients with secondary hyperparathyroidism, with chronic renal failure treated by hemodialysis. A control group was formed from 35 healthy age- and gender-matched subjects	PI, GI, PD, CAL	Secondary hyperparathyroidism does not have an appreciable effect on periodontal indices and radiographic bone height	Non-significant
Bots et al. (2006)	42 dentate chronic renal failure (CRF) patients/808 healthy subjects	PI, Decayed, Missing, and Filled Teeth (DMFT), PD, BOP, CI	All index-scores in the CRF patients were comparable with the controls except for number of teeth covered with calculus that was significantly higher ($P < 0.05$) in CRF patients (4.1 ± 2.6) than in controls (3.0 ± 2.9)	Non-significant
Duran and Erdemir (2004)	342 two subjects undergoing renal dialysis	CPITN	There was a significant positive relationship between the CPITN scores and age ($r = 0.164$, $P = 0.002$) and dialysis duration ($r = 0.240$, P = 0.000).	Significant
Chen et al. (2006)	253 hemodialysis patients, 58.8 ± 0.8 years old	PI, GI, PDI	Periodontal health status indices, including the PI, GI, and PDI, showed a significantly negative correlation with nutritional parameters, such as albumin level, blood urea nitrogen level, creatinine level, transferrin level and normalized protein catabolism rate. Multiple regression analysis revealed that age, diabetes, smoking, albumin level, and dialysis duration were associated independently with periodontitis severity in hemodialysis patients. According to the severity of periodontitis, there were higher percentiles of patients with malnutrition (chi-square = 13.055; $P = 0.005$) and inflammation (chi-square = 10.046; $P = 0.018$) in the severe group	Significant
Al-Wahadni and Al-Omari (2003)	47 patients on renal dialysis categorized into three subgroups based on their renal dialysis histories: on dialysis for <1 year; on dialysis for 1–3 years; or on dialysis longer than 3 years. No healthy controls	PI, GI, PD, DMFT	There were no statistically significant differences in PII, GI, PD, and gingival recession among the three subgroups examined. The GI revealed that 55% scored more than 2, indicating moderate to severe gingivitis.	*Non-significant*

(*continued*)

Table 5.6 (continued)

Author	Study population	Periodontal disease evaluation	Conclusion	Association
Marakoglu et al (2003)	36 cases with hemodialysis/36 healthy controls	PI, GI, PD	No statistically significant difference was observed in the clinical parameters between the two groups (PI: $t = 1.69$ $P = 0.096$; GI: $t = 1.057$ $P = 0.294$; PD: $t = 0.01$ $P = 0.99$)	Non-significant
Sakallioğlu et al. (2007)	15 cases with dialysis 1 year before /15 controls (9–11 years old)	PI, GI, PD, CAL	There were increases in the gingival crevicular fluid volume and GOP of the test group compared to those of the control group ($P < 0.01$). The PI and GI scores were higher in the test group than in the control group ($P < 0.01$)	Significant
Kshirsagar et al. (2009)	168 adult patients with end-stage renal disease; 18 months follow-up	PD, CAL	Moderate-to-severe disease was significantly associated with death from cardiovascular causes in this population	Significant
Kshirsagar et al. (2007)	35 cases with dialysis for at least 3 months /119 controls (54.6 ± 13.3 years)	GI, R, PD, BOP	Severe periodontitis was associated with low serum albumin (OR = 8.20; 95% CI: 1.61–41.82; $P = 0.01$)	Significant
Rahmati et al. (2002)	50 cases with dialysis for 22 months /36 controls	Serum IgG levels to periodontal pathogens	Elevated levels of IgG antibody to bacterial species associated with destructive periodontal diseases (*P. gingivalis* and *B. forsythus*) are associated with elevated C-reactive protein values in hemodialysis populations	Significant
Borawski et al. (2007)	106 patients: 35 on maintenance haemodialysis (HD), mean age 56 years; 33 on continuous ambulatory peritoneal dialysis (CAPD), mean age 51 years; and 38 pre-dialysis pre-dialysis chronic kidney disease (CKD) stage 2–5, mean age 51 years)	GI, PBI, PI, CAL, CPITN	Average values of the indices in hemodialysis (HD), CAPD, pre-dialysis CKD, advanced periodontitis and general population subjects were as follows: GI-1.37, 0.95, 1, 2 and 1; PBI-1.45, 0, 0, 2.20 and 1; PI-2.05, 1.59, 1, 2 and 1; and CAL loss-5.11, 3.47, 2.50, 4.68 and 1.40 mm, respectively. CPITN, analyzed separately as CPI and periodontal treatment needs, further indicated a high severity of periodontitis in all renal failure groups as compared with general population subjects	Significant
Bayraktar et al. (2007)	76 cases with hemodialysis/61 healthy controls	PD, PI, GI, CSI	A highly significant difference was found for plaque index, gingival index and calculus surface index ($P < 0.001$). There was a highly significant difference for GI and PD scores ($P < 0.001$) between subgroups receiving HD for <3 years or more. A positive correlation between time on dialysis and parameter of missing teeth ($r = 0.259$; $P = 0.024$), GI scores ($r = 0.474$; $P < 0.001$) and measurement of PD ($r = 0.481$; $P < 0.001$) was found in the hemodialysis group	Significant

CAL clinical attachment level; *CI* calculus index; *CI* confidence intervals; *CSI* calculus surface index; *CPITN* community periodontal index for treatment needs; *DMFI* decayed, missing filled index; *GI* gingival index; *PBI* papillary bleeding index; *PD* probing depths; *PDI* periodontal disease index; *OR* odds ratio; *PI* plaque index; *SOHI* simplified oral hygiene index GOP tissue osmotic pressure; CAPD continuous ambulatory peritoneal dialysis patients; CKD pre-dialysis chronic kidney disease patients.

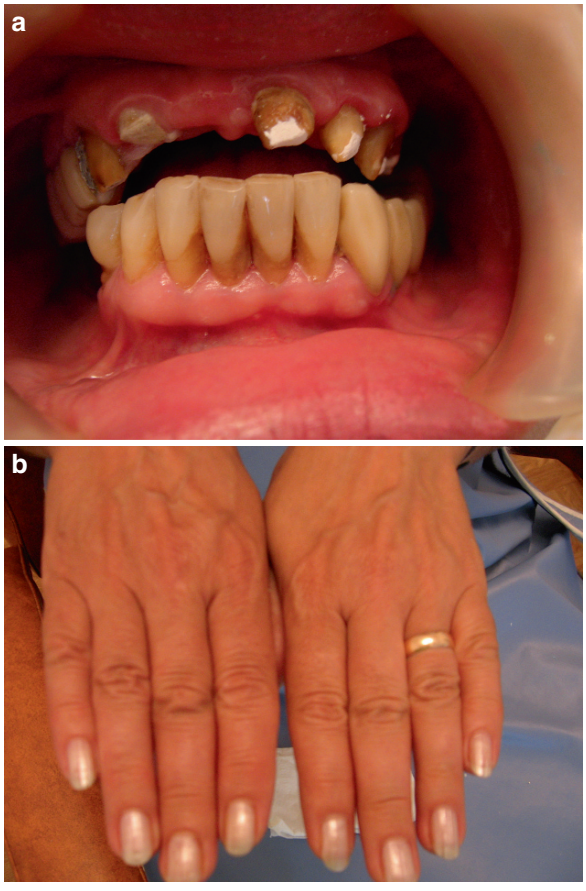

Fig. 5.2 Patient with rheumatoid arthritis Intra-oral (a) and hand (b) changes

suffering from advanced periodontitis showed a higher prevalence of RA if compared to patients with mild periodontitis (Georgiou et al. 2004). It was also showed that control of periodontal infection and gingival inflammation by scaling/root planing and plaque control in subjects with periodontal disease may reduce the severity of RA (Al-Katma et al. 2007; Ribeiro et al. 2005).

There are studies that have examined the association between RA and periodontal disease, and the results have often been conflicting (Tables 5.7 and 5.8). A major reason for these discrepancies relates to the lack of uniformity in classifying the various forms of periodontal disease and RA, the severity and duration of the disease being a very important factor in order to understand the present interrelationships (Bartold et al. 2005). The traditional drugs used to treat RA and juvenile idiopathic arthritis (JIA) include nonsteroidal antiinflammatory drugs, corticosteroids, and disease-modifying antirheumatic drugs, such as methotrexate. This intensive, systemic anti-inflammatory therapy, which is aimed at reducing RA disease activity, retarding joint erosions, and improving the patient's quality of life, probably interferes with destructive processes in the periodontium as well. Recently, Biyikoğlu et al. (2006) found no differences in the gingival crevicular fluid (GCF) levels of prostaglandin E_2 and IL-1β between periodontitis patients and RA individuals with a long history of usage of corticosteroids and methotrexate (Miranda et al. 2007). Miranda et al. (2007) analyzed markers of periodontal inflammation, IL-1β and -18, and elastase activity in the GCF of individuals with RA and compared them to controls matched for major confounders. Total amounts of IL-1β and elastase activity were significantly lower in the RA individuals; in this group, these mediators had a strong correlation.

Although the etiologies of RA and chronic periodontitis are believed to be distinctly separate, the underlying pathologic processes are of sufficient similarity to warrant postulating that individuals who are at risk for developing RA may also be at risk for developing periodontitis and vice versa (Al-Katma et al. 2007). The similarities between rheumatoid arthritis and periodontitis, as reviewed by Bartold et al. (2005) include:

Natural History

Natural history studies of periodontal disease in humans indicate the presence of three distinct subpopulations: (1) no progression of periodontal disease, in which around 10% of the population manifest very little or no disease and is of no particular consequence to the dentition; (2) moderate progression, affecting around 80% of the population and representing a very slowly progressing form of disease that can generally can easily managed via routine therapies; and (3) rapid progression, affecting approximately 8% of individuals, whereby extensive periodontal destruction that can be very difficult to control occurs. At least three types of disease manifestation can also be observed in RA populations: (1) self-limited: in these cases, individuals originally presenting for RA have no evidence of disease 3–5 years later; (2) easily controlled: the disease is relatively easily controlled with only nonsteroidal anti-inflammatory drugs; (3) progressive: these patients generally require second-line drugs, which often still do not fully control the disease (Bartold et al. 2005).

Table 5.7 Summary of papers relating to the relationship between periodontal disease and rheumatoid arthritis the appropitrate usage of

Author	Study population	Periodontal Disease Evaluation	RA treatment	Smoking status	Results	Association
Ishi et al. (2008)	39 RA patients and 22 healthy controls	PI, GBI, AL, number of teeth present	Disease-modifying antirheumatic drugs, anti-inflammatory, steroid and non-steroidal medication	Non-smokers	RA patients had fewer teeth, higher prevalence of sites presenting dental plaque and a higher frequency of sites with advanced attachment loss	Significant
Reichert et al. (2006)	78 juvenile idiopathic arthritis (JIA) and 75 controls	API, modified SBI, CAL	NSAIDs	Smokers and non-smokers	JIA patients had a significantly higher API (64.6 vs. 49.9%, $P = 0.004$) and slightly higher mean percentages of sites with CAL >3.5 mm (0.58 vs. 0.22%, $P = 0.041$)	Non-significant
Reichert et al. (2007)	110 JIA, 50 patients with generalized aggressive periodontitis, 102 patients with chronic periodontitis	PI, GBI, AL, CPITN	Not given	Smokers and Non-smokers	JIA patients had a significantly higher API (64.6 vs. 49.9%, $P = 0.004$) and slightly higher mean percentages of sites with CAL > 3.5 mm (0.58% vs. 0.22%, P = 0.041)	Significant
Miranda et al. (2003)	32 patients with JIA (mean age 15.9 ± 2.7years) and 24 controls (14.7 ±2.3years)	PI, GBI, PD, AL	Not given	Not given	The prevalence of patients with a proximal attachment loss of 2 mm or more in the JIA group was 25% and in controls it was 4.2%	Significant
Miranda et al. (2006)	18 patients with JIA (mean age 5.9 ± 2.7years) and 24 controls (14.7 ±2.3years)	PI, GBI, PD, AL	Not given	Not given	The prevalence of patients with a proximal attachment loss of 2 mm or more in the JIA group was 25% and in controls it was 4.2%	Significant
Bozkurt et al. (2000)	15 patients with RA and CP, 15 patients with CP, and 15 periodontally healthy controls	PI, GI, SBI, PD, AL, IL-6 in GCF samples	Drug therapy including prednisolone, 5 mg/day; indomethacin, 75 mg/day; and chloroquine, 250 mg/day.	Not given	No significant difference could be detected between the RA and AP groups in the mean GCF IL-6 level and mean clinical parameter data except PI. The mean percentages of sites with PD > 4 mm were significantly higher in the JIA group (3±4.7) than in the CTR group (0.4 ± 1.7) ($P = 0.012$). The mean percentages of sites with proximal CAL > 2 mm were 0.7 (± 1.4) in the JIA group and 0.001 (±0.2) in the CTR group ($P = 0.022$)	Non-significant
Bozkurt et al. (2006)	17 patients with CP, 17 patients with RA and 17 healthy controls	PI, GI, PD, AL, GBI, IL-4 and IL-10 in GCF samples	Drug therapy including prednisolone, 5 mg/day; indomethacin, 75 mg/day; and chloroquine, 250 mg/day.	Non-smokers	The mean level of IL-4 in RA group with PD >5 mm was significantly higher than that in CP group ($P < 0.05$). IL-10 mean level in the HC group was higher than those in the other groups ($P < 0.05$). In the RA group with PD <5 mm, higher IL-10 level was found compared to the CP patients ($P < 0.05$)	Significant

Sørensen et al. (2009)	Adults <35 years of age diagnosed with localized aggressive periodontitis ($n = 18$), generalized aggressive periodontitis ($n = 27$), JIA ($n = 10$), and RA ($n = 23$) and healthy controls ($n = 25$)	PI, BOP, PD, CAL, ABL, number of missing teeth, peripheral blood mononuclear cell transcripts of interleukin 1alpha, IL1beta, IL-1 receptor antagonist, IL-6, IL-10, tumor necrosis factor alpha (TNF), TNF alpha receptor I, and TNFRII	Not given	Not given	The study demonstrated only a few changes in the PBMC expression of various cytokine and cytokine inhibitor genes in aggressive periodontitis and chronic arthritis compared to controls	Non-significant
Havemose-Poulsen et al. (2005)	White adults, <35 years of age, diagnosed with localized aggressive periodontitis ($n = 18$), generalized aggressive periodontitis ($n = 27$), JIA ($n = 10$), or rheumatoid arthritis ($n = 23$) and healthy controls ($n = 25$)	AL, BOP, levels of interleukin (IL)-1a, IL-1b, IL-1 receptor antagonist (IL-1Ra), IL-6, IL-10, TNF-a, and lymphotoxin-a in peripheral blood (plasma) and unstimulated and stimulated whole blood cell cultures	No cytokine/ anti-cytokine therapies in JIA or RA patients	Not given	Patients with aggressive periodontitis and types of arthritis presented with similar components of blood cytokine profiles distinguishing them from individuals free of disease	Non-significant
Havemose-Poulsen et al. (2006)	White adults, <35 years of age, diagnosed with localized aggressive periodontitis ($n = 18$), generalized aggressive periodontitis ($n = 27$), JIA ($n = 10$), or rheumatoid arthritis ($n = 23$) and healthy controls ($n = 25$)	PI, BOP, AL, ABL, Blood samples were analyzed for erythrocyte fraction, leukocytes and differential counts, erythrocyte sedimentation rate, C-reactive protein, immunoglobulin M and IgA rheumatoid factors, and antibodies to cyclic citrullinated peptides	No cytokine/ anti-cytokine therapies in JIA or RA patients	Smokers and non-smokers	Despite treatment with antinflammatory/ antirheumatic medications, and similar levels of plaque and periodontal inflammation, young adults with RA possess a significantly higher percentage of sites with PD >4 mm, CAL >2 mm and ABL >2 mm compared to healthy control individuals. The percentage of sites with CAL >2 mm significantly correlated with the levels of IgM-reumatoid factor and IgA- reumatoid factor	Significant
Pischon et al. (2008)	57 subjects with RA and 52 healthy controls	PI, GI, PD, AL	Disease- modifying antirheumatic drugs, non-steroidal anti-inflammatory drugs, corticosteroids, and/or tumor necrosis factor-alpha antagonists	Smokers and non-smokers	Subjects with RA had a significant 8.05-fold increased odds (95% CI: 2.93–22.09) of periodontitis compared to controls	Significant
Biyikoğlu et al. (2006)	23 RA patients, 17 systemically healthy patients with periodontal disease and 17 healthy controls	PI, PD, CAL, BOP, GCF sampling	Prednisolone and methotrexate treatment	Smokers and non-smokers	There were no significant differences between the RA and periodontal disease groups in clinical periodontal parameters ($P > 0.05$)	Non-significant

ABL alveolar bone loss; *API* approximal plaque index; *BOP* bleeding upon probing; *CAL* clinical attachment level; *CI* calculus index; *CI* confidence intervals; *CPITN* community periodontal index for treatment needs; *CSI* calculus surface index; *GCF* gingival crevicular fluid; *DMFI* decayed, missing filled index ; *GI* gingival index; *OR* odds ratio; *PBI* papillary bleeding index; *PD* probing depths; *PDI* periodontal disease index; *PI* plaque index; *SBI*: sulcus bleeding index; *modified SBI* modified sulcus bleeding index; *SOHI* simplified oral hygiene index; *RA* rheumatoid arthritis

Table 5.8 Studies on the relationship between effect of periodontal therapy and disease severity in rheumatoid arthritis

Author, year	Subjects cases/ controls	Periodontitis evaluation	Smoking status	Oral intervention	Results
Al-Katma et al. (2007)	29 subjects with confirmed diagnosis of rheumatoid arthritis and mild-to-moderate chronic periodontitis of at least 3 years' duration (17 test group/12 controls)	PD, CAL, PI, GI, BOP and number of missing teeth	Non-smokers	Oral hygiene instruction + scaling/root planing	There was a statistically significant difference in DAS28 (disease activity score test) (4.3 ± 1.6 vs. 5.1 ± 1.2) and erythrocyte sedimentation rate (31.4 ± 24.3 vs. 42.7 ± 22) between the treatment and the control groups
Ribeiro et al. (2005)	16 cases/26 controls	PI, BOP, PD, CAL	Non-smokers	Oral hygiene instruction and professional tooth cleaning/oral hygiene, instruction and professional tooth cleaning, and additionally, full-mouth scaling and root planing	Erythrocyte sedimentation rate was significantly reduced after scaling and root planing although Rheumatoid factor did not show statistical reductions

BOP bleeding upon probing; *CAL* clinical attachment level; *GI* gingival index; *PD* probing depth; *PI* plaque index

Immunogenetics and Effector Mechanisms of Tissue Destruction in Rheumatoid Arthritis and Periodontitis

Periodontitis has remarkably similar cytokine profiles to RA. As for RA, disease progression seen in periodontitis consists of the continuing presence of high levels of proinflammatory cytokines including IL-1β and TNF-α, and low levels of IL-10 and transforming growth factor b, cytokines that suppress the immunoinflammatory response. Furthermore, low levels of tissue inhibitors of metalloproteinases (MMPs) and high levels of MMPs and PGE_2 secreted by macrophages, fibroblasts and other resident and inflammatory cells describe the active stages of both RA and periodontitis. In both RA and periodontitis, tissue destruction is not unidirectional, but an iterative process that is constantly being adjusted by the host response to inciting agents. The destruction of extracellular matrix in both diseases is determined by the balance of MMPs and their inhibitors. Bone destruction in periodontitis and RA is a result of the uncoupling of the normally coupled processes of bone resorption and bone formation, with PGE_2, IL-1, TNF-α, IL-6 as mediators of bone destruction. It is evident in both diseases that the host's immune response is controlled by genes that regulate differences in the monocyte/T-cell response traits to different antigens that determine both the nature of the protective antibody response and the magnitude of tissue-destructive inflammatory response (Mercado et al. 2003). In patients with RA, high plasma levels of TNF-α were related to gingival Bleeding upon Probing (BOP), more attachment loss and deeper pockets compared to those with low plasma levels (Nilsson and Kopp 2008). It was also recently showed that IL-1 and FcγR gene polymorphisms constitute a common risk factor for RA and periodontitis. However, it was suggested that the distributions of IL-1B + 3954 genotypes and IL-1A + 4845 and IL-1B + 3954 haplotypes were unique to the Japanese patients with RA and periodontitis (Kobayashi et al. 2007). In contrast, Havemose-Poulsen et al. (2007) were unable to identify any associations between the IL-1 polymorphisms and aggressive periodontitis or chronic arthritis.

Etiologic Factors

While most studies investigating the possible etiologic relationship of RA and periodontal disease have focused on shared inflammatory mechanisms, there has been limited attention given to bacterial infections that act

not only as a primary initiator of periodontal disease, but may also play a role in the etiology of RA (Mikuls et al. 2009). A growing number of epidemiologic, serologic and animal model studies provided evidence that *P. gingivalis*, the major etiological agent of periodontal disease, might be involved in the onset and progression of RA (Liao et al. 2009; Mikuls et al. 2009).

There is no question that periodontitis and RA have many pathologic features in common. Emerging evidence suggests a strong relationship between the extent and severity of periodontal disease and RA. While this relationship is unlikely to be causal, it is clear that individuals with advanced RA are more likely to experience more significant periodontal problems compared to their non-RA counterparts, and vice versa. Hence, the possibility exists that both conditions result from a common underlying dysregulation of the host inflammatory response. The precise nature of this dysregulation remains to be established (Bartold et al. 2005).

5.5 Interactions Between Periodontal Disease and Oral Cancer

In developed countries, head and neck cancer represents 5–10% of all malignant diseases. Over the past few decades, there has been a significant increase in oral cancer and related mortality in Europe, especially among young adults. In some developing countries, nearly half of cancer patients have oral cancer, largely due to exposure to carcinogens such as tobacco. In the European Union, forty-two 109 cases of oral cancer were recorded in males in 1998, with 15,744 deaths, and 11,447 cases in females, with 4,434 deaths (Campo-Trapero et al. 2008). The American Cancer Society estimated 30,990 new oral cancers and 7,320 deaths from these cancers in 2006. About 50% of those who are diagnosed this year will die within 5 years. Because of the well-recognized phenomenon of "field cancerization" in the head and neck region, persons with primary tumors of the oral cavity and pharynx are also more likely to develop cancers of the esophagus, larynx, lung, and stomach. In addition, those with oral cancer often have multiple primary lesions and have up to a 20-fold increased risk of having a second primary oral cancer (Tezal et al. 2005).

The two main risk factors for oral cancer are smoking and alcohol abuse. There is a synergistic effect among these factors and a direct relationship between the level of abuse and exposure to these substances. However, other factors have also been associated with oral and oropharyngeal cancer, among which are biologic agents such as the Human Papilloma Virus, precarious oral hygiene, previous history of respiratory or digestive tract tumor and excessive exposure to ultraviolet light (lip cancer). Most oral cancer cases are diagnosed at later stages of the disease. Self-examination and periodic visits to a trained professional are recommended for earlier diagnosis (de Rezende et al., 2008). Several studies have evaluated the possible link between tooth loss, periodontal disease and oral cancer (Table 5.9).

Chronic infections, such as periodontitis, can play a direct or indirect role in carcinogenesis (Tezal et al. 2005):

1. Direct toxic effect of microorganisms: microorganisms and their products such as endotoxins (lipopolysaccharides), enzymes (proteases, collagenases, fibrinolysin, and phospholipase A), and metabolic by-products (hydrogen sulfide, ammonia, and fatty acids) are toxic to surrounding cells and may directly induce mutations in tumor suppressor genes and protooncogenes, or alter signaling pathways that affect cell proliferation and/or survival of epithelial cells (Tezal et al. 2005);
2. Indirect effect through inflammation: chronic infection may stimulate the formation of epithelial-derived tumors through an indirect mechanism involving activation of surrounding inflammatory cells. Inflammation exposes epithelial cells to substances with mutagenic potential. Microorganisms and their products activate host cells such as neutrophils, macrophages, monocytes, lymphocytes, fibroblasts, and epithelial cells to (a) generate reactive oxygen species (hydrogen peroxide and oxy radicals), reactive nitrogen species (nitric oxides), reactive lipids and metabolites (malondialdehyde and 4-hydroxy-2-nonenal), and matrix metalloproteases, which can induce DNA damage in epithelial cells and (b) produce cytokines, chemokines, growth factors, and other signals that provide an environment for cell survival, proliferation, migration, angiogenesis, and inhibition of apoptosis. This environment may help epithelial cells to accumulate mutations and drive these mutant epithelial cells to proliferate, migrate, and give them a growth advantage (Tezal et al. 2005).
3. Increased production of carcinogenic nitrosamines is another plausible mechanism, which may explain the reported observations. The formation of endogenous nitrosamines in the oral cavity by nitrate-reducing bacteria is promoted by poor oral hygiene and periodontal disease, as well as by tobacco use

Table 5.9 Summary of studies evaluating the possible link between tooth loss, periodontal disease and oral cancer

Author, year, country	Subjects, n	Type of the study	Oral cancer diagnostic	Criteria for periodontitis	Major findings	Adjusted factors
Zheng et al. (1990) People's Republic of China	404 case/control pairs	Case-control	Cancer of the tongue and cancer of other parts of the mouth, excluding salivary gland; pharynxi ncluding nasopharynx	Missing teeth, presence of gingivitis and periodontitis	Missing teeth emerged as a strong risk factor for oral cancer: the OR for those who had lost 15–32 teeth compared to those who had lost none was 5.3 for men and 7.3 for women and the trend was significant ($P < 0.01$) in both genders	Age, gender, tobacco smoking and alcohol consumption
Bundgaard et al. (1995) Denmark	161 cases/400 controls	Case-control	Intra-oral squamous cell carcinoma	Missing teeth	Persons with less than five teeth had an OR of 2.4 (CI: 1.3–4.1) compared with persons with 15 or more teeth	Age, gender, tobacco smoking and alcohol consumption
Talamini et al. (2000) Italy	132 cases/148 controls	Case-control	Tongue, mouth, oro-pharynx and unspecified oral cancer	Missing teeth	The number of missing teeth was not significantly associated to oral cancer risk (OR = 1.4; 95% CI: 0.6–3.1), whereas a poor general oral condition was 4.5-fold (95% CI: 1.8–10.9) more frequent among cases than control subjects	Age, gender, smoking and drinking habits, prior occurrence of sexually transmitted and other infections, cancer family history, and a dietary habits
Garrote et al. (2001) Cuba	200 cases/200 controls	Case-control	Cancer of the oral cavity and oro-pharynx, prior to any cancer treatment	Missing teeth	The number of missing teeth was significantly higher among oral cancer cases compared to control subjects (OR for > 16 missing teeth = 2.7; 95% CI: 1.2–6.1). After allowance for education, smoking and drinking habits, poor general oral condition was 2.6-fold (95% CI: 1.2–5.2) more frequent among cases than control subjects	Age, sex, area of residence, education, and smoking and drinking habits

Table 5.9 (continued)

Author, year, country	Subjects, n	Type of the study	Oral cancer diagnostic	Criteria for periodontitis	Major findings	Adjusted factors
Rosenquist et al. (2005) Sweden	132 cases/320 controls	Case-control	Oral (tongue, floor of the mouth) and oropharyngeal squamous cell carcinoma	Missing teeth, height and configuration of the interproximal alveolar bone on panoramic radiographs	Average (OR = 3.0; CI: 1.7–5.1) and poor (OR = 10.0; CI: 5.1–20.1) visible plaque scores as indicators of oral hygiene were significant risk factors in unadjusted analysis; More than 20 missing teeth was a statistically significant risk factor in unadjusted (OR = 6.1; CI 2.7–14.0) and adjusted (OR = 3.4; CI: 1.4–8.5) analysis	Tobacco and alcohol consumption
Tezal et al. (2007) USA	51 cases: non-Hispanic white men/54 healthy men controls	Case-control	Primary squamous cell carcinoma of the tongue	Severity of alveolar bone loss from panoramic radiographs	Each millimeter of alveolar bone loss was associated with a 5.23-fold increase in the risk of tongue cancer (OR = 5.23; 95% CI: 2.64–10.35)	Age at diagnosis, race, ethnicity, smoking status, and number of teeth
Tezal et al. (2005b) USA	13,798 subjects aged 20 years and older with at least six natural teeth and who participated in NHANES III survey	Cohort	(1) Tumor (nonspecific); (2) precancerous lesions; and (3) any oral soft tissue lesion.	Clinical attachment level loss	CAL was not related to the presence of any soft tissue lesion (OR = 1.09, 95% CI: 0.91–1.31), but was specifically related to the presence of tumor (OR = 4.57, 95% CI: 2.25–9.30) and precancerous lesions (OR = 1.55, 95% CI: 1.06–2.27)	Age, gender, race/ethnicity, education, tobacco, alcohol, occupational hazard, and interaction term "tobacco x occupational hazard"
de Rezende et al. (2008) Brazil	50 cases/50 cancer-free subjects as controls	Case-control	Untreated oral and oropharyngeal squamous cell carcinoma	Community periodontal index of treatment needs (CPITN)	Periodontal examination and the CPITN show a significant difference among cancer patients, as 76% of them had advanced periodontal disease and periodontal pockets deeper than 6 millimeters. In contrast, only 10% of the control group members had similar degrees of disease	Age and gender

CAL: clinical attachment level; *CI* confidence intervals; *OR* odds ratio.

and certain dietary factors. Tooth loss resulting from poor oral hygiene may also contribute to greater nitrosamine production (Meyer et al. 2008).

5.6 Interactions Between Periodontal Disease and Inflammatory Bowel Disease (IBD)

IBD is characterized by chronic, relapsing intestinal inflammation producing debilitating symptoms of diarrhea, abdominal pain and malnutrition. Its main forms are ulcerative colitis, where intestinal inflammation is limited to the colon, and Crohn's disease, which can affect any part of the gastro-intestinal tract – most typically the terminal ileum and colon. As for most auto-inflammatory conditions, it is thought that genetic, immune and environmental factors combine to affect the disease predisposition and its course (Zhang et al. 2008).

Oral soft-tissue lesions associated with Crohn's disease can precede or occur concomitantly with the intestinal symptoms. Cobblestone architecture of the mucosa, aphthous-like ulcerations, nonspecific swellings of the mucosa and lips, and lymphadenopathy have been observed (Grössner-Schreiber et al. 2006). A high prevalence of caries has also been reported in Crohn's disease patients (Brito et al. 2008) (Table 5.10).

Several studies have evaluated the prevalence of periodontal lesions in IBD patients (Flemmig et al. 1991; Grössner-Schreiber et al. 2006; Brito et al. 2008). Flemmig et al. (1991) assessed the prevalence and severity of periodontal disease in patients with IBD. The periodontal status of 107 consecutive patients seeking treatment for IBD was evaluated. Examination of the mid- and mesiobuccal aspects of one quadrant on one jaw and the contralateral quadrant of the opposite jaw revealed that 93.5% of Crohn's disease patients and 95.1% of ulcerative colitis patients had at least one site with probing attachment loss of 2 mm or greater, and a mean probing attachment loss 1.4 ± 0.9 mm and 1.5 ± 1.0 mm, respectively. It was found that 28.3% of Crohn's disease patients and 29.5% of ulcerative colitis patients possessed at least one site with a pocket probing depth of 4 mm or greater; the mean pocket probing depth in these patients was 2.4 ± 0.2 and 2.3 ± 0.2 mm, respectively. Compared with the assessment of Oral Health of United States Adults, IBD patients revealed a 11.9% higher prevalence ($P < 0.01$) but 0.6 mm lower severity ($P < 0.01$) of periodontal disease. The authors suggested that there are no clinical implications for the management of periodontal disease in IBD subjects.

Grössner-Schreiber et al. (2006) compared the periodontal disease in 62 patients with IBD and 59 healthy controls. They found similar number of PD in patients with IBD (2.08 mm) and the control population (2.23 mm), although the percentage of subjects with at least one site of all sites measured with CAL > 4 and > 5 mm, respectively, demonstrated significantly higher numbers for the IBD group. The authors did not do any distinction between ulcerative colitis and Crohn's disease.

More recently, Brito et al. (2008) reported that ulcerative colitis patients had significantly fewer teeth ($P < 0.002$), deeper PD ($P < 0.0001$), more CAL ($P = 0.004$), more subjects with periodontitis ($P < 0.001$) and more sites with CAL > 3 mm than controls ($P = 0.007$). Crohn's disease patients showed less sites with plaque ($P = 0.017$) and BOP ($P = 0.038$), deeper PD ($P < 0.0001$), and there were more subjects with periodontitis ($P = 0.03$) when compared with the controls. These differences with the previous studies could be also explained by using the whole-mouth examination (six sites per tooth in all existing teeth except for the third molars), which is currently considered the gold standard. It was also revealed by Brito et al. (2008) that among the study groups smoking was an effect modifier: there was no difference in the prevalence of periodontitis among non-smoking control subjects and non-smoking subjects with Crohn's disease or ulcerative colitis, but the prevalence of periodontitis was greater among smokers with ulcerative colitis than smokers without ulcerative colitis.

It was suggested that the periodontal manifestations of IBD disease, despite the fact that the patients were taking a systemic anti-inflammatory drug therapy, may be in part attributed to the decrease in neutrophil function (a serum-mediated inhibition of neutrophil chemotaxis was demonstrated), as well as to an unusual microorganisms colonizing the oral cavity. Microbiologic studies of the periodontal flora of IBD-affected patients revealed a unique microflora composed predominantly of small, motile, Gram-negative rods, which were most consistent with the genus *Wolinella* (van Dyke et al. 1986).

Table 5.10 Summary of studies evaluating the interactions between periodontitis and inflammatory bowel disease (IBD)

Author, year	No patients cases/ controls	IBD diagnosis	Periodontal disease evaluation	Smoking status	Medical treatment of IBD patients	Results
Grössner-Schreiber et al. (2006) Germany	62/59 Mean age 38.4 ± 10.3 years	IBD	PI, BOP, PD, CAL in whole-mouth examination	Smokers and non-smokers	Corticosteroids, immunosu-pressants (azathioprine, methotrexate), aminosalicy-late), anti- tumor necrosis factor alpha and antibiotics ($n = 512$) as mono- or combination therapy	The mean PD in patients with IBD was 2.08 vs. 2.23 mm in controls ($P = 0.014$). Compared with controls, patients with IBD had more sites with CAL of at least 4 mm (81 vs. 64% in controls, $P = 0.07$) and 5 mm (63 vs. 46%, $P = 0.07$), respectively
Brito et al. (2008)	99 CD (39.0 ± 12.9 years), 80 UC (43.3 ± 13.2 years) and 74 healthy controls (40.3 ± 12.9 years)	Crohn's disease and ulcerative colitis	PI, BOP, PD, CAL assessed at four sites of all teeth in two quadrants	Smokers and non-smokers	Aminosalicylates, immunomodu-lators and corticosteroids as mono- or combination therapy	Significantly more patients with UC (90.0%; $P < 0.001$) and CD (81.8%; $P = 0.03$) had periodontitis than controls (67.6%). Among smokers, UC had greater median PD (2.2 vs. 1.7 mm; $P < 0.0001$) than controls. Among non-smokers, CD (2.4 mm; $P < 0.0001$) and UC showed deeper pockets (2.3 mm; $P < 0.0001$) compared with controls (1.5 mm)

BOP bleeding on probing; CAL clinical attachment level; PD probing depth; PI:plaque index

5.7 Interactions Between Periodontal Disease and Alzheimer Disease (AD)

AD is one of the leading causes of dementia afflicting the elderly. In the United States, approximately 4.5 million patients are currently diagnosed with AD. The prevalence of AD increases with age from 4% in the 65 to 75 years age group to 19% in the 85 to 89 years age group, and the incidence of AD increases from 7/1000 in the 65 to 69 years age group to 118/1000 in the 85 to 89 years age group (Kamer et al. 2008).

AD is a chronic progressive neurodegenerative disorder characterized by three primary groups of symptoms. The first group (cognitive dysfunction) includes memory loss, language difficulties, and executive dysfunction (that is, loss of higher level planning and intellectual coordination skills). The second group comprises psychiatric symptoms and behavioral disturbances – for example, depression, hallucinations, delusions, agitation – collectively termed non-cognitive symptoms. The third group comprises difficulties with performing activities of daily living (deemed "instrumental" for more complex activities such as driving and shopping, and "basic" for

dressing and eating unaided). The symptoms of AD progress from mild symptoms of memory loss to very severe dementia. Increasingly, the coexistence of vascular disease and AD is being recognized clinically, pathologically and epidemiologically (Burns and Iliffe 2009).

The cause of AD is unknown, but case-control studies have linked several risk factors with the disease, including age, family history, apolipoprotein E4 status, head injury, depression, hypertension, diabetes, high cholesterol, atrial fibrillation, presence of cerebral emboli and low physical and cognitive activity (Burns and Iliffe 2009).

On the basis of the contribution of moderate to severe periodontitis to systemic inflammation and the potential role of inflammation in the etiology and progression of AD, Kamer et al. (2008) proposed that chronic periodontitis might be a risk factor in the incidence and progression of AD. Periodontitis is a chronic inflammatory disease resulting in years of locally increased proinflammatory molecules that surround the trigeminal cranial nerve endings. Periodontitis also results in years of systemic host exposure to proinflammatory cytokines and other systemic markers of inflammation such as CRP. Therefore, hypothetically, periodontal-derived cytokines could reach the brain by both systemic and neural pathways and amplify brain cytokine pools. Periodontal pathogens associated with moderate to severe periodontitis are Gram-negative anaerobic species, rich in endotoxin/LPS that can stimulate proinflammatory cytokines and CD14 activity. In addition, several bacteria associated with severe or progressive periodontitis are capable of invading tissues, including *A. actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Treponema* (*T.*) *denticola. T. denticola* is from the same class as *Treponema palidum*, known to invade brain tissue and to induce chronic inflammation, cortical atrophy and amyloid deposition in subjects with syphilis. In fact, *Treponema sp*. have been detected in the trigeminal ganglia, brainstem, and cortex of the human brain, and AD donors were more likely to have *Treponema* and more *Treponema sp*. than controls, suggesting that oral bacteria are capable of invading brain tissue perhaps via peripheral nerve fibers (Kamer et al. 2008).

Inflammation is a prominent component of both AD and chronic periodontitis. Hyper-inflammatory genotypes, as evidenced by IL-1α-889 and IL-1β+3953 polymorphisms, have been associated with both AD and chronic periodontitis. Another possibility exists that periodontitis and AD, although separate entities, converge to a common pathogenic base (effector), a hyper-inflammatory response to β-amyloid peptide in AD and to periodontal pathogens in periodontitis (Kamer et al. 2008).

5.8 Assessing Confounding and Effect Modification in Research Involving Periodontal Disease and Systemic Diseases

Although confounding and effect modification are basic concepts in quantitative research, they are not always treated in an appropriate manner in epidemiological studies on the association between periodontal and systemic diseases (Ylöstalo and Knuuttila 2006). Two recent papers have explored the concepts of confounding and effect modification and illustrated the importance of recognizing and properly accounting for them in the design and analysis of studies of periodontal diseases (Hyman 2006; Ylöstalo and Knuuttila 2006).

The term confounding refers to a situation where the categories of the exposure variable are different in relation to extraneous determinants. Confounder is defined as "an extraneous determinant of the outcome parameter in terms of which there is lack of comparability of the effects and/or populations." For example, gender, age, health behavior, and socioeconomic status are often associated with the outcome and unevenly distributed among exposed and unexposed subjects causing confounding (Ylöstalo and Knuuttila 2006).

The following criteria to identify actual confounders can be used: (1) it must be predictive for the disease, (2) it must be associated with the exposure under study, and (3) it must not be a link in the causal path between the exposure and the outcome. As the underlying causal models can be highly complex, the selection of confounders requires theoretical knowledge about their relation. Confounding can also be evaluated and controlled in the analysis by using multivariate models, stratification or, in some cases, by standardization. A change in the magnitude of a parameter estimate in models with and without a potential confounder could be used to assess the magnitude of confounding. In the study design, confounding can be controlled by randomization (experimental studies), restriction or by matching either at an individual level or at a group level (observational studies) (Ylöstalo and Knuuttila 2006).

Effect modification is the inconstancy in the magnitude of the effect across levels of another subject characteristic, while an effect modifier is a subject characteristic on which the effect depends. Effect modification can be assessed using regression models or statistical tests such as a test of heterogeneity (Ylöstalo and Knuuttila 2006). Although confounding and effect modification are basic concepts in quantitative research, they are not always treated in an appropriate manner in epidemiological studies on the association between periodontal and systemic diseases.

Four lines of evidence suggests that the observed periodontitis–systemic disease associations are, in part, a result of confounding by smoking – the inability to distinguish the effect of smoking on periodontitis from the effect of smoking on systemic diseases. First, no periodontitis–systemic disease associations have been identified among never-smokers. Second, periodontitis and smoking mimic one another with respect to the types of diseases with which they are associated (e.g. lung cancer and Parkinson's disease). Third, only studies with inadequate adjustment for smoking report significant periodontitis–systemic disease associations. Finally, elimination of dental infection, unlike smoking cessation, does not reduce coronary heart disease risk (Hujoel et al. 2002).

A systematic evaluation of the periodontitis–systemic associations among healthy never-smokers would help to indicate in which direction, if any, periodontitis-systemic disease research should progress (Hujoel et al. 2002; Spiekerman et al. 2003).

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Genetic Variability and Periodontal Disease

6

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There are estimated to be 25,000–50,000 different genes in the human genome. Evidence for a genetic predisposition to periodontitis comes from four areas of research: (1) the study of inherited diseases and genetic syndromes; (2) family studies; (3) twin studies; and (4) population studies. Genetic polymorphisms thus far studied with gingivitis, chronic and aggressive periodontitis are presented.

6.1 Evidence for the Role of Genetic Variants in Periodontitis

There are estimated to be 25,000–50,000 different genes in the human genome. Genes can exist in different forms or states. Geneticists refer to different forms of a gene as *allelic* variants or *alleles*. Allelic variants of a gene differ in their nucleotide sequences. When a specific allele occurs in at least 1% of the population, it is said to be a genetic *polymorphism*. Two or more alleles for a given locus may exist in nature throughout evolution, but may develop at any time. A polymorphic locus is one whose alleles are such that the most common, normal variant (*N-allele*) among them occurs with 99% frequency in the population. Thus, if a locus is for example bi-allelic, the rarer allele (designated *R-allele*) must occur with a frequency of 41% in the population. In this way, when different alleles of a given gene coexist in the human population, we speak about genetic polymorphisms (Loos et al. 2005).

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Polymorphism arises as a result of mutation. The different types of polymorphism are typically referred to by the type of mutation that created them. The simplest type of polymorphism results from a *single base* mutation, which substitutes one nucleotide for another. The polymorphism at the site harboring such changes has recently been termed a "*single nucleotide polymorphism (SNP),*" although previously, in some instances, such variation was referred to by the particular methods used to detect it. Digestion of a piece of DNA containing the relevant site with an appropriate restriction enzyme could then distinguish alleles or variants based on resulting fragment sizes via electrophoresis, and this type of polymorphism was thus referred to as "*restriction fragment length polymorphism (RFLP)*" (Schork et al. 2000; Loos et al. 2005).

The SNP may have no effects or may have some important biological effects. For example, if a transition has taken place within the coding region of a gene, it may result in an amino acid substitution and therefore an altered protein structure, which may then alter its function. Or, when such mutations have taken place in the promoter region of the gene, it may alter gene regulation, for example, resulting in (completely) inhibited or reduced gene expression or, alternatively, resulting in over-expression of the gene, perhaps with biological consequences. SNPs occur more frequently than any other type of genetic polymorphism; the frequency of SNPs across the human genome is estimated at every 0.3–1 kilobases (kb) (Schork et al. 2000; Loos et al. 2005).

Other types of genetic polymorphism result from the insertion or deletion of a section of DNA. The most common type of such "*insertion: deletion*" polymorphism is the existence of variable numbers of repeated base or nucleotide patterns in a genetic region. Repeated base patterns range in size from several hundreds of base pairs, known as "*variable number of tandem repeats*"

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(*VNTRs or "minisatellites"*), to the more common "microsatellites" consisting of two, three or four nucleotides repeated some variable number of times. Microsatellites are often referred to as "*simple tandem repeats*" (*STRs*). Repeat polymorphisms often result in many alleles or variants (e.g., several different repeat sizes) within the population and are thus considered "*highly polymorphic*." This can be extremely useful for population genetic studies since the probability that two individuals from different populations (ethnic groups, diseased vs. non-diseased populations, etc.), will have the same number of repeats can be quite low. The genome-wide frequency estimates for STRs are difficult to come by, though a range of figures of one STR every 3–10 kb seems reasonable (Schork et al. 2000; Loos et al. 2005).

Another type of insertion: deletion polymorphism involves the presence or absence of Alu segments at a genetic location. Alu segments are named according to the restriction enzyme used to detect them (e.g., AluI), and contain two sequences, approximately 120–150 bases in length, separated by an A base-rich segment. Insertions of this type occur approximately every 3 kb on average. Large insertion:deletion polymorphism such as Alu insertions are easy to identify and genotype, given the large differences in resulting amplified fragments (Schork et al. 2000; Loos et al. 2005).

Genetic polymorphisms are very useful in studies of population genetics. After genotyping individuals and assessing genotype frequencies among groups of interest, one can also calculate the frequency of the N-allele and the R-allele among the groups or populations under study. Frequencies of genotypes and alleles may differ between a diseased group and a healthy group. Subsequently, when a given allele is identified to be associated with disease, functional studies can be started to investigate the possible role of that gene in the aetiology and pathogenesis of the disease (Loos et al. 2005).

Evidence for a genetic predisposition to periodontitis comes from four areas of research: (1) the study of inherited diseases and genetic syndromes; (2) family studies; (3) twin studies; and (4) population studies.

6.1.1 Study of Inherited Diseases and Genetic Syndromes

Evidence for the role of specific genes in disease may be gleaned from the study of inherited conditions or genetic disorders, in which the disease is pathognomonic. A number of monogenic syndromes with accompanying severe periodontal disease have been reported in the literature (acatalasia, hypophosphatasia, Chédiak-Higashi syndrome, chronic neutropenia, leukocyte adhesion deficiency, cyclic neutropenia, Ehlers-Danlos syndrome, Papillon-Lefèvre syndrome) (Hodge and Michalowicz 2001).

A commonality of these conditions is that they are inherited as Simple Mendelian traits and are usually due to genetic alterations of a single gene locus. The significance of these conditions is that they clearly demonstrate that a genetic mutation at a single locus can impart susceptibility to periodontitis. Additionally, these conditions illustrate that this genetic susceptibility may segregate by different transmission patterns. The fact that the altered proteins function in different structural and immune pathways indicates that genetic modulation of a variety of different genes can affect a variety of different physiological and cellular pathways, imparting susceptibility to pathological consequences in the periodontium in individuals with appropriate microbial challenges. These conditions illustrate that genetic contributions to periodontitis susceptibility are multifaceted and may potentially involve many different gene loci. However, in contrast to nonsyndromic forms of periodontitis, these conditions have periodontal disease manifestations as part of a collection of syndromic manifestations. In most cases of aggressive periodontitis, individuals present with clinical manifestations of periodontitis, but do not appear to have any other clinical disease manifestations (Kinane and Hart 2003).

6.1.2 Family Studies

There is literature reporting familial aggregation of periodontal diseases, but, due to different terminology, classification systems and lack of standardized methods of clinical examination, it is difficult to compare reports directly. Although periodontal disease nosology has changed many times over the timeframe of these reports, most familial reports for periodontitis are for early-onset forms now called aggressive periodontitis (Stabholz et al. 1998). This aggregation within families strongly suggests a genetic predisposition. It must be borne in mind that familial patterns may reflect

exposure to common environmental factors within these families. Thus it is important to consider the shared environmental and behavioral risk factors in any family. These would include education, socio-economic grouping, oral hygiene, possible transmission of bacteria, diseases such as diabetes and environmental features such as passive smoking, sanitation, etc. Some of these factors, such as lifestyle and behavior and education, may be under genetic control and may influence the standard of oral hygiene. The complex interactions between genes and the environment must also be considered in the evaluation of familial risk for the periodontal diseases (Kinane and Hart 2003).

In chronic periodontitis, the phenotype or disease characteristics do not present significantly until the third decade of life, whereas in the aggressive forms of periodontal disease, the presentation can occur in the first, second, third, and fourth decades. This variability in presentation of significant signs of disease makes diagnosis difficult, not only in declaring if a patient suffers from the disease but also in detecting patients who do not suffer from the disease, and differentiating between adult and aggressive forms of periodontitis (Kinane and Hart 2003).

6.1.3 Twin Studies

Studying phenotypic characteristics of twins is a method of differentiating variations due to environmental and genetic factors. Monozygous twins arise from a single fertilized ovum and are therefore genetically identical and always the same sex. Dizygous twins arise from the fertilization of two separate ova and share, on average, one half of their descendent genes in the same way as siblings do. Any discordance in disease between monozygous twins must be due to environmental factors. Any discordance between dizygous twins could arise from environmental and/or genetic variance. Therefore, the difference in discordance between monozygous and dizygous twins is a measure of the effects of the excess shared genes in monozygous twins when the environmental influence is constant (Hodge and Michalowicz 2001).

Based on 110 paires of adult twins, a significant genetic component was identified, suggesting that 38–82% of the population variance for probing depth (PD), attachment loss and dental plaque may be attributed to genetic factors (Michalowicz et al. 1991). A study by Corey et al. (1993) of self-reported periodontal health among 4,908 twin pairs found a history of reported periodontal disease in 420 individuals who were members of 116 monozygotic (MZ) and 233 dizygotic (DZ) twin pairs. The mean age at diagnosis in this sample was 31.4 ± 0.7 years and was significantly earlier in females than males (30.1 vs. 33.0 years, $P < 0.025$). Proband-wise concordance rates were 0.38 for MZ and 0.16 for DZ twins. A subsequent study on 117 pairs of adult twins (64 MZ and 53 DZ pairs) revealed that approximately half of the variance in disease in the population is attributed to genetic variance. PD, clinical attachment level (CAL), plaque, and gingivitis (GI) were assessed on all teeth by two examiners. Measurements were averaged over all sites, teeth and examiners. Extent of disease in subjects was defined at four thresholds: the percentage of teeth with CAL ≥ 2, CAL ≥ 3, PD ≥ 4, or PD ≥ 5 mm. Genetic and environmental variances and heritability were estimated using path models with maximum likelihood estimation techniques. MZ twins were more similar than DZ twins for all clinical measures. Statistically significant genetic variance was found for both the severity and extent of disease. Adult periodontitis was estimated to have approximately 50% heritability, which was unaltered following adjustments for behavioral variables including smoking. In contrast, while MZ twins were also more similar than DZ twins for GI scores, there was no evidence of heritability for GI after behavioral covariates such as utilization of dental care and smoking were incorporated into the analyses (Michalowicz et al. 2000).

6.1.4 Population Studies

Environmental or behavioral risk factors for a disease are often first detected in large epidemiological or population-based studies. In genetic epidemiology, similar approaches can be used to identify genetic risk factors for disease. The frequencies of polymorphisms of candidate genes, whose protein products play a role in the inflammatory or immune response, can be compared between cases and controls. A genetic polymorphism is the long-term occurrence in a population of two or more genotypes that could not be maintained by recurrent mutation. A significant difference in the frequency

of a specific polymorphism between a disease group and a control group is the evidence that the candidate gene plays some role in determining susceptibility to disease. An association indicates that either the candidate gene directly affects disease susceptibility or that it is in linkage disequilibrium with (very close to) the disease locus. This method can help to elucidate the pathogenesis of a disease process, identify causal heterogeneity and ultimately identify individuals most at risk for disease. In population studies, it is important to clearly define disease status. Likewise, because of the possibility of racial heterogeneity, it is important to insure that patient and control groups are racially matched (Hodge and Michalowicz 2001).

6.2 A Gene Mutation of Major Effect on Human Disease and its Association with Periodontitis

Diseases that follow predictable and generally simple patterns of transmission have been called "Mendelian" conditions. The name reflects the fact that these diseases occur in simple patterns in families, and in most cases, a single gene locus is the major determinant of the clinical disease phenotype. These diseases follow a classic Mendelian mode of inheritance (autosomal-dominant, autosomal-recessive, or X-linked). Usually, the prevalence of these Mendelian conditions is rare (typically, much less than 0.1%), with the exception of some unique populations that have been isolated from other human populations. When the genetic basis of a Mendelian condition is identified, it is often found that the condition results from the effect of a genetic mutation at a single gene locus. The disease phenotype usually occurs over a broad range of environments, and although environmental factors and other genes can modify them, in many cases, they manifest in a remarkably similar way (Kinane and Hart 2003).

Table 6.1 lists a series of genetic syndromes known to be associated with either premature tooth loss due to periodontitis or a phenotype resembling aggressive periodontitis. The significance of these conditions is that they clearly demonstrate that a genetic mutation at a single locus can impart susceptibility to periodontitis. Additionally, these conditions illustrate that this genetic susceptibility may segregate by different transmission patterns. The fact that the altered proteins function in different structural and immune pathways indicates that genetic modulation of a variety of different genes can affect a variety of different physiological and cellular pathways, imparting susceptibility to pathological consequences in the periodontium in individuals with appropriate microbial challenges. These conditions illustrate that genetic contributions to periodontitis susceptibility are multifaceted and may potentially involve many different gene loci. However, in contrast to nonsyndromic forms of periodontitis, these conditions have periodontal disease manifestations as part of a collection of syndromic manifestations. In most cases of aggressive periodontitis, individuals present with clinical manifestations of periodontitis, but do not appear to have any other clinical disease manifestations. This is not inconsistent with a genetic disease etiology. Expression of genes can vary in different tissues, and mutations of a ubiquitously expressed gene can result in a tissue specific condition (Kinane and Hart 2003).

6.3 Modifying Disease Genes in Relation to Periodontitis

Most common diseases have a complex genetic etiology. In contrast to the relatively simple monogenetic diseases discussed previously, complex genetic diseases are not due to a single gene defect. In complex diseases, genetic variants at multiple gene loci contribute to overall disease liability. As such, a cause-and-effect relationship between a particular genetic allele and a disease is not possible. In these cases, a genetic allele is found to be statistically associated with disease more than is found in unaffected individuals. This mathematical association is not necessarily biological or physiological. Studies reporting association vary in design and rigor from reports of an association in only a few individuals in a family (which are not statistically validated to be associated in a general population) to large population-based studies. Association studies ideally evaluate large numbers in population- based studies, and thus have the power to detect a significant association. Issues of allele frequency in the population studied, case-control design, and population stratification are very important, but unfortunately are often omitted from dental studies (Kinane and Hart 2003).

Table 6.1 Examples of syndromic forms of periodontitis in which inheritance is Mendelian and due to a genetic alteration at a single gene locus

Disease	Genetic defect	Phenotype	Inheritance	References regarding periodontal condition
Papillon-Lefèvre syndrome	Cathepsin C	Prepubertal periodontitis	Autosomal recessive	Kressin et al. 1995; Hattab et al. 1995; Ghaffer et al. 1999; Lundgren et al. 1998; Velazco et al. 1999; Ishikawa et al. 1994; Rudiger and Berglundh 1999
Haim-Munk syndrome	Cathepsin C	Prepubertal periodontitis	Autosomal recessive	Hart et al. 2000a, b, 1997; Deas et al. 2003
Ehlers-Danlos syndrome	Collagen	Early-onset periodontitis/ localized juvenile periodontitis	Autosomal dominant	Perez et al. 2002
Cyclic neutropenia	Neutrophil elastase	Early-onset periodontitis	Autosomal dominant	Kinane 1999; Deas et al. 2003
Chronic familial neutropenia	Defect unknown	Early-onset periodontitis	Autosomal dominant	Kinane 1999
Chediak-Higashi syndrome	Lysosomal trafficking regulator gene	Severe periodontitis	Autosomal recessive	Steenberghe 1997
Leukocyte adhesion deficiency type 1	Leukocyte chain adhesion molecule CD18	Prepubertal periodontitis	Autosomal recessive	Dixon et al. 2004; Majorana et al. 1999; Waldrop et al. 1995
Leukocyte adhesion deficiency type II (LADII)/ congenital disorder of glycosylation type IIc	GDP-fucose transporter-1		Autosomal recessive	

Currently, very little is known about which genes may be involved in periodontitis as disease modifying genes (Loos et al. 2005)

6.3.1 Heritability of Gingivitis

Periodontal diseases (gingivitis and periodontitis) are inflammatory processes of the gingival and supporting structures of the teeth induced by a microbial biofilm, but individual differences in the host immune response to infection may affect the susceptibility and severity of disease. Gingivitis (GI) is mainly related to plaque and calculus and leads to a local inflammatory response, which, however, is unable to eliminate the microbial products completely, and chronic progression may turn into periodontitis (Vokurka et al. 2009).

Growing evidence suggests that gingival inflammation may represent a true risk factor for attachment loss and tooth loss, and that an association may exist between susceptibility to GI and susceptibility to periodontitis. In this context, it is feasible that genes implicated in the regulation of inflammatory process of periodontal tissues associated with plaque accumulation may play a role in explaining the individual variability in the severity of both plaque-induced GI and destructive periodontitis (Dashash et al. 2007).

The degradation of collagen fibers and extracellular matrix components results from the activity of matrix metalloproteinases (MMPs). MMPs, structural and functional family of proteolytic enzymes, may play an important role in tissue remodeling and repair associated with development of inflammatory response. Periodontal disease development and progression can be caused by MMPs produced by both infiltrating and

resident cells of the periodontium. One of the most important MMPs, MMP-9 (also known as gelatinase B or 92-kD type IV collagenase), is active against collagens and proteoglycans. The coding gene was located on chromosome 20q11.2–q13.1 and several polymorphisms have been detected in the MMP-9 gene (Vokurka et al. 2009). In a study group of 298 Caucasian children, aged 11–13 years, Vokurka et al. (2009) found significant differences in −1562T allele frequencies for MMP-9 polymorphism (−1562C > T), but the allele frequencys were not significant for IL-18 variant (−607A > C). Furthermore, a highly significant association of the composite genotype (formed by the variants of the both genes) with GI was found ($P = 0.004$, $p_{corr} < 0.05$).

Because of the reported inter-individual differences in the gingival response to plaque accumulation, several investigators tested the association between IL-1 genotype and the severity of gingival Inflammation, but contrasting results were obtained (Jepsen et al. 2003; Cullinan et al. 2001; Goodson et al. 2000; Scapoli et al. 2005a; Müller and Barrieshi-Nusair 2007).

Interleukin-1 receptor antagonist (IL-1Ra) is an endogenous receptor and an anti-inflammatory cytokine, which is able to block the action of IL-1α and IL-1β by modulating their biological effects and preventing signal transduction. A significant association was observed between IL-1Ra gene polymorphism (a variable number of 86 bp tandem repeat [VNTR] located in the second intron of IL-1Ra gene) and GI in children (Dashash et al. 2007). The IL-1RN*2 allele (A2; two repeats of VNTR) was significantly more frequent in controls (37%) compared to children with GI (22%). In addition, the carriage of A2 seemed to be protective against GI, and it was more frequent in controls (60%) compared to children with GI (40%; $P = 0.008$). Moreover, multiple logistic regression analysis showed that the association between IL-1Ra gene polymorphism of VNTR and GI in children remained significant ($P = 0.014$) regardless of the significant influence of plaque ($P = 0.013$).

Genetic polymorphisms thus far studied with GI are listed in Table 6.2.

6.3.2 Heritability of Aggressive Periodontitis

Aggressive periodontitis is less common than chronic periodontitis and principally affects young patients. It occurs in localized and generalized forms that differ in many respects with regard to their etiology and pathogenesis. Localized aggressive periodontitis (LAP) and generalized aggressive periodontitis (GAP) were previously called "*localized and generalized juvenile periodontitis*" or "*early-onset periodontitis*", respectively. Features of aggressive periodontitis that are common to both the localized and generalized forms of the disease are (Armitage 2004):

- *Primary features:* Except for the presence of periodontitis, patients are otherwise clinically healthy; Rapid attachment loss and bone destruction; Familial aggregation.
- *Secondary features (often present):* Amounts of microbial deposits are inconsistent with the severity of periodontal tissue destruction; Elevated proportions of *Aggregatibacter actinomycetemcomitans* and, in some populations, *Porphyromonas gingivalis* may beelevated;Phagocyteabnormalities;Hyperresponsive macrophage phenotype, including elevated levels of prostaglandin E_2 (PGE_2) and interleukin-1β (IL-1β); Progression of attachment loss and bone loss may be self-arresting (Armitage 2004).

Some types of aggressive periodontitis seem to be inherited in a Mendelian manner, and both autosomal modes and X-linked transmission have been proposed. Genetic segregation analyses have been carried out using family pedigrees and the distribution of family members with aggressive periodontitis. Most of the evidence for a genetic predisposition to aggressive periodontitis comes from segregation analyses of families with affected individuals in two or more generations, and the results in different sets of families are consistent with both autosomal-dominant and autosomal-recessive inheritance, as well as X-linked dominant inheritance, but no single inheritance mode that would include all families has been established (Meng et al. 2007).

In a family study, 39 sibships (116 individuals, aged 13–48) were evaluated for clinical indices, neutrophil chemotaxis and serum antibodies to *A. actinomycetemcomitans*. In 14 sibships, all affected persons had localized form of juvenile periodontitis; 14 other sibships had all affected individuals with generalized form of juvenile periodontitis; and 11 had at least one sib with each form. For probands with decreased chemotaxis, 71% of affected sibs and 36% of clinically healthy sibs had decreased chemotaxis. The associations of disease with these risk factors were stronger in localized form of juvenile periodontitis only sibships. Some affected

Table 6.2 Genes associated with gingivitis (GI) risk

Gene	Locus	Negative reports			Positive reports		
		Study populations	Number	References	Study populations	Number	References
Interleukin-1 cluster	ILA + 4845 IL-1B + 3953, IL-1B-511, IL-1B + 3954 bp Combination of alleles 2 of interleukin (IL)-1A (−889) and IL-1B(+3954) IL-1RN *2 allele (A2); IL-1RN, intron two variable number tandem repeats	Caucasian population	1	Jepsen et al. 2003	Arabic population Caucasian population	5	Müller et al. 2007 Dashash et al. 2007 Scapoli et al. 2005a Lang et al. 2000 Goodson 2000
Interleukin-6	−174, −572, and −597	Caucasian population	1	Scapoli et al. 2007	Caucasian population	1	Holla et al. 2008b
Interleukin-10	−1082 (G/A) −819 (C/T) −592 (C/A)	–	0	–	Caucasian population	2	Dashash et al. 2006 Dashash et al. 2005
Interleukin-12	1188 A/C	–	0	–	Caucasian population	1	Reichert et al. 2008
Interleukin-18	−607 A/C	Caucasian population	1	Vokurka et al. 2009	–	0	–
MMP-9	−1562 C/T	–	0	–	Caucasian population	1	Vokurka et al. 2009
Tumor necrosis factor	−308	Caucasian population	1	Scapoli et al. 2007	–	0	–
Lymphotoxin alpha (LT-A)	+252	Caucasian population	1	Scapoli et al. 2007	–	0	–
Fibrinogen	−β455 G/A	–	0	–	Caucasian population	1	Ge et al. 2008

sibs had neither risk factor, while many currently healthy sibs had one or both (Boughman et al. 1992). Mixed model segregation analyses of 100 families, ascertained through 104 probands with early-onset periodontitis, were carried out by Marazita et al. (1994) to test major locus and multifactorial hypotheses for the etiology of early-onset periodontitis. The segregation analysis results were consistent with an autosomal major locus being sufficient to explain the family patterns of early-onset periodontitis in the entire dataset. A dominant mode of transmission was most likely, with penetrance of about 70%.

The advent of more advanced molecular techniques enabled scientists to investigate genetic polymorphisms in population, case-control and functional studies. Reports on the association between gene polymorphisms and AP are summarized in Table 6.3.

6.3.3 Heritability of Chronic Periodontitis

Most patients with plaque-induced periodontitis will have the chronic form. The main clinical features and

Table 6.3 Genes associated with aggressive periodontitis risk

Gene	Locus	Negative reports			Positive reports		
		Study populations	Number	References	Study populations	Number	References
Interleukin-1 cluster	IL-1A (G[4845]T), IL-1A (C[−889]T) IL-1B (C[3953/4]T), IL-1B (T[−511]C) Composite IL-1A (G[4845]T)/IL-1B (C[3953/4]T) Composite IL-1A (C[−889]T)/IL-1B (C[3953/4]T)	Brazilian population Caucasian population, African-American population, Central American Hispanics	11	Droździk et al. 2006 Fiebig et al. 2008 Gonzales et al. 2003 Hodge et al. 2001 Hodge et al. 2001 Maria de Freitas et al. 2007 Papapanou et al. 2001 Parkhill et al. 2000 Sakellari et al. 2006 Scapoli et al. 2005b Walker et al. 2000	Caucasian population African-American population, Japanese population, Chinese population, Chilean population, New Zealand population, Turkish population	12	Berdeli et al. 2006 Brett et al. 2005 Diehl et al. 1999 Havemose-Poulsen et al. 2007 Krátká et al. 2007 Li et al. 2004 Li et al. 2005 Quappe et al. 2004 Ren et al. 2008 Shimomura-Kuroki et al. 2009 Tai et al. 2002 Thomson et al. 2001
Interleukin-4	IL-4 (C[−590]T), IL-4 70bp repeat in intron 2	Iranian population, Japanese population, Caucasian population	3	Hooshmand et al. 2008 Gonzales et al. 2004 Michel et al. 2001	Caucasian population	1	Gonzales et al. 2007
Interleukin-6	IL-6 (G[−174]C), IL-6R (A[48892]C), IL-6R (G[−183]A)	–	0	–	Japanese population, Caucasian population	5	Galicia et al. 2006 Nibali et al. 2009 Nibali et al. 2008a Nibali et al. 2008b Nibali et al. 2007
Interleukin-10	IL-10 (C[−627]A) IL-10 (G[−1082]A)	Caucasian population, Iranian population	4	Brett et al. 2005 Gonzales et al. 2002 Kinane et al. 1999 Mellatiet et al. 2007	Taiwanese population, Caucasian population	2	Hu et al. 2009 Reichert et al. 2008
Interleukin-12	1188 A/C	Caucasian population	1	Reicher t et al. 2008	–	0	–
Interleukin-13	IL-13 (C[−1112]T), IL-13 (A[−1512]C)	Caucasian population	1	Gonzales et al. 2007	–	0	–
Interleukin-18	c. −368G > C, c. −838C > A −656, −607, −137, +113 +127 and +105 (third position of codon 35)	Caucasian Population	2	Noack et al. 2008b Folwaczny et al. 2005	–	0	–
Fc gamma receptor	FcγRIIA: 494A/G(R131H) *FcγRIIB*: 695 T-C FcγRIIIB: 141 G-C (NA antigen), 266 C-A (SH antigen), NA1/NA2, NA1/NA2, NA2/NA2, NA1, NA2	Taiwanese population	1	Chung et al. 2003	African-American population, Caucasian population, Japanese population, Chinese population	6	Fu et al. 2002 de Souza and Colombo 2006 Kobayashi et al. 2000a An et al. 2009 Nibali et al. 2006
Tumor necrosis factor α	TNFA (G[−238]A), TNFA (T[−1031]C), TNFA (C[−863]A), TNFA (C[−857]T)	Turkish population, Brazilian population	4	Menezes and Colombo 2008 Guzeldemir et al. 2008 Maria de Freitas et al. 2007 Schultz et al. 2008	Chinese population, Chilean population	2	Zhu et al. 2007 Pérez et al. 2004

Table 6.3 (continued)

Gene	Locus	Negative reports			Positive reports		
		Study populations	Number	References	Study populations	Number	References
Transforming Growth Factor beta TGFβ	+915G/C, Thr263Ile and 713/8delC	Turkish population	1	Atila et al. 2006	–	0	–
Vitamin D receptor	*RFLP: BsmI, ApaI, TaqI, and FokI*	Japanese population Caucasian population	2	Yoshihara et al. 2001 Nibali et al. 2008	Chinese population, Japanese population, Korean population, Caucasian population	6	Sun et al. 2002 Park et al. 2006 Hennig et al. 1999 Tachi et al. 2001 Wang et al. 2008b Li et al. 2008
Estrogen receptor	*RFLP: XbaI and PvuII*	Chinese population	2	Zhang et al. 2004 Wang et al. 2008b	–	0	–
RANK/RANKL/OPG	OPG: A163G, T245G, T950C, G1181C, C4441T, A6833G/A6890G	Japanese population	1	Soedarsono et al. 2006	Korean population	1	Park et al. 2008
Lactoferrin	Lys/Arg polymorphism of the lactoferrin gene at position 29 [reference sequence (rs) 1126478], 11 (T11A), A/G nucleotide mutation causing a threonine/alanine substitution at position 11 (T11A)	Caucasian population	1	Jordan et al. 2005	African-American population Taiwanese, population	3	Jordan et al. 2005 Velliyagounder et al. 2003 Wu et al. 2009
MMP matrix metalloproteinases −1, −2, −3, −9, −12, TIMP	MMP-1: 1G/2G (−1607) MMP-2: −1306C/T, −735C/T MMP-3: 5A/6A (−1171) MMP-12: 357Asn/Ser TIMP-2: −418-G/C	Chinese population Japanese population Turkish population	4	Chen et al. 2007 Itagaki et al. 2004 Gürkan et al. 2008 Gürkan et al. 2007a (MMP-2,MMP-12)	Chinese population Turkish population	2	Cao et al. 2005 Gürkan et al. 2007a (MMP-9)
Human leukocyte antigen	HLA-A: A1, A2, A3, A9, A23 (A9), A24 (A9), A10, A11, A29 (A19), A30 (A19), A31 (A19), A28 HLA-B: B51 (B5), B52 (B5), B12, B44 (B12), B45 (B12), B13, B14, B15, B18, B27, B35, B40 HLA-Cw: Cw1, Cw2, Cw3, Cw4, Cw5, Cw6, Cw7, Cw8 HLA-DR: DR1, DR2, DR3, DR4, DR5, DR6, DR7, DR8, DR9, DR10	Caucasian population	1	Hodge et al. 1999	Caucasian population, Japanese population	8	Bonfil et al. 1999 Ohyama et al. 1996 Takashiba et al. 1994 Machulla et al 2007 Reichert et al. 2007 Stein et al. 2003 Shimomura-Kuroki et al. 2009 Roshna et al. 2006
CD 14	C(−159)T and G(−1359)T	Caucasian population	2	Schulz et al. 2008 James et al. 2007	–	0	–
Toll-like receptor, TLR-2, -4	TLR2: Arg677Trp, Arg753Gln TLR4: Asp299Gly, Thr399Ile	Caucasian population, Chinese population, Turkish population	3	Schröder et al. 2005 Zhu et al. 2008 Emingil et al. 2007a	Caucasian population	2	James et al. 2007 Brett et al. 2005

(*continued*)

Table 6.3 (continued)

Gene	Locus	Negative reports			Positive reports		
		Study populations	Number	References	Study populations	Number	References
CARD15	c.2104 C > T, c.2722 G > C c.3020insC (p.R702W, p.G908R, and p.L1007fs × 1008)	Caucasian population	1	Noack et al. 2006	–	0	–
Cathepsin C	−1209_−1219 del, −932 G > A, −18 T > C, 458 C > T, 1357 A > G, 386T > A, 935A > G, 1235A > G, 1040A– > G, D11S1365	–	0	–	Caucasian population, Jordanian population, Mixed population	5	Noack et al. 2004 Noack et al. 2008 Toomes et al. 1999 Hewitt et al. 2004 Hart et al. 2000a, b
FMLP formyl–methionyl–leucyl–phenylalaine receptor	FPR1: c.329T > C and c.378C > G	Mixed population (African-American; Brazilian; Turkish)	1	Zhang et al. 2003	Mixed population (Caucasian and African-American)	1	Gwinn et al. 1999
Tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1)	4G/5G polymorphism in the promoter region of the PAI-1 gene and Alu-repeat insertion/deletion (I/D) polymorphism in intron 8 of the TPA gene	–	0	–	Turkish population	1	Emingil et al. 2007b
Interferon IFNγ	874 AA, TA, and TT 5644GA	Caucasian population, Iranian population	2	Reichert et al. 2008 Hooshmand et al. 2008	–	0	

characteristics of chronic periodontitis are (Armitage 2004):

- Most prevalent in adults, but can occur in children and adolescents
- Amount of destruction is consistent with the presence of local factors
- Subgingival calculus is a frequent finding
- Associated with a variable microbial pattern
- Slow to moderate rate of progression, but may have periods of rapid progression
- Can be associated with local predisposing factors (e.g., tooth-related or iatrogenic factors)
- May be modified by and/or associated with systemic diseases (e.g., diabetes mellitus)
- Can be modified by factors other than systemic disease such as cigarette smoking and emotional stress

The typical patient is over 30 years of age with substantial deposits of plaque and calculus associated with the presence of gingival inflammation, periodontal pockets and attachment loss. In most cases, the disease is slowly progressing, but short periods of rapid attachment loss can occur. Chronic periodontitis was once called "*adult periodontitis*" since it was believed that only adults developed the disease. However, epidemiologic data clearly show that the disease can also be found in children and adolescents. Although chronic periodontitis can occur in localized or generalized patterns, the two forms appear to be identical with regards to their etiology and pathogenesis (Armitage 2004).

Genes have also been implicated to play a role in chronic periodontitis, but in contrast to aggressive periodontitis, chronic periodontitis does not typically follow a simple pattern of familial transmission or distribution. The twin study is probably the most popular method that supports the genetic aspects of chronic periodontitis. This study substantiates the contribution that genes make vs. the environment in a phenotypic expression. Monozygous twins, in contrast to dizygous

twins, come from a single ovum and therefore share exactly the same genes. Discordance in the disease experience of monozygous twins must be caused by environmental determinants as seen in twins reared apart. In dizygous twins, differences could be a result of both genetic and environmental differences (Yoshie et al. 2007). Studies conducted on twins provide us with interesting points on the genetic influences in diseases to what extent this is influenced by the environment (Yoshie et al. 2005).

Michalowiccz et al. (1991) examined the relative contribution of environmental and host genetic factors to clinical measures of periodontal disease through the study of both reared-together twins and monozygous twins reared apart. PD, clinical attachment loss, GI and plaque were assessed from the Ramfjord teeth in 110 pairs of adult twins (mean age 40.3 years), including 63 monozygous and 33 dizygous twin pairs reared together and 14 monozygous twin pairs reared apart. Heritability estimates indicated that between 38 and 82% of the population variance for these periodontal measures of disease may be attributed to genetic factors. Genetic and environmental variances and heritability for GI and adult periodontitis using data from twins reared together were evaluated using path models with maximum likelihood estimation techniques in 117 pairs of adult twins (64 MZ and 53 DZ pairs). Adult periodontitis was estimated to have approximately 50% heritability, which was unaltered following adjustments for behavioral variables including smoking (Michalowiccz et al. 2000). Corey et al. (1993) revealed that approximately half of the variance in disease in the population is attributed to genetic variance.

Nikolopoulos et al. (2008) conducted a systematic review and a meta-analysis in order to investigate the potential association of cytokine gene polymorphisms with either aggressive or chronic periodontal disease. Six polymorphisms were included in the meta-analysis, which are the following: IL-1A G[4845]T, IL-1A C[-889]T, IL-1B C[3953/4]T, IL-1B T[-511]C, IL-6 G[-174]C and TNFA G[-308]A. Using random effect methods the authors found statistically significant association of IL-1A C[-889]T and IL-1B C[3953/4]T polymorphisms with chronic periodontal disease without any evidence of publication bias or significant statistical heterogeneity. A weak positive association was also found concerning IL-1B T[-511]C and chronic periodontal disease. No association was found for all the cytokines examined as far as the aggressive form of the disease was concerned.

Genetic polymorphisms thus far found to be associated with chronic (adult) periodontitis are listed in Table 6.4.

6.3.4 Heritability of Peri-Implantitis

Endosseous implants present high survival rates within a 10-year observation time; however, implant failure and biologic complications are not completely avoidable. Although specific bacteria, dental plaque and environmental factors are associated with peri-implant disease, there are currently no reliable predictors of peri-implantitis occurrence and severity. Disagreement about which clinical measures of peri-implant health are of diagnostic value continues because of the complexity of the disease process. Thus, identification of genes that control or modify aspects of the host response may provide a method to identify individuals at an elevated risk for peri-implant infections. Elevated levels of the inflammatory cytokine interleukin-1 (IL-1) in the crevicular fluid around diseased implants seem to play an important role in the pathogenesis and severity of peri-implantitis (Andreiotelli et al. 2008). As reviewed by Andreiotelli et al. (2008), several studies revealed that the diagnostic value of both IL-1 genotyping and genetic tests for implant failure or peri-implantitis should be reconsidered before altering treatment planning, regimens and maintenance in implant dentistry (Montes et al. 2009; Laine et al. 2006; Jansson et al. 2005; Gruica et al. 2004; Feloutzis et al. 2003; Shimpuku et al. 2003). In a partially edentulous group treated for periodontal disease before implant treatment, a synergistic effect between the IL-1 genotypes and smoking was detected (Jansson et al. 2005), characterizing individuals with these two conditions together as a high-risk population for implant failure (Montes et al. 2009). Negative results were reported as well, showing that the interleukin-1 polymorphism exerted only little influence on the peri-implant crevicular immune response, and this influence appeared to be of limited impact in sites with established peri-implantitis lesions (Lachmann et al. 2007; Rogers et al. 2002; Campos et al. 2005; Wilson and Nunn 1999).

Table 6.4 Genes associated with chronic periodontitis risk

Gene	Locus	Negative reports			Positive reports			
		Study population	Number	References	Study population	Number	References	
Interleukin-1 cluster	IL-1A (G[4845]T), IL-1A (C[−889]T) IL-1B (C[3953/4]T), IL-1B (T[−511]C) Composite IL-1A (G[4845]T)/IL-1B (C[3953/4]T) Composite IL-1A (C[−889]T)/IL-1B (C[3953/4]T) IL-1 receptor antagonist (RN) + 2018 T/C	Caucasian population Chinese, population Japanese, population, South Indian population, Thai population, Mixed population	13	Anusaksathien et al. 2003 Armitage et al. 2000 Droździk et al. 2006 Galbraith et al. 1999 Guzman et al. 2003 Imamura et al. 2008 Jansson et al. 2006 Kaarthikeyan et al. 2009 Mark et al. 2000 Nastri and Caruso 2003 Papapanou et al. 2001 Sakellari et al. 2003 Sakellari et al. 2006	Brazilian population, Caucasian population, Chilean population, Japanese patients, Maharashtrian ethnicity, Turkish population	24	Agrawal et al. 2006 Berdeli et al. 2006 Brett et al. 2005 Cullinan et al. 2001 Ferreira et al. 2008 Gore et al. 1998 Goteiner et al. 2008 Kobayashi et al. 2007 Komatsu et al. 2008 Kornman et al. 1997 Kowalski et al. 2006 Laine et al. 2001	Lopez et al. 2005 McDevitt et al. 2000 Meisel et al. 2002 Meisel et al. 2003 McGuire and Nunn 1999 Meisel et al. 2004 Moreira et al. 2005 Moreira et al. 2007a Socransky et al. 2000 Struch et al. 2008 Trevilatto et al. 2002 Wagner et al. 2007
Interleukin-2	−330 (T− > G)	–	0	–	Brazilian population	1	Scarel-Caminaga et al. 2002	
Interleukin-4	IL-4 (C[−590]T), IL-4 70bp repeat in intron 2, IL-4 RA Q551R	Iranian population, Turkish population, Brazilian population	4	Hooshmand et al. 2008 Kara et al. 2007 Pontes et al. 2004 Scarel-Caminaga et al. 2003	Caucasian population, Korean population	2	Holla et al. 2008a Kang et al. 2003	
Interleukin-6	IL-6 (G[−174]C), IL-6R (A[48892]C), IL-6R (G[−183]A)		0	–	Caucasian population, Japanese population, Brazilian population, Mixed population, Chinese Hans population	12	Trevilatto et al. 2003 Komatsu et al. 2005 Holla et al. 2004b D'Aiuto et al. 2004 D'Aiuto et al. 2005 Babel et al. 2006	Galicia et al. 2006 Tervonen et al. 2007 Moreira et al. 2007b Guan et al. 2008 Nibali et al. 2009 Babel et al. 2006
Interleukin-10	IL-10 (C[−592]A) IL-10 (C[−597]A) IL-10 (C[−627]A) IL-10 (C[−819]T) IL-10 (C[−824]T) IL-10 (G[−1082]A) IL-10 (G[−1087]A)	Caucasian population	4	Savarrio et al. 2007 Brett et al. 2005 Gonzales et al. 2002 Yamazaki et al. 2001	Turkish population, Caucasian population, Brazilian population, Taiwanese population	8	Sumer et al. 2007 Babel et al. 2006 Berglundh et al. 2003 Scarel Camingga et al. 2004	Hu et al. 2009 Claudino et al. 2008 Cullinan et al. 2008 Donati et al. 2008
Interleukin-12	1188A/C	Caucasian population	1	Reichert et al. 2008	–	0	–	–
Interleukin-16	(T[−295]C)	Caucasian population	1	Folwaczny et al. 2005	–	0	–	–
Interleukin-18	−656, −607, −137, +113, +127 and +105 (third position of codon 35)	Caucasian population	1	Folwaczny et al. 2005	–	0	–	–
Fc gamma receptor	*FcγRIIA*: R/R131, R/H131, H/H131, H131, R131 *FcγRIIB*: 232I/T, 646–184A/G *FcγRIIIA*: −158V/F, −158V/V, −158F/F *FcγRIIIB*: NA1/NA2, NA1/NA2, NA2/NA2	Taiwanese population, Japanese population, Caucasian population	6	Chung et al. 2003 Kobayashi et al. 1997 Colombo et al. 1998 Kobayashi et al. 2007 Komatsu et al. 2008 Kobayashi et al. 2000b An et al. 2009	Japanese population, Caucasian population,	8	Yamamoto et al. 2004 Meisel et al. 2001 Yoshihara et al. 2001 Kobayashi et al. 2001	Kobayashi et al. 1997 Sugita et al. 2001 Yasuda et al. 2003

Table 6.4 (continued)

Gene	Locus	Negative reports			Positive reports			
		Study population	Number	References	Study population	Number	References	
Tumor necrosis factor	TNFA (G[−238]A), TNFA (T[−1031]C), TNFA (C[−863]A), TNFA (C[−857]T), TNFA (G[−308]A), TNFA (G[−376]A), TNFA (G[−489]A)	Caucasian population, Brazilian population,	5	Folwaczny et al. 2004d Craandijk et al. 2002 Menezes and Colombo 2008 Schultz et al. 2008	Japanese population, Caucasian population, Turkish population, Mixed population	7	Soga et al. 2003 Fassman et al. 2003 Shimada et al. 2004 Akman et al. 2008	Babel et al. 2006 D'Aiuto et al. 2004 Trombone et al. 2008
Transforming growth factor beta TGFβ	−988 (C/A), −800 (G/A), −509 (C/T), codons 10 (L10P) and 25 (R25P) of exon 1, +915G/C, Thr263Ile and 713/8delC	Caucasian population	1	Holla et al. 2002b	Caucasian population, Turkish population	3	de Souza et al. 2003b Babel et al. 2006 Atila et al. 2006	
Vitamin D receptor	*RFLP: BsmI, TaqI, ApaI and FokI*	Chinese population, Caucasian population, Brazilian population	4	Sun et al. 2002 Nibali et al. 2008 de Souza et al. 2007 Gunes et al. 2008	Chinese population, Japanese population, Caucasian population, Brazilian population	8	Wang et al. 2009 Wang et al. 2008a Zhang et al. 2005 Naito et al. 2007	Inagaki et al. 2003 Tachi et al. 2003 Tachi et al. 2001 de Brito Júnior et al. 2004
Estrogen receptor-α	*RFLP: XbaI and PvuII*	–	0	–	Chinese population	2	Zhang et al. 2004 Wang et al. 2008a	
RANK/RANKL/ OPG	OPG: -223 (C/T), Lys3Asn and Met256Val, 245 T>G, A163G, T245G, T950C, G1181C, C4441T, A6833G/ A6890G	Caucasian population Brazilian population	3	Wohlfahrt et al. 2006 Wagner et al. 2007 Baioni et al. 2008	Korean population	1	Park et al. 2008	
Lactoferrin	Lys/Arg polymorphism of the lactoferrin gene at position 29 [reference sequence (rs) 1126478] in the N-terminal alpha-helical region	Taiwanese population	1	Wu et al. 2009	–	0	–	
MMP matrix metalloproteinases −1, −2, −3, −9, −13	*MMP-1*: −1607 1G/2G, −519A/G, and −422A/T *MMP-2*: −1575G/A, −1306C/T, −790T/G, and −735C/T *MMP-3*: 5A/6A (−1171) *MMP-9*: −1562C/T and R279Q *MMP-13*: -77A/G	Caucasian population, Brazilian population, Japanese population Turkish population	7	Holla et al. 2005 Astolfi et al. 2006 Holla et al. 2006 De Souza et al. 2005 Itagaki et al. 2004 Ustun et al. 2008 Gürkan et al. 2008	Caucasian population, Brazilian population, Japanese population, Turkish population, Chinese population	8	Holla et al. 2004a de Souza et al. 2003a Astolfi et al. 2006 Pirhan et al. 2008 Pirhan et al. 2009	Gürkan et al. 2008 Keles et al. 2006 Cao et al. 2006
Human leukocyte antigen	*HLA*-A: A1, A2, A3, A9, A10, A11, A29 (A19), A28 *HLA-B*: B15, B18, B5 *HLA-DR*: DR1, DR2, DR3, DR4, DR5, DR6, DR7, DR8, DR9, DR10 *HLA-DQ*: DQ1, DQ6 (DQ1), DQ2, DQ3	–	0	–	Caucasian population, Japanese population	5	Alley et al. 1993 Machulla et al. 2002 Reichert et al. 2007	Stein et al. 2003 Suzuki et al. 2004
CD 14	c.−159C > T, C-260T, −159C-to-T	Japanese population Caucasian population	3	Yamazaki et al. 2003 Schulz et al. 2008 Donati et al. 2008	Caucasian population	6	Holla et al. 2002a Folwaczny et al. 2004a Donati et al. 2005	Tervonen et al. 2007 Laine et al. 2005 Raunio et al. 2009

(*continued*)

Table 6.4 (continued)

Gene	Locus	Negative reports			Positive reports		
		Study population	Number	References	Study population	Number	References
Toll-like receptor: TLR-2, −4	TLR2: Arg753Gln, Arg677Trp, −183 A/G, −148 C/T, −146 T/G, +1350 T/C, +2343 G/A TLR4: Asp299Gly, Thr399Ile, +3725 G/C, +3528 C/G, +4022 C/G, +4529 G/C	Caucasian population, Mixed population, Chinese population, Turkish population	9	Izakovicova Holla et al. 2007 Laine et al. 2005 James et al. 2007 D'Aiuto et al. 2004 Berdeli et al. 2007 Zhu et al. 2008 Folwaczny et al. 2004b Imamura et al. 2008 Emingil et al. 2007a, b	Japanese population, Caucasian population	3	Fukusaki et al. 2007 Brett et al. 2005 Schröder et al. 2005
CARD15	CARD15: 3020insC and 2104T mutations	Caucasian population	2	Laine et al. 2004 Folwaczny et al. 2004c	–	0	–
Tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1)	4G/5G polymorphism in the promoter region of the PAI-1 gene and the Alu-repeat insertion (I)/deletion (D) polymorphism in intron 8 of the t-PA gene	Caucasian population	1	Gürkan et al. 2007b	Caucasian population	1	Izakovicová Holla et al. 2002
NAT2 N-acetyltransferase	Mutations at positions $G^{191}A$, $C^{282}T$, $C^{481}T$, $G^{590}A$, $A^{803}G$, and $G^{857}A$	Caucasian population	1	Kocher et al. 2002	Caucasian population	1	Meisel et al 2000
Fibrinogen	−b455 G/A, fibrinogen-like 2 (*FGL2*) gene polymorphism	–	0	–	Caucasian population, Japanese population, Chinese population	3	Sahingur et al. 2003 Suzuki et al. 2004 Ge et al. 2008
Cytochrome P450	CYP1A1: 6235T/C, 4889A/G CYP2E1: PstI-RFLP	–	0	–	Korean population	1	Kim et al. 2004
RAGE receptor of advanced glycation end-products	RAGE: (1704G/T), (2184A/G), (G82S)	–	0	–	Caucasian population	1	Hollá et al. 2001
CCR5 crotonyl coenzyme reductase 5	CCR5: 59653 C > T, CCR5: Delta32 mutation, CCR5: wt/wt genotype	Caucasian population	3	Savarrio et al. 2007 Folwaczny et al. 2003 Wohlfahrt et al. 2006	–	0	–
Calcitonin gene receptor	CALCR: SNP (C/T) in intron 3	–	0	–	Japanese population	1	Suzuki et al. 2004
Interferon IFNγ	874 AA, TA, and TT 5644GA +874 AA, TT + AT	Caucasian population, Iranian population	3	Babel et al. 2006 Reichert et al. 2008 Hooshmand et al. 2008	–	0	–

6.4 Common Guidelines for Association Studies

The candidate gene approach tries to identify one allele of a gene that is more frequently seen in subjects with the disease than in subjects without the disease. Candidate genes are chosen on the basis of their known or presumed functions that are thought to have some plausible role in the disease. There are three types of candidate genes: functional candidate genes; positional candidate genes; and expressional candidate genes. Functional candidate genes are derived from an existing knowledge of the phenotype and the potential function of the gene

involved after clinical or physiological studies of affected individuals. Positional candidate genes are based on the involvement of the gene to a marked location after genetic linkage analyses. Expressional candidate genes are determined through differences in gene expression using microarrays (Yoshie et al. 2007; Hodge 1993).

For several genes, which have been individually sequenced for association with periodontitis, we see a scattered picture from different studies of varied populations and ethnicities. To produce scientifically sound and meaningful disease-association studies, there are? some issues and concerns that should be addressed (Yoshie et al. 2007).

6.4.1 Ethnic Heterogeneity

In designing a case–control study, subjects should be carefully matched by ethno-geographic origin in addition to other potential confounding factors in order to avoid systematic differences in genetic composition between the two groups. Failing to do so could result in different frequencies of SNP alleles, and the unsuspecting investigator might then draw unwarranted conclusions about localizations of susceptibility genes. There is also a clear statement that in the pre-sence of large biological and environmental variability, genetic effects can differ across different populations, or even among generations within the population. Variation in genotype frequencies across diverse populations may affect the number of individuals at increased risk for a disease, and population substructure imbalances may create spurious differences in genotype frequencies of the compared groups in gene disease association studies. Considering the issues mentioned, it was suggested to select a more homogenous population (age- and race-matched), and to study, with caution, the applicability of a certain gene marker before commencing with any attempts to replicate the same study in the population under investigation(Yoshie et al. 2007).

6.4.2 Clinical Classification

Classifying periodontal diseases has been a long-standing dilemma largely influenced by paradigms that reflect the understanding of the nature of periodontal diseases during a given historical period. As a result of its familial tendency, aggressive periodontitis generally appears in individuals before the age of 35 years, but age alone is not sufficient to establish diagnosis. On the other hand, chronic periodontitis is quite complex and much more dependent on environmental factors that confront the patient during his lifetime. In addition, microbial plaque deposition, smoking and systemic diseases largely influence the phenotypic expression of the disease. For these combined reasons, chronic periodontitis is considered to appear later in life. The periodontist is therefore challenged regarding which classification a patient would properly fall into. Therefore, investigators should strictly adhere to the classification set during the American Academy of Periodontology workshop in 1999. Moreover, subjects falling into the gray zone between aggressive and chronic periodontitis should be excluded in the study (Yoshie et al. 2005, 2007).

6.4.3 Functional Polymorphisms

Structural gene defects can affect the qualitative response and regulatory polymorphisms can alter the response, quantitatively. However, many studies fail to provide functional evidence for gene polymorphisms and periodontal diseases. The majority only statistically demonstrated the association between polymorphisms and periodontitis (Yoshie et al. 2007). Kinane and Hart (2003) outlined the requirements in providing a disease-polymorphism association:

- The polymorphism must influence the gene product
- Biases in the study population should be recognized and controlled for
- Confounders such as smoking and socio-economic class must be sorted out; and the
- Affected gene product should be part of the disease etiopathology

6.4.4 Sample Size of the Study Subjects

Owing to limited number of samples, most sample sizes in genetics are small. This scenario describes very well the small number of cases in aggressive periodontitis association studies. The number of subjects in studies of

chronic periodontitis tends to be larger, but variations do occur. The size of subjects clearly contributes to the differences in statistical power of the results, especially in a complex disease like periodontitis (Yoshie et al. 2007).

6.4.5 Choice of Controls

Defining the appropriate controls for a case–control study in periodontitis still lacks clarity. Some reports generally described their control as healthy, while others specifically characterized controls as patients with GI or slight periodontitis (Yoshie et al. 2007).

6.4.6 Data Presentation

Expressing the results in P-values only is extremely popular in all types of studies in periodontitis. It was suggested that the data should be presented be evaluated using CI and relative risk (RR) values, as these portray the effect size with a description of its precision. This is in contrast to the P-value, which tests against the null hypothesis of no association and could provide false-positive conclusions. Furthermore, Relative risk and 95% Confidence Intervals provide readers with more useful information that does use hypothesis testing (Yoshie et al. 2007).

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Implication of Systemic Osteoporosis on Oral Health

7

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Osteoporosis is defined as a skeletal disorder characterized by low bone mass and micro-architectural deterioration with a resulting increase in bone fragility and susceptibility to fracture (Cummings and Melton 2002; NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy 2001). Increases in the elderly population worldwide will likely cause a dramatic rise in osteoporotic fractures. Both vertebral and hip fractures contribute to an increased risk of mortality as well as morbidity and a rapid increase of medical costs in older populations (Kado et al. 2003; Farahmand et al. 2005). In 1994, Osteoporosis was defined as a bone mineral density (BMD) T-score of −2.5 or less at either the lumbar spine or the femoral neck, in accordance with the WHO classification [World Health Organization (WHO) Studying Group (1994)].

However, does osteoporosis really represent a severe oral health problem? In many phases of dentistry, healthy bone with normal regenerative capacity is essential for a successful outcome. It is important to know both bone quantity and quality of the jaws in planning prosthetic and pre-prosthetic surgical treatment as well as periodontal disease treatment because alveolar bone loss is a prominent feature of periodontitis (von Wowern 2001b).

7.1 Diagnostic Methods In Vivo

Several investigators have dealt with developing methods suitable for assessing osteoporotic changes in the jaws. The only part of the jaws, which reasonably fulfills the demands of a standard site (smallest possible inter- and intra-individual variations in anatomical size, shape, bone structure, and function) is *the basal area of the mandible posterior to the mental foramen*. These considerations have resulted in the use of this area of the mandible as the standard site (von Wowern 2001b).

Bras et al. (1982) have measured the thickness of the mandibular angular cortex with a marking gauge on *panoramic radiographs*. Benson et al. (1991) have made bilateral measurements on panoramic radiographs, identifying the shortest distance between the inferior border of the mandible and the superior and inferior margins of the mental foramen. These indices were calculated as a ratio of the cortical thickness to the relatively constant distance between the mental foramen and the inferior mandibular border.

- Panoramic mandibular index (PMI) (superior) = Thickness of cortex/Distance from superior margin of mental foramen to inferior border of mandible (PMI superior margin = C/A).
- PMI (inferior) = Thickness of cortex/Distance from inferior margin of mental foramen to inferior border of mandible (PMI inferior margin = C/B).

Using PMI on a sample of 355 postmenopausal women aged 48–56 years, Klemetti et al. (1993c) have found that PMI did not significantly correlate with the BMD values of the spongiosa and cortex in the edentulous area of the mandible.

Klemetti et al. (1994) first proposed cortical shape classification of the mandible on panoramic radiographs. In this classification, the inferior cortex, distally from the mental foramen, was observed on both sides of the mandible. Subjects were classified into three groups (C1–C3), according to the following criteria: (C1) the endosteal margin of the cortex was even and sharp on both sides; (C2) the endosteal margin

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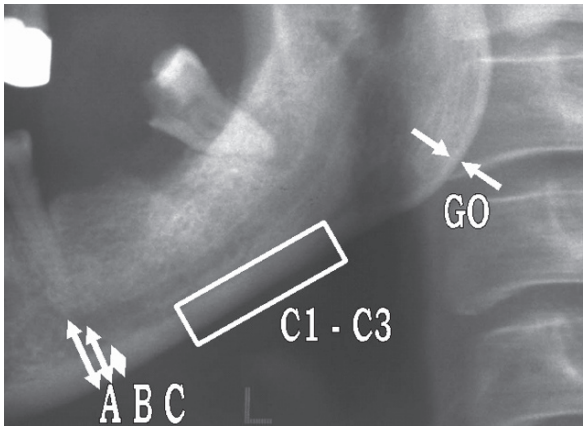

Fig. 7.1 Regions for measuring mandibular bone changes in vivo on panoramic radiographs: Calculation of panoramic mandibular index (PMI superior margin = C/A; PMI inferior margin = C/B) (Benson et al. 1991). Thickness of the mandibular angular cortex with a marking gauge (GO) (Bras et al. 1982). Changes in inferior cortex (C1–C3) detected on both sides of the mandible, distally from the mental foramen (Klemetti et al. 1994). Mandibular Cortical Width (C) below the mental foramen (Taguchi et al. 1995a, b, 1999)

showed semilunar defects (lacunar resorption) or seemed to form endosteal cortical residues (one to three layers) on one or both sides; and (C3) the cortical layer formed heavy endosteal cortical residues and was clearly porous (Fig. 7.2).

Other method assessing the mineral status of the mandible was the mandibular cortical width (MCW) below the mental foramen on panoramic radiographs (Taguchi et al. 1995a, b, 1999) (Fig. 7.1).

Dental evaluation of mandibular bone mass on *periapical radiographs* with an aluminum step wedge attached to the occlusal surface used microdensitometry. In the dentate subjects, the bone was scanned between the mandibular second premolar and first molar from the alveolar crest to the inferior border of the film. In the edentulous mandibles, the trabecular bone was scanned in an anterior to posterior direction distal to the mental foramen. The bone-mass measurement was compared with the step-wedge standard to compensate for radiographic and processing variations and to provide a unit for quantifying bone mass in

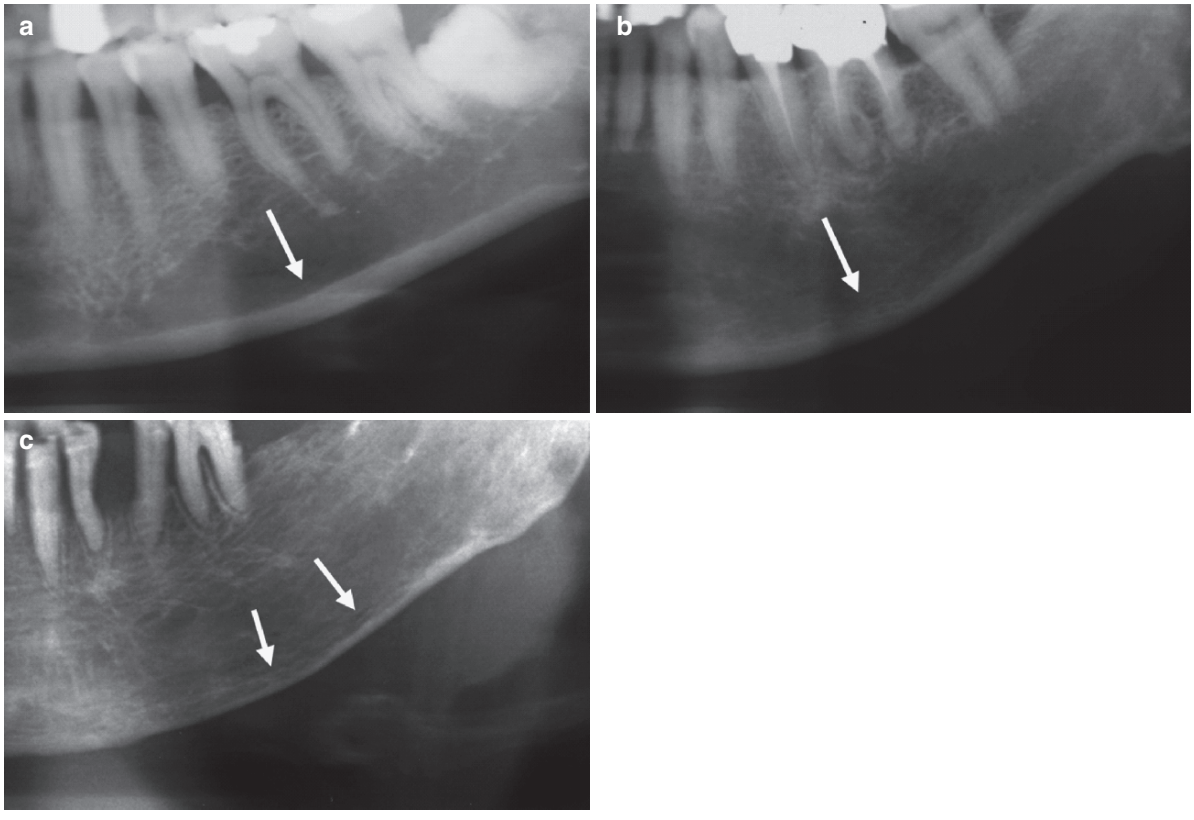

Fig. 7.2 **a**. (C1) Normal cortex: the endosteal margin of the cortex is even and sharp on both sides. **b**. (C2) Mildly to moderately eroded cortex: the endosteal margin shows semi-lunar defects (lacunar resorption) or appears to form endosteal cortical residues.**c**. (C3) Severely eroded cortex, with the cortical layer exhibiting heavy endosteal residues and obvious porosity

standardized aluminum equivalents (Kribbs et al. 1989, 1990a; Kribbs 1990b). Recently, more precise periapical radiographic method with aluminum step wedge has been used in multi-center study group, named OSTEODENT project (Nackaerts et al. 2006, 2007, 2008; Geraets et al. 2007).

Intraoral radiographs were also evaluated for alveolar bone density change using computer-assisted densitometric image analysis (CADIA) (Payne et al. 1999, 2000; Bragger et al. 1988) and radiographic digitization (Shrout et al. 2000; Southard and Southard 1992, 1994; Southard et al. 2000; Jacobs et al. 1996).

Single photon or dual photon absorptiometry utilizes a gamma source to measure bone mass in grams (approximate ash weight) per cm along the axis of the bone. Single photon absorptiometry (SPA) requires a means to assure that the soft tissue equivalent material overlying the bone is of constant thickness. The original technique involved submerging the forearm or leg in a water bath limiting its use to peripheral sites, such as the distal radius (Jeffcoat et al. 2000). Von Wowern et al. (1994) estimated the bone mineral content (BMC) of the mandible in vivo at the standard site on the mandible (the base and body in the left molar region) with the dual-photon scanner GT_{45} with a ^{153}Gd, especially developed for scanning of the jaws (von Wowern 1985a, b; von Wowern et al. 1992, 1994, 2001a). This dual photon absorptiometry (DPA) bone scanner fulfills the demands for short- and long-term precision (2–1%), accuracy (6%), minimal radiation dosage, effective correction to account for varying soft thickness, and can also be used for forearm BMC measurements (von Wowern 2001b).

Dual energy X-ray absorptiometry (*DXA*) uses an X-ray source for measurements of bone mass, as "areal density" in units of g/cm^2. DXA may be used to measure BMD in central sites such as the spine or hip or peripheral sites such as radius (Jeffcoat et al. 2000). Using Hologic QDR-1000 bone densitometer, designed to measure lumbar spine and hips, ex vivo and in vivo measurements were made in selected areas of the mandible (Corten et al. 1993). However, in vivo, the reproducibility of the measurements was poorer for the dental patient and for the three denture wearers. The difference of reproducibility between ex vivo and in vivo measurements can partly be explained by the rigidity of the fixation equipment, the movement of the patient, and the mobility between the denture base and the underlying soft alveolar tissue (Corten et al. 1993). The technique was applied for both maxilla, and mandible as well (Horner et al. 1996; Devlin et al. 1998; Drage et al. 2007).

Quantitative computed tomography (*QCT*) is a relatively new method that permits direct measurement of either trabecular or total bone density by further analysis of the information obtained from special computed tomography protocols. QCT provides measures of bone "apparent" density in units of g/cm^3 (Jeffcoat et al. 2000). After the first scanning of a lateral projection view, Klemetti et al. (1993b) located and selected the jaw slices to be measured. With a tomogram for localization, 4 mm-high slices (with a shift of 1 mm) were measured through the mandible, with the scanner tilted so that the slices lay parallel to the inferior margin of the mandible (Klemetti et al. 1993b). The cortical BMD (BMDC) was measured in two regions, bucally and distally from the foramen mentale and labially between the mental foramina. The BMD of the trabecular portion of the mandible was measured distally from the mental foramen (Klemetti et al. 1993b). The disadvantages of total body QCT scanners are the relatively high radiation dose, the uncomfortable position of the patients in a closed box and their extremely high price while QCT scanners are much more acceptable from this point of view (von Wowern 2001a). Further shortcoming of QCT is that both collagen and fat components in the jaws may influence the results. To resolve this, dual energy QCT (DEQCT) has been developed and applied to the measurement of BMD of the jaw (Taguchi et al. 1996). Recently, some investigators started to apply cone beam CT (CBCT) for the measurement of BMD of the jaws because radiation dose of CBCT is relatively smaller than that of conventional CT (Lee et al. 2007; Veyre-Goulet et al. 2008; Lagravère et al. 2008). However, further investigation would be necessary to conclude that CBCT may be used for the BMD assessment of the jaws.

Taking everything into account, the most preferable method for jaw BMD assessment seems to be a QCT thin slice bone scanner, as it allows separate cortical/cancellous analyses and useful anatomic bone measurements as well. However, a suitable reference standard for jaw BMD assessments and the appropriate software will be required (von Wowern 2001b).

7.2 Relationship Between Systemic and Mandibular Bone Mineral Density

It has long been postulated that mandibular BMD may be indicative for systemic BMD (Jeffcoat et al. 2000).

Kribbs et al. (1989) explored the relationship between skeletal bone mass and bone mass of the mandible in a group of 85 postmenopausal women with osteoporosis. Mandibular bone mass was determined by microdensitometry on periapical radiographs. One occlusal radiograph was made of each side of the mandible for measuring the thickness of the arch at the site where bone mass was measured. Mass was divided by width to derive bone density. Mandibular bone mass was significantly correlated with total body calcium (TBC) and bone mass of the radius, determined by SPA. Mandibular bone mass was correlated with vertebral bone mass, measured at the lumbar spine by the DPA ($r = 0.33$) and by QCT ($r = 0.30$). Mandibular bone density correlated with TBC ($r = 0.31$) and SPA of the radius ($r = 0.34$) to a lesser degree than did the mandibular mass (Table 7.1).

When analyzing the same parameters in normal women ($n = 50$), 23 women younger than 50 years of age and 27 older than 50 years of age, Kribbs et al. (1990a) reported that mandibular bone was significantly correlated with vertebral BMC ($r = 0.39$) with the use of DPA and with radial BMC ($r = 0.33$). Compared with the younger group, the older group had significantly greater bone density, thinner mandibular width, a thinner cortex and fewer teeth.

In a normal population ($n = 27$ women), mandibular bone mass and density were significantly greater ($P < 0.001$) than in an osteoporotic population ($n = 85$ women) with radiographic evidence of vertebral compression fractures (Kribbs 1990b).

In a later study, von Wowern et al. (1994) compared the bone loss in elderly women, including both the patients with osteoporotic fractures ($n = 12$) and the age-matching normal women ($n = 14$). The BMC of the mandible was estimated in vivo at the standard site of the mandible (the base and body in the left molar region) with the dual-photon scanner GT_{45} with a ^{153}Gd, as well as the BMC of the forearm bones. Significant differences in BMC of the mandible ($P < 0.01$) and the distal forearm ($P < 0.01$) were demonstrated, with the lowest mean BMC values in the osteoporotic group.

The BMD of the mandibular cortex (BMDC) was measured in 77 postmenopausal women, aged 48–56 years, by QCT. The BMD in the buccal cortex was significantly higher than that in the lingual side ($P < 0.001$). The BMD of the mandible, bucally and distally from the foramen mentale correlated well with the densities of the femoral neck and lumbar spine, while the correlations of the lingual cortical BMD of the mandible values with these densities were somewhat weaker. When patients were divided into groups based on the BMD of their femoral collum, in the groups with the lowest bone density and with average bone density the BMDC values correlated significantly with trabecular BMD (BMDT). In those groups, the buccal values for the cortex did not show any correlations with BMDT. The amount of cortical bone in the femoral collum, about 75%, was closer to that in the mandible than the amount in the vertebrae (Klemetti et al. 1993a).

Jacobs et al. (1996) examined the relationship between bone mass of the jaw and the axial skeleton on a group of 69 female subjects who received hormone replacement therapy for an average period of 5 years. DPA was applied to determine the BMD of the lumbar spine. The jaw bone of the posterior region of the mandible was evaluated by means of standardized long-cone radiographs. A densitometric wedge in the film holder provided a reference to quantify bone mass. Absolute values of the jaw bone mass were moderately correlated with the BMC of lumbar spine (Jacobs et al. 1996).

Using dual-energy QCT, the mandibular and third lumbar (L_3) vertebral BMD were evaluated in 21 women within 5 years after the menopause (recent postmenopause) and the other 23 women more than 5 years after the menopause (long-term menopause). The mandibular cortical BMD in the recent postmenopausal group was significantly higher than that in the long-term group ($P < 0.01$). There was no significant difference between the two groups for mandibular trabecula, L_3 vertebra cortical and trabecular BMD. When spearman correlation coefficients were calculated, there were significant relationships between mandibular cortical BMD and L_3 vertebral BMD ($P < 0.01$) in the recent postmenopausal group, and between the mandibular BMD and trabecular L_3 BMD ($P < 0.05$) in the long-term postmenopausal group (Taguchi et al. 1996).

The Women's Health Initiative is an unprecedented study of women's health after menopause in the United States. Specific risk factors for diseases including heart disease and osteoporosis were being addressed

Table 7.1 Relationship between systemic and mandibular bone density

Authors	Type of study	Population	Osteoporosis assessment	Mandibular BMD assessment	Association
Kribbs et al. (1989)	Cross-sectional	85 postmenopausal (50–84 years age) women with osteoporosis	Total body calcium; SPA of the radius; DPA of the vertebrae	Mandibular bone mass was determined by microdensitometry on periapical radiographs; Mandibular bone density = MB mass/ width of the arch; Cortical thickness at the gonion	Significant
Kribbs et al. (1990a)	Cross-sectional	50 normal women (20–90 years age)	Same as above	Same as above	Significant
Kribbs (1990b)	Cross-sectional	85 women with osteoporo-sis(50–84 years age) 27 normal women	Radiographic evidence of vertebral compression fractures	Same as above	Significant
von Wowern et al. (1994)	Cross-sectional	12 women with osteoporosis, 14 normal women	DPA of the forearms	Bone mineral content of the mandible estimated in vivo at the standard site of the mandible (the base and body in the left molar region) by DPA	Significant
Klemetti et al. (1994)	Cross-sectional	77 postmenopausal women (48–56 years)	DPA of the femoral neck and lumbar spine (L_2–L_4)	BMD of the mandibular cortex measured by quantitative computed tomography (QCT)	Significant
Jacobs et al. (1996)	Longitu-dinal (5 years)	69 female subjects who received hormone replacement therapy (32–64years age)	DPA of the vertebrae	BMD of the mandible on radiographs	Moderate correlation
Taguchi et al. (1996)	Cross-sectional	21 women within 5 years after the menopause (recent post-menopause) and the other of 23 women more than 5 years after (long-term menopause)	Vertebral BMD by QCT	BMD of the mandibulae measured by QCT	Significant
Taguchi et al. (1999)	Cross-sectional	90 Japanese women (40–68 years)	BMD of the lumbar spine measured by dual energy computed tomography	Mandibular inferior cortical width; Morphology of mandibular inferior cortex (MIC); Alveolar bone height measured on panoramic radiographs	Significant
Jeffcoat et al. (2000)	Cross-sectional	158 post menopausal women	DPA of the hip	Areal bone density on quantitative digital intraoral radiographs	Significant
Devlin and Horner (2002)	Cross-sectional	74 postmenopausal British women, aged 43–79 years (mean age, 62 years)	DXA of the right femoral neck and lumbar spine (L1–L4) SPA of the proximal and distal forearm	Mandibular inferior cortical width (MCW) below the mental foramen (MCW) on panoramic radiographs	Significant

(*continued*)

Table 7.1 (continued)

Authors	Type of study	Population	Osteoporosis assessment	Mandibular BMD assessment	Association
Drozdzowska et al. (2002)	Cross-sectional	30 healthy postmenopausal edentulous Polish women, aged from 48 to 71 years old (mean age, 59 years)	BMD of the hip (neck-BMD, Ward's-BMD, trochanteric BMD) and mandible (m-BMD) measured by DXA; Calcaneus using Achille and hand phalanges were assessed by Quantitative Ultrasound	Mandibular Cortical Index (MCI), The height of MIC (mm), Panoramic Mandibular Index (PMI), Mandibular Ratio-MR were evaluated on panoramic radiographs	Non-significant
Taguchi et al. (2006)	Cross-sectional	158 healthy Japanese postmenopausal women, aged 46 –64 years	BMD at the lumbar spine and the femoral neck determined by DXA	Mandibular cortical shape (erosion) and width evaluated on dental panoramic radiographs	Significant
Yaşşar and Akgünlü (2006)	Cross-sectional	48 postmenopausal Turkish women, aged 40–64 years	Spine BMD measured by DXA	Cortical width, cortical index, PMI and mandibular crest resorption degree on panoramic radiographs; Fractal dimension was calculated from the direct digital periapical radiographs of the mandibular premolar-molar region in box-counting method	Non-significant
Devlin et al. (2007)	Cross-sectional	671 postmenopausal European women 45–70 years of age	DXA scans of the left hip and lumbar spine	Mandibular cortical width (MCW) and porosity in the mental foramen region of the mandible on panoramic radiographs	Significant
Taguchi et al. (2007)	Cross-sectional	450 postmenopausal Japanese women (mean age, 57.2 years)	BMD of the lumbar vertebrae (L2–L4) measured by DXA	MCW and shape on panoramic radiographs	Significant
Okabe et al. (2008)	Cross-sectional	659 Japanese subjects (262 men and 397 women)	Heel bone density measured by ultrasound densitometry	Cortical width and shape (normal cortex, mildly to moderately eroded cortex, and severely eroded cortex) evaluated on panoramic radiographs	Significant

BMD bone mineral density; *CW* thickness of the mandibular cortex; *DPA* dual-photon absorptiometry; *M/M ratio* index of the mandibular alveolar bone resorption degree (the total mandibular height is divided by the height from the center of the mental foramen to the inferior border of the mandible); *PMI* the ratio of the thickness of the mandibular cortex to the distance between the mental foramen and the inferior mandibular cortex; *SPA* single-photon absorptiometry

nationwide. All first 158 subjects enrolled in the study were postmenopausal females, with a hip BMD confirmed by DXA to be within or below one standard deviation of young subjects. Intraoral radiographs of the area of the first mandibular basal bone were digitized and corrected for contrast and angulation errors, and areal bone density was calculated relative to a reference wedge in the film holder. The population had a mean age of 62.2 ± 7.6 years; 66% of subjects reported taking hormone replacement therapy, and 57.1% of subjects classified themselves as Caucasians, 42.5% were African-Americans, and 0.4% were American Indian. Significant correlations were found between mandibular basal BMD and hip BMD (r = 0.74; P < 0.001) (Jeffcoat et al. 2000).

Mandibular inferior cortical width, morphology of mandibular inferior cortex (MIC), alveolar bone height measured on panoramic radiographs were compared with the third lumbar vertebral BMD (L_3 BMD), measured by DEQCT in 90 Japanese women, aged 40–68 years (mean age 54.1), by means of multiple regression analysis, controlling for body mass index, menopausal status, years since menopause and self-reported periodontal condition. As L_3 BMD decreased, MCW decreased and the MIC value significantly increased. A significant difference in MCW between the high and low L_3 BMD groups was found (4.3 ± 0.8 vs. 3.7±0.9mm) (P < 0.01) (Taguchi et al. 1999).

Devlin and Horner (2002) reported that the mandibular inferior cortical width below the mental foramen (MCW) was significantly correlated with the BMD T-score at the lumbar vertebrae (r = 0.52, P < 0.01) in 74 postmenopausal British women aged 43–79 years (mean age, 62 years).

Okabe et al. (2008) also reported the significant correlation between the MCW and heel bone density measured by ultrasound (r = 0.44, P < 0.001) in 659 Japanese subjects (262 men and 397 women).

Yaşşar and Akgünlü (2006) found no significant association between the MCW and spine BMD measured by DXA in 48 postmenopausal Turkish women aged 40–64 years, although a marginally significant relation was noted on binary regression analysis.

Drozdzowska et al. (2002) also failed to find a significant association between the MCW and hip BMD and ultrasound measurements of calcaneus and hand phalanges in 30 healthy postmenopausal edentulous Polish women aged from 48 to 71 years (mean age, 59 years). The small sample sizes may have contributed to the lack of significant associations in these two studies.

In the recent study in 450 postmenopausal Japanese women (mean age, 57 years), spine BMD measured by DXA was significantly correlated with the MCW (r = 0.44, P < 0.001) (Taguchi et al. 2007) The adjusted odds ratios for low spine BMD (T-score ≤−1.0) associated with the second, third, and lowermost quartiles of cortical width were 1.71, 2.30, and 5.43, respectively, compared to the uppermost quartile. In this study, the lowermost quartile was below 2.9 mm (corrected for vertical magnification error). In other study of 157 healthy younger postmenopausal women <65 years old, the respective likelihood ratios for identifying women with low BMD (at the spine or proximal femur) and osteoporosis were 13.90 and 6.40 for thin cortical width (<3.0 mm) (Taguchi et al. 2006)

Devlin et al. (2007) reported that for three observers, a MCW of < 3 mm (corrected for magnification error) provided diagnostic odds ratios of 6.51, 6.09, and 8.04 in screening of osteoporosis in 671 postmenopausal European women aged 45–70 years. They concluded that only dental patients with a thinner MCW (i.e., < 3 mm) should be referred for further osteoporosis investigation.

As Jacobs et al. (1996) observed, mandibular bone mass seems to be more related to bone mass of highly corticalized bone (e.g., second metacarpal, femur, forearm) than to bone mass of trabecular skeletal bones (e.g., iliac crest, lumbar spine). The metabolism of trabecular bone is faster and more susceptible to osteoporotic changes (Jacobs et al. 1996). It was found, in a group of 40 healthy edentulous patients between 40 and 80 years of age, that maxillary BMD was significantly lower (mean = 0.55 g/cm^2) than that of the mandibular body (mean = 1.11 g/cm^2) as determined by dual-energy X-ray absorptiometry (Devlin et al. 1998).

7.3 Effects of Osteoporosis Therapy on Oral Health

The degree of bone changes in the jaws caused by the systemic treatment cannot be precisely predicted from the concomitant bone changes elsewhere, as the rate of bone remodeling is site specific and this applies particularly to the jaws. Nor can these bone changes be predicted from experimental studies on rats, as the function of their jaws and cortical bone remodeling differ from those in humans (von Wowern 2001b).

Several studies have examined the benefits of hormone replacement therapy on oral health (Table 7.2).

Norderyd et al. (1993) examined the association between supplementary estrogen intake and periodontal and gingival status in a total of 228 women, 50–64 years of age. The average percent of gingival units with bleeding was significantly lower ($P = 0.009$) in the estrogen group (30 ± 19%) compared to the control (39 ± 21%). The percent of surfaces with visible plaque was also significantly lower ($P = 0.03$) in the estrogen group, 60

Table 7.2 Effects of estrogen (hormone) replacement therapy (HRT) for osteoporosis on the jaws

Authors	Type of study	Population	Treatment	Major results
Norderyd et al. (1993)	Cross-sectional	57 women constituted estrogen group; 171 women constituted control group (mean age 56 ± 4.4 years)	HRT	Fever bleeding sites in estrogen group
Paganini-Hill (1995)	Ten years longitudinal study	3,921 women (age range 52 to 109 years)	HRT	Tooth loss and edentia significantly lower in estrogen users than in nonusers
Jacobs et al. (1996)	Five years longitudinal study	69 Caucasian female subjects (mean age 32–64 years)	HRT	Positive effect of ERT on the bone mass of the mandible
Ferreira et al. (1996)	Cross-sectional	327 premenopausal women (25–49 years old), 314 postmenopausal women (50–74 years old)	HRT	HRT is associated with significant less tooth loss in postmenopausal women
Grodstein et al. (1996)	Prospective study	761 postmenopausal women	HRT	Estrogen use may reduce tooth loss
Krall et al. (1997)	Cohort study	488 women (72–95 years old)	HRT	Estrogen users had more teeth remaining than nonusers; duration of estrogen use was an independent predictor of the number of teeth remaining ($P = 0.015$)
Reinhardt et al. (1999)	Two years longitudinal study	59 moderate/advanced adult periodontitis patients 16 non-periodontitis subjects	HRT	In non-smoking osteopenia/osteoporotic periodontits patients, E_2 supplementation is associated with reduced gingival inflammation and frequency of CAL loss
Ronderos et al. (2000)	Cross-sectional	116,555 adults	HRT	Inverse association between the use of estrogen RT and the mean CAL among postmenopausal women
López-Marcos et al. (2005)	Cross-sectional	210 menopausal women aged 40–58 years patients, divided into two groups: One group ($n = 134$) received HRT administered in patches and the other group ($n = 56$) did not received this therapy	HRT	HRT acts as a protecting factor against tooth mobility and improves the depth of periodontal probing. Regarding gingival recession and the dental pain, no significant results were obtained either for patients not receiving HRT or for patients being treated with dermal patches
Giuca et al. (2009)	Cross-sectional	95 female patients; 14 were the control group and received no hormone replacement therapy, while 81 patients underwent two types of therapy: 38 were prescribed estrogen therapy and 43 phytotherapy	HRT	It was observed that the patients receiving treatment had an improvement or disappearance of symptoms in the oral cavity: salivary change, gingivitis, bleeding and taste changes

CAL clinical attachment level

± 30 s. 69 ± 28% in the control group. Conversely, the percent of teeth with subgingival calculus (38 ± 37 vs. 29 ± 34%), mean probing pocket depth (2.2 ± 0.6 vs. 2.0 ± 0.5 mm), mean clinical attachment loss (2.3 ± 0.9 vs. 2.1 ± 0.7 mm) and mean crestal bone height (2.8 ± 1.3 vs. 2.7 ± 0.9mm) was greater in the control group than in the estrogen group, whereas the number of remaining teeth (21.3 ± 5.8 vs. 22.9 ± 5.3) was less in the control group. Statistically significant differences between the groups were also observed for dental care habits ($P < 0.001$) and education ($P = 0.022$). After overall effect adjustments with ANCOVA, the values for mean percent gingival bleeding units were closer for the two groups (31 vs. 38%), but those women taking estrogen still had statistically significantly fewer bleeding sites than the controls ($P = 0.04$).

A 10-year longitudinal study of a cohort sample of 3,921 women (age range 52–109 years) revealed that the risk of tooth loss and the proportion of edentulous women decreased with increasing duration of estrogen use ($P < 0.001$). The age-adjusted ridge resorption for <25 teeth was 0.76 (95%CI: 0.67–0.87) for estrogen users compared with nonusers. Fewer estrogen users than nonusers were edentulous in all age groups, except for those less than 70 years old (Paganini-Hill 1995).

Sixty-nine Caucasian female subjects (mean age 32–64 years) received hormone replacement therapy for an average period of 5 years. Bone mass of the lumbar spine was determined by DPA. The jaw bone of the posterior region of the mandible was evaluated by means of standardized long cone radiography using individual bite-blocks. Bone measurements were clearly dependent on the quantity of hormonal drugs administered during the study period. At both examinations, lumbar BMC and BMD were positively related to the normalized amounts of drugs administered (Spearman rank correlation $r = 0.39–0.73$, $P < 0.01$). A similar relationship was also established for jaw-bone mass measured at the second examination (Spearman rank correlation $r = 0.41$, $P < 0.01$) (Jacobs et al. 1996).

The Nurses' Health Study was conducted in a random sample of postmenopausal nurses who reported no tooth loss, 300 current users of estrogen, 300 past users and 300 women who had never used estrogen. Of the 900 women, 761 responded to the questionnaire, which asked about personal and professional care in three time periods: 1981–1986, 1987–1990, and 1991. An inverse association was observed between current use of postmenopausal hormones and the loss of one or more teeth. The age-adjusted relative risk (RR) in current users was 0.73 (95%CI: 0.69–0.77), compared with never-users. The risk of tooth loss did not appear to change according to the duration of current or past estrogen use. Compared with never-users, current users of less than 1 year had a RR of 0.80 (95%CI: 0.67–0.94), and those who had used hormones for 15 years or more had a risk of 0.73 (95%CI: 0.65–0.83). Similarly, among past users, the RR was 0.93 for those who had used estrogen for <1 year and 0.98 for those who had used estrogen for 15 years or more. For the current users of conjugated estrogen, the impact of dosage was also examined and a decreased risk of tooth loss associated with all dosages of conjugated estrogen was found. For women using 0.3 mg/day of estrogen, the RR was 0.69 (95%CI: 0.59–0.81); for those taking 1.25 mg or more, this estimate was 0.75 (95%CI: 0.66–0.85). Similarly, there was a reduced risk of tooth loss associated with all dosages of current progestin (Grodstein et al. 1996).

Krall et al. (1997) estimated the effect of duration of estrogen use on the number of teeth remaining and likelihood of being edentulous on 488 women, aged 72–95 years, who participated in the twenty-third examination cycle (1994–1995) of the Framingham Heart Study, a population-based study begun in 1948. The number of teeth remaining and their location were recorded by a trained observer. History and duration of ERT were obtained from records, kept since cycle 10 (1960–1963). The difference in mean number of teeth between estrogen users and nonusers remained after adjustment for age, smoking status and level of education (12.5 ± 0.8 vs. 10.7 ± 0.6, $P = 0.046$). The association with estrogen was present among three of the four tooth types (incisors, canines and premolars, but not molars)/ and was unchanged by adjustment for covariates. Duration of estrogen use was a predictor of the number of teeth present among all women (slope ± SE = 24±0.10 teeth/year of estrogen use, $P = 0.015$). Long-term use of estrogen (more than 8 years) was associated with significantly greater retention of all types of teeth except molars. The results were unchanged when current smoking status or duration of smoking was substituted from history of ever having smoked or when years of schooling were substituted for high school education. Prevalence of edentulism was inversely related to estrogen duration. Only 21% of the long-term users were edentulous compared with 27% of women who used it for? 1 to 8 years, and 33% of non-users ($P = 0.06$). When controlled for age, smoking status and education, the odds of being

edentulous were reduced by 6% for each year of estrogen use (OR = 0.94, 95%CI: 0.90–0.99).

Reinhardt et al. (1999) evaluated the influence of serum estradiol levels and osteopenia/osteoporosis on common clinical measurements over a 2-year period on 59 moderate/advanced adult periodontitis patients and 16 non-periodontitis subjects, all within 5 years after menopause at baseline. Serum 17β-estradiol was measured with a solid-phase ^{125}I radioimmunoassay. DXA was used to measure BMD of the lumbar spine in periodontitis patients only. Clinical measurements in periodontitis patients were taken at four posterior interproximal sites per tooth at biannual appointments to determine incidence of progressive periodontitis, gingival inflammation and supragingival plaque. E_2 status did not influence the percentage of sites loosing CAL for either periodontitis or non-periodontitis groups, but when non-smoking osteopenia/osteoporotic periodontits patients were evaluated, E_2 deficient subjects had more BOP (bleeding on probing) (43.8vs. 24.4%, $P < 0.04$) and a trend toward a higher frequency of ≥ 2.0 mm CAL loss vs. 1.2%, $P < 0.01$) than E_2 sufficient subjects.

In a large sample of U.S. adults (116,555 subjects), Ronderos et al. (2000) has reported an inverse association between the use of estrogen replacement therapy and the mean CAL among postmenopausal women ($P = 0.00001$). After adjustment for the effects of age, race-ethnicity, smoking, poverty-income ratio and frequency of dental visits, postmenopausal women who reported having used estrogen supplementation presented significantly lower mean CAL than those who never used estrogen. The difference in the mean CAL between the never-users and those women who reported using estrogen either for 1 year or less, for 2–5 years, or for more than 5 years were 0.16 mm ($P = 0.031$), 0.33 mm ($P = 0.0003$), and 0.36 mm ($P < 0.0001$), respectively. After adding several covariates: BOP, calculus, BMD diagnosis, to a multiple regression model, the strength of the association was reduced ($P = 0.01$) and estimated differences between the estrogen users and non-users were narrowed.

Relationships among number of teeth remaining (total, anterior, and posterior teeth), oral bone height, oral bone porosity, BMD of the lumbar spine and the femoral neck, estrogen use status, and the duration of estrogen use were evaluated in 330 Japanese postmenopausal women (mean age ± SD, 56.8 ± 7.6 years). Analysis of covariance adjusted for confounding variables revealed that estrogen users (66 subjects) tended to have more posterior teeth than did non-users (264 subjects) ($P = 0.065$), although there were no significant differences in number of total ($P = 0.196$) and anterior ($P = 0.751$) teeth remaining, oral bone height ($P = 0.970$), oral bone porosity ($P = 0.745$), BMD of the lumbar spine ($P = 0.459$) and the femoral neck ($P = 0.749$) between estrogen users and non-users. Multiple regression analysis showed that the duration of estrogen use was significantly associated with number of total ($P = 0.019$) and posterior ($P = 0.007$) teeth remaining independent of age and oral bone height (Taguchi et al. 2004)

The effect of calcium supplementation on tooth loss and periodontal status has also been assessed and there is limited evidence that calcium supplementation may be beneficial (Jeffcoat et al. 2000).

Sixty-six persons with periodontal problems were prescribed at random calcium (1 g) or placebo tablets daily during a trial period of 180 days. Their periodontal status was examined at day 0 and at day 180. No statistically significant change in the number of probing depths >3 mm could be found between the groups. Similar results were obtained for plaque index (PI), mobility and furcation involvement. A comparison between pairs of bitewing radiographs taken before and after the trial failed to show any changes in marginal bone level and density (Uhrbom and Jacobson 1984).

Krall et al. (1996) conducted a 7-year longitudinal study on 189 healthy, white, postmenopausal women who were not taking estrogens. In Study I, women were randomly assigned to one of three arms of intervention: two groups took 5000 mg/day of elemental calcium either in the form of calcium citrate, malate, or calcium carbonate for 2 years, and the third group took a placebo. In Study II, which was 1 year in duration, the women were randomly assigned to 400 IU of vitamin D or placebo and all received calcium supplements (377 mg/day). Study III was a randomized 2-year trial comparing 2 doses of vitamin D (100 IU or 700 IU) each in combination with 500 mg/day of calcium. During Study I, a greater proportion of the placebo group lost teeth than the calcium supplemented group (11 vs. 4%, $P = 0.054$), and the association was pronounced among nonsmokers, where 12% of women in the placebo group lost teeth compared with 3% in the supplemented group ($P = 0.04$). However, in Studies II and III, in which all women were taking calcium supplements, neither of the vitamin D interventions was associated with the likelihood of loosing teeth.

It was showed that lower dietary intake of calcium increased the risk of periodontal disease. The odds ratios were 1.84 (95%CI: 1.36–2.48) for young males (20– 39 years), 1.99 (95%CI: 1.34–2.97) for young females and 1.90 (95%CI: 1.41–2.55) for middle-aged males (40–59 years), after adjusting for gingival bleeding and tobacco consumption. A dose-response relationship was seen. In females, there was a 54% greater risk of periodontal disease in subjects who had the lowest levels of dietary calcium intake (2–499 mg) and a 27% greater risk in subjects who took moderate levels of dietary calcium (500–799 mg), as compared to those who took 800 mg or more dietary calcium per day (Nishida et al. 2000).

Data from a prospective study of oral health in men showed a similar association between higher calcium intake and reduced alveolar bone loss. The number of teeth with progression of alveolar bone loss over a 7-year period was significantly lower among men whose calcium intake was at least 1000 mg/ day, compared to men with a calcium intake below the level (Krall et al. 2001).

The bisphosphonates have been shown to prevent alveolar resorption and preserve mandibular bone mass in animals (Yaffe et al. 1997; Reddy et al. 1995), but their clinical use in the treatment of periodontal bone loss in humans needs to be established (Wactawski-Wende 2001). In a pilot clinical trial, the efficacy of the bisphosphonate drug alendronate in slowing alveolar bone loss due to periodontits has been investigated. This double-blind placebo-controlled randomized clinical trial measured loss of bone height and density using digital substraction radiography over a 9-month period. Alendronate reduced the risk of progressive loss of alveolar bone. The relative risk of progressive loss of bone height and density was 0.45 for the alendronate-treated patients compared with placebo-treated patients (Jeffcoat et al. 2000). However, the caution should be noted about the use of bisphosphonates on oral health because a large number of reports alarm the presence of bisphosphonate-related osteonecrosis of the jaws since 2003 (Edwards et al. 2008)

Largely, the above-mentioned studies have found a positive association between hormone replacement therapy and the oral health. One potential source of bias in these important studies (and in fact, addressed in the reports) is the fact that the same patients who seek to prevent osteoporosis may seek preventive dental care as well. The populations from the cohort studies (Grodstein et al. 1996; Paganini-Hill 1995) are large, but composed of relatively well-educated, higher socio-economic groups (Jeffcoat 1998). Furthermore, while these studies showed a relationship between tooth loss and estrogen deficiency, the cause of the tooth loss was not defined (Payne et al. 1999).

7.4 Correlation of Tooth Loss with Skeletal Bone Mineral Density

Numerous studies have looked at the relationship between osteoporosis or bone density and tooth loss (Wactawski-Wende 2001).

Two hundred eight white women, aged 60–69 years, who visited a private practice during 12-month interval, have acquired 218 upper or lower full dentures. Each woman's smoking habits and current osteoporosis severity (percent cortical area at metacarpal midshaft – PCA) were compared with the age at which she had acquired each full denture. Among osteoporotic women (PCA < 70%) who still had their natural teeth at the age of 50 years, 44% had required a new full denture before age 60 compared with 15% of non-osteoporotic women (PCA > 80%) (Daniell 1983).

Variables of mandibular bone mass, mandibular bone density, cortical thickness at the gonion, number of mandibular teeth present and age were all evaluated statistically to determine which variables best distinguished an osteoporotic population of 85 women diagnosed with radiographic evidence of vertebral compression fractures from a normal population (n = 27 women). Only mandibular bone mass and the number of teeth were useful parameters (Kribbs 1990b).

Lumbar BMD (lumbar BMD) and metacarpal cortical thickness (MCT) were measured in 286 female volunteers between 46 and 55 years of age. Compared to dentate subjects, the lumbar BMD and MCT of the edentulous women were not significantly different. No correlation was found between lumbar BMD, MCT and the number of missing teeth (Elders et al. 1992).

Klemetti and Vainio (1993) examined clinically, the jaws of 355 postmenopausal women, and measured by DXA their bone mineral status, the BMD at the femoral neck and the lumbar spine. When the number of teeth was compared with the BMD of the femoral neck and lumbar spine, no correlation was found (correlation values 0.03 and 0.01, respectively). The sample of 355 women was then divided into three groups based on dentition: group 1, teeth in both

jaws (n = 125); group 2, teeth only in the mandible (n = 102); and group 3, with no teeth (n = 128). When the femoral and lumbar BMD values of the groups were compared, no dependencies were observed either [significance (p) of dependence: 0.98 and 0.85, respectively] (Table 7.3).

Taguchi et al. (1995a) evaluated 64 women aged between 50 and 70 years for several oral signs on panoramic radiographs: the number of teeth present, MCW below the mental foramen, the degree of mandibular alveolar bone resorption and morphologic classification of MIC. Osteoporotic signs consisted of thoracic spine fracture as demonstrated on lateral chest radiographs. The number of teeth present in the osteoporotic group was significantly lower than those in non-fracture group (6.2 ± 9.3 vs. 19.0 ± 8.3, $P < 0.01$). The number of teeth present (N) was highly related to the probability of thoracic spine fracture and was used to derive the probability equation for the presence of thoracic spine fracture: probability value = $1\ (1 + e^{-Z})$, $Z = 18.68 - 0.29$ age $-0.27\ N$, where age range was 50–70 years. A probability value below 0.5 indicated low probability of fracture, and above 0.5 indicated a high probability.

The relationship between mandibular bone mass and tooth loss was studied in 269 patients who had neither metabolic disease nor local lesions affecting the mandibular cortex. The mineral status of the mandible was assessed by measuring the MCW below the mental foramen. In male subjects, there was no significant correlation between the number of teeth present and the MCW. Among women in seventh decade, number of teeth present in those in whom MCW was higher than mean was significantly greater than that for those in whom MCW was less than mean (Taguchi et al. 1995b).

Krall et al. (1996) evaluated if rates of bone loss in the whole body, femoral neck and spine are accelerated in women who are concurrently losing teeth, and has estimated the risk of tooth loss associated with systemic bone loss in 189 healthy, white, dentate, postmenopausal women who participated in a 7-year period longitudinal study. BMD of the lumbar spine, femoral neck and whole body were measured by DPA or DXA. The rates of BMD changes at all three sites were independent predictors of tooth loss in a multivariate models: for each 1% per year decrement in whole body BMD, the risk of tooth loss was more than quadrupled, and for the same magnitude of BMD loss at the femoral neck and spine, the risk of tooth loss was increased by 50 and 45%, respectively. Other independent predictors of tooth loss risk were years since menopause and number of teeth at baseline (Krall et al. 1996).

Mohammad et al. (1997) assessed bone densities of the spine by DXA in a group of 44 non Hispanic white women (aged 50–75 years). All bone densities above the age-adjusted mean population bone density were classified as HBD group, while those below the age-adjusted population mean bone density were classified as a LBD group. In a comparison of the group mean of missing teeth, no significant difference was found between the HBD group (3.81 ± 0.9 teeth) and the LBD group (4.90 ± 0.89 teeth). The mean tooth loss in the maxillae or mandible was not different between those two groups. In the stepwise linear regression equation, tooth loss was not significantly associated with spinal bone density ($P = 0.679$) (Mohammad et al. 1997).

Vertebral and proximal femoral BMD were measured by DXA in a cross-sectional study of 135 postmenopausal women (age range 41–70 years). Although the number of remaining teeth was correlated with attachment loss ($r = -0.26$, $P < 0.01$), none of the BMD measurements (expressed either as raw values, T scores or Z scores) was significantly correlated with the number of remaining teeth in this population (Hildebolt et al. 1997).

Bando et al. (1998) examined the effect of dentate state on skeletal BMD in postmenopausal women. The periodontally healthy group (group H) consisted of 14 women who had at least 25 teeth with no radiographic evidence of alveolar bone loss (mean age: 64.0 ± 5.5 years). The edentulous group (group E) consisted of 12 women who had been edentulous for an average of 15 years (mean age: 67.1 ± 2.9 years). BMD of the lumbar spine was determined by DXA. The BMD in the group H was 1.07 ± 0.21 g/cm^2, and that of group E was 0.89 ± 0.17 g/cm^2 ($P < 0.05$). Furthermore, the BMD of group H subjects expressed as a percentage of normal BMD in age-matched Asian women was significantly higher than that of group E subjects ($P < 0.05$).

Mandibular cortical bone mass, alveolar bone height and number of teeth present (total, anterior and posterior) were compared with the L_3BMD, measured by DEQCT (dual energy computed tomography), in 90 Japanese women (age range 40–60 years) by means of multiple regression analysis, controlling for body mass index, menopausal status, years since menopause and self-reported periodontal condition. There were significant relationships between the number of total and posterior teeth present and L_3BMD, but years since

Table 7.3 Relationship between tooth loss and bone mineral density

Authors	Type of study	Population	BMD assessment	Association with tooth loss
Kribbs (1990b)	Cross-sectional	85 women with osteoporosis; 27 normal women	Mandibular bone mass; Mandibular bone density; Cortical thickness at the gonion	Significant
Elders et al. (1992)	Cross-sectional	286 women (46–55 years)	Lumbar BMD bar BMD); Metacarpal cortical thickness	Non-significant
Klemetti and Vainio (1993)	Cross-sectional	355 postmenopausal women	BMD of the femoral neck and the lumbar spine	Non-significant
Taguchi et al. (1995a)	Cross-sectional	64 women (50–70 years)	Thoracic spine fracture as demonstrated on lateral chest radiographs	Significant
Taguchi et al. (1995b)	Cross-sectional	269 patients who had neither metabolic disease nor local lesions affecting the mandibular cortex	Lower border mandibular cortical width in the mental foramen region measured on panoramic radiographs	Significant
Krall et al. (1996)	Longitudinal 7 years	189 healthy, white, dentate, postmenopausal women	BMD of the lumbar spine, femoral neck and whole body measured by dual-photon or dual energy X-ray absorptiometry (DXA)	Significant
Mohammad et al. (1997)	Cross-sectional	44 non-Hispanic white women (aged 50 to 75 years)	Bone densities of the spine by DXA	Non-significant
Hildebolt et al. (1997)	Cross-sectional	135 postmenopausal women (age range 41–70 years)	Vertebral and proximal femoral BMD measured by dual-energy X-ray absorptiometry	Non-significant
Bando et al. (1998)	Cross-sectional	14 periodontally healthy women (mean age: 64.0 ± 5.5 years); 12 edentulous women (mean age: 67.1 ± 2.9 years)	BMD of the lumbar spine	Significant
Taguchi et al. (1999)	Cross-sectional	90 Japanese women (age range 40–60 years)	Third lumbar vertebral bone mineral density measured by dual energy computed tomography	Significant
Inagaki et al. (2001)	Cross-sectional	190 Japanese women (89 premenopausal, 101 postmenopausal)	Metacarpal bone mineral density measured by computed X-ray densitometry	Significant
Mohammad et al. (2003)	Cross-sectional	30 postmenopausal, dentate, Asian-American women	BMD of the os calcis, composed primarily of trabecular bone, was assessed by DXA	Significant
Gur et al. (2003)	Cross-sectional	1,171 postmenopausal osteoporotic women, aged 40–86 years (mean age, 61.19±7.28 years)	BMD of the spine and femur were measured by DXA	Significant

(*continued*)

Table 7.3 (continued)

Authors	Type of study	Population	BMD assessment	Association with tooth loss
Bollen et al. (2004)	Cross-sectional	Cases (n = 93) were individuals reporting osteoporotic fractures (fractures occurring after minor impact). Controls (n = 394) were individuals reporting traumatic fractures (n = 105) or no fractures (n = 289). All were older than 60 years.	History of self-reported osteoporotic fractures	Non-significant
Yoshihara et al. (2005)	Cross-sectional	460 Japanese subjects70-year-old	BMD of the os calcis using an ultrasound bone densitometer	Significant
Vescini et al. (2005)	Cross-sectional	455 healthy women	BMD measured both by DXA and quantitative ultrasound measurements	Significant
Inagaki et al. (2005)	Cross-sectional	356 Japanese women (171 premenopausal, mean age 37.9 + /−8.0 years; 185 postmenopausal, mean age 63.3 + /−7.7 years)	Metacarpal BMD measured by computerized X-ray densitometry	Significant
Famili et al. 2005	Two-year period	398 women (mean age 75.5 years)	BMD of the total hip and its subregions was measured using DXA	Non-significant
Drozdzowska et al. (2006)	Cross-sectional	30 healthy postmenopausal edentulous Polish women, aged from 48 to 71 years (mean age, 59 years)	BMD of the hip (neck-BMD, Ward's-BMD, trochanteric BMD) and mandible (m-BMD) measured by DXA; Calcaneus using Achille and hand phalanges were assessed by Quantitative Ultrasound	Significant
Vlasiadis et al. (2007)	Cross-sectional	133 postmenopausal women 38–80 years-of-age	BMD at the lumbar spine was measured by DXA	Significant
Nicopoulou-Karayianni et al. (2009)	Cross-sectional	665 females, aged 45–70 years	Bone density was measured at the total hip, femoral neck and lumbar spine	Significant

BMD bone mineral density; *DXA dual energy X-ray absorptiometry*

menopause was the only independent predictor of number of anterior teeth present (Taguchi et al. 1999).

Association between self-reported number of remaining teeth and BMD of the spine and the femoral neck was investigated in a cohort of 1,914 Japanese subjects aged 48–95 years, who were recruited from the Adult Health Study conducted by the Radiation Effects Research Foundation in Hiroshima. BMD of the spine and the femoral neck was measured by DXA. Tooth count was self-reported in response to a simple question to subjects about the number of remaining teeth they had at the time of the survey. Multiple regression analysis adjusted for age, weight, height, smoking, estrogen use and years since menopause revealed a

significant association between number of remaining teeth and BMD of the femoral neck in both men and women; however, no association was found between number of remaining teeth and BMD of the spine in both sexes. Retention of four teeth was significantly associated with a 0.004 g/cm^2 increase in femoral neck BMD in men ($P < 0.05$), which was similar to that observed in women ($P < 0.01$) (Taguchi et al. 2004).

Among 460 subjects with 70-year-old who have approximately the same number of each gender, an association between the BMD of the os calcis measured by an ultrasound bone densitometer and number of remaining teeth excluding the third molars was investigated. The mean number of remaining teeth for the osteopenia group (OG) and the no-osteopenia group (NOG) was 15.97 ± 9.98 and 18.31 ± 8.06, respectively in females, and 16.32 ± 9.93 and 18.12 ± 9.33, respectively in males (OG vs. NOG, $P = 0.047$ by two-way ANOVA). In addition, stiffness was significantly associated with the number of remaining teeth (CV = −0.157, $P = 0.005$) using linear multiple regression analysis adjusted by four other variables. In this study, there was a significant relationship between the number of remaining teeth and BMD of the os calcis (Yoshihara et al. 2005).

In the recent OSTEODENT study, the number of teeth was counted for 651 subjects aged 45–70 years. The BMD at the total hip, femoral neck and lumbar spine were measured by DXA. The mean number of teeth in the osteoporotic subjects was 3.3 fewer than normal subjects and 2.1 fewer if those with no teeth were excluded. The association between osteoporosis and having <6 or having <28 teeth remained significant after adjusting for covariates with *P*-values of 0.016 and 0.011, respectively. A single regression model for tooth count with normal errors would not fit all the data. By fitting mixture regression models to subjects with tooth count >0, three clusters were identified corresponding to different degrees of tooth loss. The overall effect of osteoporosis was as follows: −1.8 teeth before and after adjusting for smoking, −1.2 teeth after adjusting for age, and −1.1 teeth after adjusting for both age and smoking (Nicopoulou-Karayianni et al. 2009).

Cross-sectional studies correlating skeletal BMD with tooth count have not shown similar results. It was suggested that they may provide a basis for the design of future studies, but are not likely in themselves to provide a definitive answer to the temporal relationship between the onset of loss of skeletal BMD and teeth. At least three reasons contribute to this assertion. First, it is impossible to determine the cause of a lost tooth from a single examination. Teeth may be lost for many reasons other than decreased bone support including, but not limited to caries, endodontic involvement, fractures, trauma, and restorative considerations. Second, few teeth actually exfoliate, rather dentists extract them for a variety of sound diagnostic, prognostic, esthetic, patient preference and financial reasons. Therefore, unless very large populations are studied where these effects may be random among different groups, practice patterns and patient preference have the potential to bias the results. Third, patient recall of reasons for extraction is not always reliable, and if records of treatment are secured, they do not uniformly contain the information required to determine the reason for extraction (Jeffcoat 1998).

7.5 Relationship Between Osteoporosis and Residual Ridge Resorption

Several studies have investigated the influence of osteoporosis on residual ridge resorption (RRR).

In a cross-sectional study on 459 random panoramic radiographs of edentulous patients, the RRR in the mandible was expressed as a ratio IC/IM, that is, distance from the inferior border of the mandible to the crest of the residual ridge (IC) to the distance from the inferior border of the mandible to the crest of the inferior border of the mental foramen (IM) (Fig. 7.3). There was a

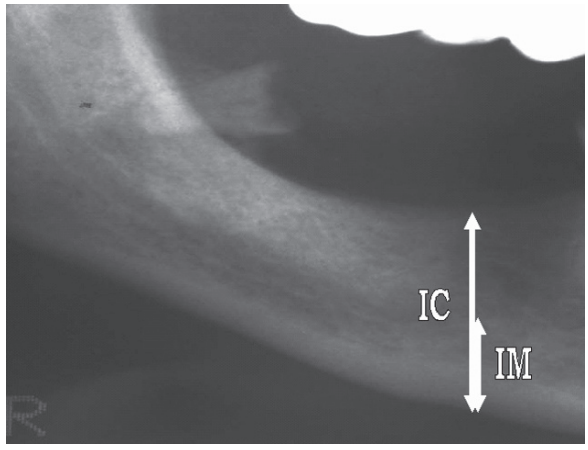

Fig. 7.3 Measuring method for the height of ridge on panoramic radiograph – from the inferior border to the superior edge of the alveolar crest in the region of the mental foramen (IC), divided by the distance from the inferior border to the lower edge of the mental foramen (IM) to calculate the percent of bone remaining

significantly larger percentage of women than men aged 55 years or greater in the edentulous class 3 (severe) resorption group, but this difference could not be related to the occurrence of menopause (Ortman et al. 1989).

The height of the edentulous ridge (RRR = IC/IM) was found to be correlated significantly ($P < 0.05$) with TBC ($r = 0.55$) and with mandibular bone mass ($r = 0.56$). Cortical thickness at the angle of the mandible was correlated with SPA of the radius ($r = 0.57$) and mandibular bone mass ($r = 0.54$) (Kribbs et al. 1989) in a group of edentulous postmenopausal women with osteoporosis (Table 7.4).

The BMC in edentulous mandibles in a group of young and older women after vestibule-lingual sulcoplasty with free skin grafts was measured in a 2-year follow-up study at the standard site of the mandible (the mandibular base and body in the left molar region) and in the D-site (the denture-wearing site of the mandible, the part of the mandible anterior to the mandibular rami). A significant negative correlation was found between the initial BMC values in the standard site and the BMC loss (in percent) in the denture-wearing site, and a significant positive relationship between the BMC loss (in percent) in the two sites of the mandible in the elder group of women (67–81 years) (von Wowern and Hjorting-Hansen 1991).

von Wowern and Kollerup (1992) has quantified the RRR atrophies of edentulous maxillae and mandible on lateral cephalogrames as sagittal areas measured in square millimeters in two groups of edentulous women. A significant difference in the size of sagittal maxillary area was demonstrated with the smallest mean value in the osteoporotic group. No significant difference in the size of the mandibular sagittal area was found between the two groups, but the smallest mean value was observed for the osteoporotic group.

The relationship between the height of the mandibular residual ridge and the severity of osteoporosis in elderly edentulous patients was investigated by Hirai et al. (1993). Frontal and lateral radiographs of the vertebrae were taken for evidence of ostoporosis. Height of the mandibular residual ridge was measured on panoramic radiographs from the inferior border to the superior edge of the alveolar crest in the region of the mental foramen. The height of the residual ridge decreased according to the severity of osteoporosis with a correlation coefficient $r = -0.42$ ($P < 0.01$). The PTH blood level was high in the patients with the lower residual ridges while the calcitonin value was low. Similar results were reported by Klemetti et al. (1993c).

A close relationship between osteoporosis and RRR has been strongly suggested (Hirai et al. 1993)

7.6 Osteoporosis and Implants-Supported Overdentures

The characteristics of the bone at the implantation site and the anatomical locations are among the factors, which seem to profoundly influence implant failure rates, independently of whether implants are located or not (Esposito et al. 1998).

Based on its radiographic appearance and the resistance at drilling, bone quality has been classified in four categories: (1) "type 1 bone," in which almost the entire jaw is composed of homogenous compact bone; (2) "type 2 bone," in which a thick layer of cortical bone surrounds a core of dense trabecular bone of favorable strength; (3) "type 3 bone," in which a thin layer of cortical bone surrounds a core of dense trabecular bone of favorable strength, and (4) "type 4 bone," characterized by a thin layer of cortical bone surrounding a core of low-density trabecular bone of poor strength (Esposito et al. 1998). In an attempt to isolate Branemark implants failure by bone type in a 5-year analysis, Jaffin and Berman (1991) showed that Type 4 bone was the single greatest determinant in predicting fixture failure. In types 1, 2, and 3, total fixture failure rate was 3% (29/952), however, in type 4 bone, 36 of 102 implants did not integrate (35%) (Jaffin and Berman 1991). Similar results were reported by Hutton et al. (1995) in a 3-year multicenter study undertaken on 120 subjects to provide data on implant and overdenture survival. Multivariate analysis suggested that, after compensating for confounding, dental arch and bone quality remained significantly ($P < 0.05$) related to overdenture treatment failure. The risk of overdenture treatment failure was much greater for the maxilla than for the mandible (OR = 7.33, 95%CI: 1.81–29.62). The overdentures fabricated for arches with bone quality four had a failure rate of 35.71% compared to 0.00%, 7.00 and 5.26% for arches with bone quality 1, 2, and 3, respectively. The odds of overdenture treatment failure for bone quality four was much greater (OR = 5.55, 95%CI: 1.20–25.68) than for the other quality groups.

Table 7.4 Relationship between osteoporosis and residual ridge resorption (RRR)

Authors	Type of study	Population	Osteoporosis assessment	RRR assessment
Ortman et al. (1989)	Cross-sectional	459 adult edentulous patients	Not done	RRR = IC/IM, where *IC* distance from the inferior border of the mandible to the crest of the residual ridge; *IM* distance from the inferior border of the mandible to the crest of the inferior border of the mental foramen
Kribbs et al. (1989)	Cross-sectional	85 edentulous postmenopausal women (50–84 years) with radiographic evidence of vertebral compression fractures for a diagnosis of osteoporosis	Total body calcium; SPA of the radius; DPA of the vertebrae cortical thickness at the gonion	The height of the edentulous ridge was measured from the inferior border to the superior edge of the alveolar crest in the region of mental foramen and was divided by the distance from the inferior border to the lower edge of mental foramen
Kribbs et al. (1990a)	Cross-sectional	50 normal women 20–90 years	SPA of the radius; DPA of the vertebrae; Mandibular bone mass on radiographs made distal to the mental foramen	Same as above
von Wowern and Hjorting-Hansen (1991)	Longitudinal, 2-year follow-up	25 normal edentulous women: $n = 11$, age 37–54 years; $n = 14$, age 67–81 years. All have had vestibulo-lingual sulcoplasty with free skin grafts	Bone mineral content(BMC) measured at the standard site of the mandible (the mandibular base and body in the left molar region) measured by DPA	BMC in the D-site (the denture- wearing site of the mandible, the part of the mandible anterior to the mandibular rami) measured by DPA
von Wowern and Kollerup (1992)	Case-control	12b osteoporotic edentulous women (test group) and 16 normal long-term edentulous women (control group)	BMC at the standard site of the mandible and the forearm bones measured by DPA	Sagittal maxillae and mandibles areas measured in square millimeters on lateral cephalogrames
Klemetti et al. (1993c)	Cross-sectional	355 postmenopausal women, aged 48 to 56 years	DPA of the femoral neck and vertebrae; Mandibular BMD measured by QCT	Same as above
Hirai et al. (1993)	Cross-sectional	44 in elderly edentulous patients (mean age 81.1 years)	Severity of osteoporosis by examining frontal and lateral radiographs of the vertebrae; Serum Ca, Phosphor, Parathormone, Calcitonine	Same as above

(*continued*)

Table 7.4 (continued)

Authors	Type of study	Population	Osteoporosis assessment	RRR assessment
Bollen et al. (2004)	Cross-sectional	Cases (n = 93) were individuals reporting osteoporotic fractures (fractures occurring after minor impact). Controls (n = 394) were individuals reporting traumatic fractures (n = 105) or no fractures (n = 289). All were older than 60 years	History of self-reported osteoporotic fractures	The residual ridge height of the edentulous mandible was measured at the site of the mental foramen

BMD bone mineral density; *DPA* dual-photon absorptiometry; *QCT* quantitative computed tomography; *SPA* single-photon absorptiometry

A clear correlation between implant failures and the complexity of the grafting procedure has been observed (Esposito et al. 1998). In retrospective analysis of 49 patients who received bone graft augmentation to the maxillary sinuses, the relative bone mass density (BMD%) differed significantly between the group who lost significantly more implants and their controls matched for age and sex (Blomqvist et al. 1996). The author suggested that in addition to local complications, general disorders, such as osteoporosis must be considered in excessive implant loss.

Toronto implant study patient series was done to determine whether age and sex, which have been recognized as important risk factors for osteoporosis, are also risk factors for patients with dental implants. No statistical difference in implant failure rates between women over and under age 50 could be shown. The lack of association of implant failure with gender (P = 0.22), as well as the opposite age trends observed in the implant failure rate (a decrease with age) and the prevalence of osteoporosis (an increase with age), suggest that it is unlikely that osteoporosis has had any influence on implant failure (Dao et al. 1993).

No substantial associations between DXA bone density T-scores at both distal and proximal radius and distal implant failure were observed in a controlled clinical trial performed on two groups of 49 patients. Bone quality appeared to substantially influence the risk for a patient to lose at least one implant. Implant placement in sites with thin cortical bone and low cancellous bone density (bone quality 3 and 4) increased the risk for a patient to lose at least one implant by 160%, when compared to implant placement in sites with good bone density (bone quality 1 and 2) (OR = 2.6; 95%CI: 1.1–6.2). After adjustments for age and gender, the odds ratio was 2.3. Bone quantity did not appear to have a substantial relationship to implant failure risk (Becker et al. 2000).

After loss of tooth, the edentulous mandible supports a continuous process of alveolar ridge resorption. It has been showed that the use of overdentures on osseointegrated implants in mandible improves the oral functions of patients (biting forces and chewing) (Haraldson et al. 1988). Reddy et al. (2002) assessed whether placement of implants in anterior mandible and fixed detachable restoration prevents or reverses ridge resorption in 60 consecutively treated patients. Panoramic radiographs were made at baseline (following implant surgical placement) and longitudinally at 1, 2, 3, and 4 years, post restoration. Using a computer enhancement method, the height of the mandible was measured at 5, 10, 15, and 20 mm distal to the most posterior implant on each film. The study indicates that implant placement in the mandibular arch prevents ridge resorption. In addition, a small amount of bone gain or growth of the mandible was observed both clinically and radiographically. Overall, the mean mandibular bone height at surgical placement was 7.25 ± 0.25mm. Four years post restoration, the mean mandibular bone height was 8.18 ± 0.18 mm. The growth of the mandible appeared to take place during the first year of restored function with a fixed dentition. The dimensions of the mandible were sustained for the following 3 years. The authors explained the growth of the mandible by following Wolff's law, often simply stated as "form follows function." This would indicate that

the growth in the mandible is a result of increased work by the mandible after restoration with the fixed cantilever restoration. The fact that the amount of vertical growth in this study was found to be approximately equal at all measurement stations gives further support to the hypothesis that shear stress is the major determinant of mandibular growth in this configuration.

The beneficial effect of ITI implants with overdenture on bone loss of edentulous mandible was also reported by von Wowern et al. (1990). BMC measurements in the mandible were performed by DPA, at the ITI sites, premolar region and standard site (i.e., the mandibular base and body in the left molar region) at 3 weeks, postoperatively and at the 2-year follow-up visit. Each patient had three to five implants placed in the anterior mandibular residual ridge between the mental foramina. The BMC analyses demonstrated that BMC changes at the ITI site and the premolar region (i.e., just behind the distal implants) were significantly smaller than at the standard site of the mandible. Four persons even showed BMC gain in the premolar region. The relationship between the percentage BMC change at the ITI site and the premolar region was $r = 0.87$; $y = 1.8x + 3.1$ ($n = 9$, $P < 0.001$).

We can conclude that:

1. The use of dental implants, overdentures improve oral function of the patients and leads to a load-related positive bone remodeling that minimizes, or in some cases may counteract, the physiologic age-related changes of alveolar ridge resorption (von Wowern et al. 1990).
2. A theoretical or practical basis was not provided to expect osteoporosis to be a risk factor for osseointegrated dental implants (Dao et al. 1993).
3. Local bone quality classified during implant placement is the best predictor for implant risk (Dao et al. 1993).

7.7 Relationship Between Systemic Osteoporosis and Periodontal Disease

Osteoporosis and osteopenia are characterized by reductions in bone mass and may lead to skeletal fragility and fracture. Periodontitis is an inflammatory disease characterized by loss of connective tissue and alveolar bone. Like osteoporosis, it is a silent disease, not causing symptoms until late in the disease process when mobile teeth, abscesses and tooth loss may occur. While the etiologic agent in periodontitis is a pathogenic bacterial plaque in a susceptible patient, periodontitis and osteoporosis have several risk factors in common. They include an increased prevalence with increasing age, smoking and influence of disease or medications that may interfere with healing. In addition, the pathophysiology in both diseases appears to have hereditary or, at least, familial component (Reddy 2001). It is unknown whether the rate of progression of periodontitis is related to systemic osteopenia (Jeffcoat 1998) (Table 7.5).

A possible correlation between alveolar and skeletal bone loss coincident to osteoporosis was investigated in several studies. In an animal model, Johnson et al. (1997) has evaluated the effect of estrogen deficiency on bone metabolism after ovariectomy in sheep. The distance from the cemento-enamel junction to the alveolar crest (A), from the root apex to the alveolar crest (B) and the percentage of root embedded in bone [(B/A + B) × 100)] was assessed from radiographs. The periodontal probing depth was measured using a manual periodontal probe. Sheep from both groups (test group: 16 animals ovariectomy OVX; control: 12 animals sham-operated (C) had areas of horizontal and vertical alveolar bone loss; however, the OVX sheep had more generalized and more severe alveolar bone loss. Mean gingival sulcus depths were significantly greater in OVX than C sheep after 3 months (4.6 ± 0.2 vs. 3.9 ± 0.3 mm) and became progressively deeper during the subsequent year (6.8 ± 0.6 vs. 3.7 ± 0.4 mm). In addition, the percentage of total sites exhibiting sulcular depths of 4 to 6 mm and >6mm was greater in OVX than C after 3 months and became progressively greater during the subsequent year. Also, the percentage of the root embedded in bone was significantly less in OVX (65.3 ± 2.8 mm at 3 months; 42.4 ± 4.1 mm at 1 year). The alveolar bone loss was not a direct result of inflammation and may have been enhanced by estrogen deficiency. The author also observed the relatively high percentage of sites with >6 mm of gingival sulcus depth, and the number of teeth with a relatively low percentage of root embedded within bone in both groups may reflect the effects of aging on the sheep dentition in addition to the effects of estrogen deficiency. More recently, Orrico et al. (2007) revealed that the period after ovariectomy influenced the femoral BMD and the vertical bone loss in induced periodontal disease.

Studies in humans present conflicting results about the influence of osteoporosis on oral bone loss. In a

Table 7.5 Relationship between osteoporosis and periodontitis

Authors	Type of study	Population	Osteoporosis assessment	Periodontitis evaluation	Association
Brennan et al. (2007)	Cross-sectional	1,329 postmenopausal women	lumbar spine, hip, forearm, whole body BMD	CAL, calculus	Significant
Elders et al. (1992)	Cross-sectional	286 women (46 to 55 years)	Lumbar BMD (lumbar BMD); metacarpal cortical thickness	Alveolar bone loss on vertical bitewing radiographs, PD, BOP	Non-significant
Famili et al. (2004)	2 years longitudinal	398 women (mean age 75.5 years)	BMD of the total hip and its subregions was measured using DXA	PD, CAL	Non-significant
Gomes-Filho et al. (2007)	Cross-sectional	139 postmenopausal women: 48 in the case group (with periodontal disease) and 91 in the control group (without periodontal disease)	Densitometry	PD, CAL, BOP, ABL	Significant
Hildebolt et al. (1997)	Cross-sectional	135 postmenopausal women (age range 41–71 years)	DXA of postcranial (vertebral and proximal femur) BMD	CAL, PD, GR	Non-significant
Inagaki et al. (2001)	Cross-sectional	190 Japanese women (89 premenopausal, 101 postmenopausal).	Metacarpal BMD measured by computed X-ray densitometry	Community Periodontal Index of Treatment Need (CPITN)	Significant
Inagaki et al. (2005)	Cross-sectional	356 Japanese women (171 premenopausal, mean age 37.9±8.0 years; 185 postmenopausal, mean age 63.3±7.7 years)	Community Periodontal Index of Treatment Needs (CPITN)	metacarpal BMD	Significant
Kribbs (1990b)	Cross-sectional	85 women with osteoporosis(50–84 years age) 27 normal women	Cortical thickness at the gonion Radiographic evidence of vertebral compression fractures Mandibular bone mass and density	PD, GR, BOP	Non-significant
Kribbs et al. (1989)	Cross-sectional	85 edentulous postmenopausal women (50–84 years) with radiographic evidence of vertebral compression fractures for a diagnosis of osteoporosis	Total body calcium; SPA of the radius; DPA of the vertebrae Cortical thickness at the gonion	PD GR, BOP	Significant
Kribbs et al. (1990a)	Cross-sectional	50 normal women, aged 20–90 years	SPA of the radius; DPA of the vertebrae Mandibular bone mass on radiographs made distal to the mental foramen Cortical thickness at the gonion	PD, GR, BOP	Significant
Lundström et al. (2001)	Cross-sectional	210 women, aged 70 years	BMD of the hip	Number of remaining teeth, dental plaque, gingival bleeding, probing pocket depths, gingival recession	Non-significant
Mohammad et al. (1996)	Cross-sectional	Experimental group: 22 women with low mean spine bone density; Control group: 20 women with high mean spine bone density	Spine BMD (DPA)	PD, GR, CAL	Significant
Mohammad et al. (1997)	Cross-sectional	44 non Hispanic white women (aged 50 to 75 years)	BMD of the spine (DXA)	DMFT, GI, CAL, PD, GR	Significant
Mohammad et al. (2003)	Cross-sectional	30 postmenopausal, dentate, Asian-American women	BMD of the os calcis, composed primarily of trabecular bone, was assessed by DXA	tooth loss, PD, CAL	Significant
Payne et al. (1999)	Longitudinal 2 years follow-up	21 women with normal lumbar spine BMD; 17 women with osteoporosis or osteopenia of the lumbar spine	DXA of the lumbar spine	Alveolar bone height loss; Computer-assisted densitometric image analysis (CADIA) for changes in bone density at the crestal and subcrestal regions of interproximal bone	Significant

Table 7.5 (continued)

Authors	Type of study	Population	Osteoporosis assessment	Periodontitis evaluation	Association
Payne et al. (2000)	2 year longitudinal	59 postmenopausal women as subjects: 38 non-smokers, 21 smokers	BMD of the lumbar spine (L2-L4) (DXA)	Interproximal alveolar bone density and changes in alveolar bone height evaluated with CADIA	Significant
Persson et al. (2002)	Cross-sectional	1,084 subjects 60–75 years	Mandibular cortex index (MCI) on panoramic radiographs (PMX); Self-reported history of osteoporosis	Composite periodontal index (0–3) after identifying the frequencies of mesial and distal vertical bone defects > 3 mm	Significant
Phipps et al. (2007)	2.7 years longitudinal	1347 (137 edentulous) older men	BMD measured at the hip, spine, and whole-body, by dual-energy X-ray absorptiometry, and at the heel by ultrasound	CAL, PD, BOP	Non-significant
Pilgram et al. (2002)	Cross-sectional	35 patients with estrogen replacement	BMD at the lumbar spine (anterior-posterior and lateral) and proximal femur (neck, trochanter, intertrochanter, Ward's triangle, and total area).	PD,CAL	Weak association
Ronderos et al. (2000)	Cross-sectional	11,655 adults (5733 males, 5922 females)	BMD of the proximal femur (DXA)	PD,CAL, BOP	Non-significant
Shen et al. (2004)	Cross-sectional	Thirty-four patients (18 in the osteoporotic and 16 in the non-osteoporotic group) were selected from 329 postmenopausal Taiwanese women	Radiographic measurements of spinal bone mineral density	PD, CAL, R	Significant
Takaishi et al. (2006)	Cross-sectional	40 postmenopausal Japanese women aged 50–69 years	Lumbar spine BMD was measured by dual X-ray absorptiometry (DXA) and calcaneus speed of sound (SOS) by quantitative ultrasound (QUS).	PD, tooth mobility, mandibular alveolar BMD	Significant
Tezal et al. (2000)	Cross-sectional	70 postmenopausal Caucasian women aged 51 to 78	BMD at lumbar spine and femur (DXA)	BOP, PD, CAL, alveolar bone loss on bitewing radiographs	Significant
Von Wowern and Kollerup (1992)	Longitudinal	17 acute nephritic dentate patients undergoing intensive long-term (12 months) high-dose steroid treatment	DPA measurements of BMC of mandible and forearm	Visible plaque, gingival bleeding and CAL on 6 selected teeth	Non-significant
Von Wowern et al. (1994)	Cross-sectional	12 women with osteoporotic fractures and 14 normal women	BMC of the mandible at the standard site (DPA) BMC of the forearm (DPA)	Same as above	Significant
von Wowern et al. (2001a)	10 years longitudinal	24 young patients with severe periodontitis (age range 22 to 42 years)	Bone mineral content or density (BMD at the standard site of the mandible (DPA), lumbar spine and the left femoral neck (DXA)	PD, CAL, alveolar bone loss	Significant
Wactawski - Wende et al. (1996)	Cross-sectional	70 postmenopausal white women aged 51 to 78	DXA of the lumbar spine and femur	CAL; Interporximal alveolar crest height (ACH) on radiographs	Significant
Weyant et al. (1999)	Cross-sectional	292 dentate women (average age 75.5 years)	systemic BMD at 8 anatomic sites (hip, radius, spine, calcaneus) by SPA, DPA	BOP, CAL, PD from 3 sites on the buccal aspects of all remaining natural teeth	Non-significant
Yoshihara et al. (2004)	3 years longitudinal	184 Japanese, aged 70 years	BMD of the heel measured using an ultrasound bone densitometer.	CAL	Significant

PD probing depths; *R* gingival recession; *CAL* clinical attachment level; *BOP* bleeding on probing; *DXA* dual energy *X* ray absorptiometer; *DPA* dual-photon absorptiometry; *SPA* single-photon absorptiometry; *PI* Plaque Index; *GI* Gingival Index; *BMD* bone mineral density

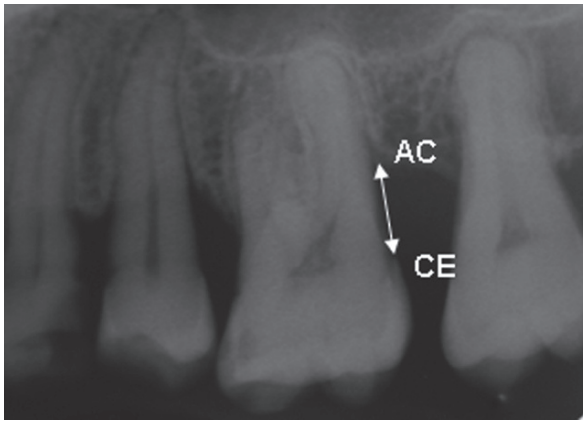

Fig. 7.4 The measurement of the mean alveolar bone loss (D_{alv}; mm) between the bone level of the alveolar crest (AC) and the cemento-enamel junction (CEJ), perpendicular to the occlusal surface of the tooth.

cross-sectional study, Kribbs et al. (1989) explored the relationship between skeletal bone mass (SPA of the radius, DPA of the vertebrae, CT of the vertebrae) and bone mass in the mandible in the group of postmenopausal women with osteporosis. The pockets depths around each tooth were measured in the standard six locations, as well as gingival recession. Presence or absence of BOP? was recorded for evaluating inflammation in the marginal gingival. Mandibular bone mass, assessed on periapical radiographs by microdensitometry, was correlated with mean periodontal pocket depth ($r = 0.38$) and BOP ($r = 0.33$) (Figs. 7.4).

When age-related changes of mandibular bone mass and density were evaluated in a group of normal women, it was found that the group older than 50 years had significantly greater bone density, thinner mandibular width, a thinner cortex, fewer teeth, more BOP

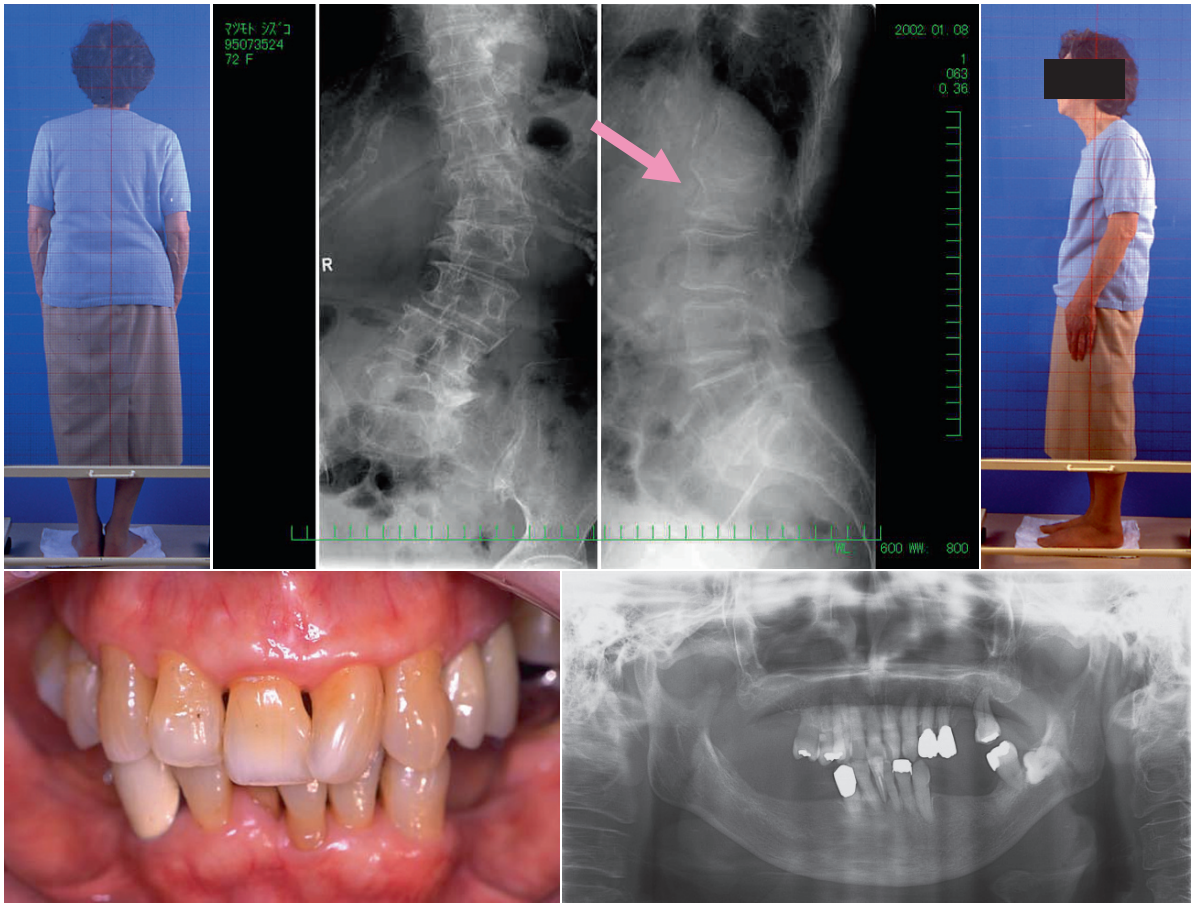

Fig. 7.5 Periodontal aspect of a 74 years old woman with BMD = 0.686 g/cm2 (bone mass assessed as 65.9 % of the young adult mean (YAM) bone mineral density): 21 remaining teeth, mean Probing Depth = 2.5 mm, mean Clinical Attachment Level = 3.6 mm, Bleeding on Probing = 26.2%, Alveolar Bone loss =38.5%

and more CAL loss. No significant differences were found in mandibular bone mass or in the periodontal pocket depth (Kribbs et al. 1990a).

In order to evaluate a possible relationship between systemic bone mass and periodontitis, Elders et al. (1992) performed an intra-oral examination and measurements of alveolar bone height, lumbar BMD and MCT in a large group of women between 46 and 55 years of age. The alveolar bone loss (D_{alv}; mm) was measured on vertical bitewing radiographs, distally and mesially to each premolar and molar tooth, between the bone level of the alveolar crest and the cemento-enamel junction, perpendicular to the occlusal surface of the tooth. Linear regression analysis was used to examine relations between the continuous variables. In dentate subjects, mean D_{alv}, lumbar BMD and MCT correlated significantly with age and years since menopause. No significant correlation was found between parameters of periodontitis (PD, BOP, number of missing teeth) or mean D_{alv} and the bone mass parameters (lumbar BMD and MCT) (Figs. 7.5 and 7.6).

No significant differences in any periodontal measures were found in normal ($n = 27$) and osteoporotic women ($n = 85$) with radiographic evidence of vertebral compression fractures. The periodontal measurements included mean pocket depth, the clinical attachment level and the percent of pockets that bleed on probing around each mandibular tooth in six locations (Kribbs 1990b).

Von Wowern et al. (1992) reported that long-term (12 months), high-dose steroid treatment induces mandibular bone loss similar to that of the other cortical bones of the skeleton, without affecting the marginal periodontal bone significantly. No relation was found between the BMC loss (%) within the mandible and the concomitant changes of loss of attachment ($P > 0.10$).

In a cross-sectional study, von Wowern et al. (1994) analyzed the possible relations between osteoporosis and the degree of periodontal bone loss in elderly women, including both the patients with osteoporotic fractures and the age-matching normal women, taking the age at menopause and smoking habits into account. No significant differences were found with respect to plaque (O: 46.67 ± 10%; N: 36.67 ± 6.67%) and gingival bleeding (O: 46.67 ± 11.67%; N: 43.33 ± 10%), whereas significantly greater loss of attachment was seen in osteoporotic women (O: 3.65 ± 0.18 mm; N: 2.86 ± 0.19 mm, $P < 0.01$). Loss of attachment was registered at three sites (buccal, mesial and lingual) on six selected teeth: 16, 21, 24, 36, 41, and 44.

Mohammad et al. (1996) assessed the periodontal status in two groups of women that had received spinal bone density measurements: 20 women in the bottom tertile of bone density (with a mean spine bone density of 0.753 ± 0.039 gm/cm^2) formed the experimental group, and 22 women in the top tertile of bone density (with a mean spine bone density of 1.032 ± 0.028 gm/cm^2) formed the control group. Periodontal examination included measurement of pocket depths, gingival recession, periodontal attachment loss, There were no significant differences in Plaque Index, Gingival Index, and probing depth in both groups; however, there were significant differences in gingival recession components of periodontal attachment level in both groups. Recession in the low-density group was significantly greater than in the high-density group ($P < 0.05$). A significant negative correlation (−0.348) was found between CAL and spine bone density. Of the periodontal variables measured, recession showed the most significant negative correlation (−0.4111) with spine bone density.

The relationship between osteopenia and periodontal disease was studied in a cohort of 70 postmenopausal white women aged 51–78 years, exhibiting established periodontitis, defined as having 6 mm or more interproximal CAL and at least one site with probing depth of 5 mm or greater. Periodontal disease was determined using both CAL (measured with a Florida Probe) and interproximal alveolar crest height (ACH), using the method of Hausmann et al. (1992). Skeletal BMD was assessed by using DXA at the anterior-posterior lumbar spine (AP-L2) and five regions of the femur (trochanter, inter-trochanter, Ward's triangle, femoral neck, and total femur). After adjusting for contributions of other independent variables that have been shown to be predictors of periodontal disease or osteopenia (age, years since menopause, estrogen use, body mass index, smoking level), a positive correlation was found between mean ACH and BMD of the AP spine ($r = 0.24$), trochanter ($r = 0.28$), Ward's triangle ($r = 0.26$), and total femur ($r = 0.26$). The relationship between CAL and BMD did not reach statistical significance for any areas of the spine or femur (Wactawski-Wende et al. 1996).

In a cross-sectional study on a group of 135 postmenopausal women (age range 41–71 years), no significant correlation was found between CAL and postcranial (vertebral and proximal femur) BMD measured by DXA. Multivariate analysis showed current smoking ($P = 0.01$), years since menopause ($P = 0.02$) and the

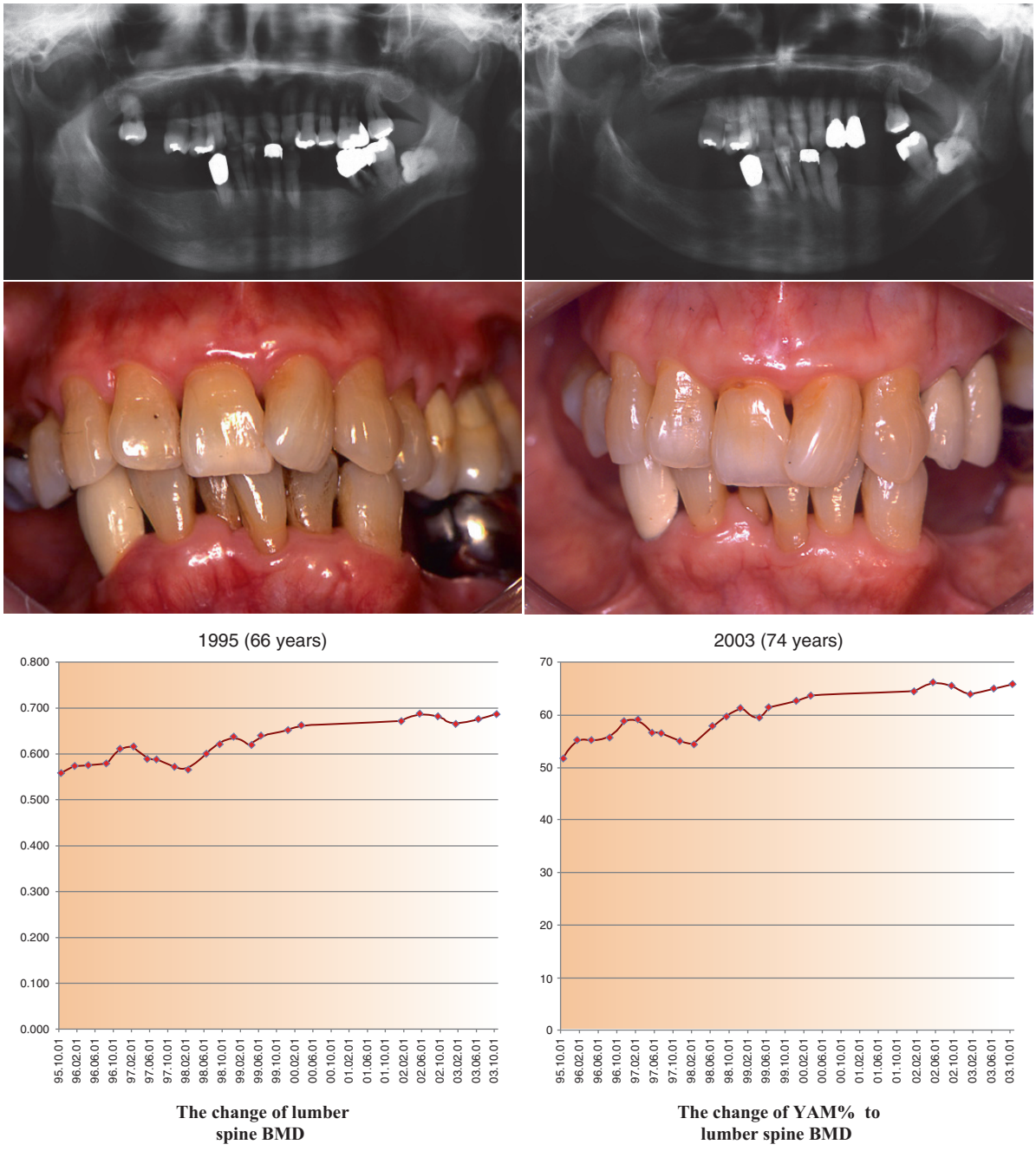

Fig. 7.6 Longitudinal change of lumber spine BMD, YAM (young adult mean) % to lumber spine BMD and periodontal status (66y to 74y).

interaction of age and current smoking ($P < 0.01$) to be statistically significant predictors of CAL. Because the study was aimed at determining the pathogenic role of systemic bone loss independent of periodontal disease, subjects were selected with periodontal pockets no deeper than 5 mm and were excluded if they had overt periodontal disease. Thus, the relatively small variability of CAL in the enrolled study population may have limited to detect more effects (Hildebolt et al. 1997).

Mohammad et al. (1997) assessed BMD at the spine by DXA in a group of 44 non Hispanic white women (aged 50 to 75 years). All bone densities above the

age-adjusted population mean bone density were classified as a HBD group, while those below the age-adjusted population mean bone density were classified as a LBD group. Both dental PI and the GI of the two groups were not significantly different, suggesting a similar oral hygiene condition in both groups. However, the loss of attachment in the LBD group was significantly higher (31%) than that in the HBD group ($P < 0.05$). The difference mainly resulted from gingival recession ($P < 0.05$) because the gingival pocket depths were not significantly different between the two groups.

Alveolar (oral) bone height and density changes in osteoporotic/osteopenic women compared with women with normal lumbar spine BMD were investigated in a 2-year longitudinal clinical study. Subjects entered into the study if they had nine or more posterior teeth consisting of two or more sites with established periodontitis, namely probing depth >5mm, >6 mm clinical attachment loss, radiographic alveolar crestal bone loss. Women entered the study either already receiving estrogen replacement therapy or not. Current smokers were excluded. Subjects were in periodontal maintenance programs and had not received any active periodontal therapy. Posterior interproximal supragingival plaque and presence or absence of bleeding within 30 s of probing with a pressure-sensitive probe was recorded. Four vertical bitewing radiographs of posterior sextants were taken at baseline and 2-year visit. Radiographs were examined using CADIA for changes in bone density at the crestal and subcrestal regions of interproximal bone. Changes in alveolar bone height were also measured. Osteoporotic/osteopenic women with a history of periodontitis exhibited a statistically significantly higher frequency of alveolar bone height loss ($P < 0.05$) and crestal ($P < 0.025$) and subcrestal ($P < 0.03$) density loss relative to women with normal BMD of the lumbar spine. Osteoporotic/osteopenic women had a statistically significantly higher frequency of BOP. Plaque frequency did not differ between groups. For the overall patient population, Estrogen-deficient subjects experienced a statistically significantly increased frequency of crestal density loss ($P < 0.05$) compared with estrogen-sufficient subjects (Payne et al. 1999).

A subsequent 2-year longitudinal study demonstrated that smoking is a risk factor for progressive alveolar bone height and density loss in postemenopausal women in supportive periodontal treatment programs. In addition, a significant interaction was noted between BMD status and smoking for combined alveolar bone crestal and subcrestal density change ($P < 0.05$). Only the non-smoking, normal BMD subjects manifested mean alveolar bone density gain over the 2-year study; smokers and osteoporotic/osteopenic subjects demonstrated mean alveolar bone density loss. Plaque and BOP did not differ between smokers and non-smokers (Payne et al. 2000).

No statistically significant association between five indicators of periodontal disease (average CAL loss per subjects, sites per person with at least 4 mm CAL loss, sites per person with at least 6 mm CAL loss, n teeth per person exhibiting BOP, average deepest pocket per person) and measures of systemic BMD at 8 anatomic sites (hip, radius, spine, calcaneus) were found in a cross-sectional study on 292 old dentate women (average age 75.5 years), after controlling for age, smoking and number of remaining teeth. There was one suggestive trend found between average periodontal attachment loss and BMD at the trochanteric region. Also, the deepest pocket per patient was associated with BMD measured at 5 bone sites: total lumbar, femoral neck, trochanteric and intertrochanteric region, total hip. The assessment for periodontal destruction was made from three sites on the buccal aspect (mesio-buccal, mid-buccal, disto-buccal) of all remaining natural teeth. Also, a time difference of 2– 5 years between BMD and periodontal measurements was reported, but due to a BMD decrease rate of about 1% per year for postemenopausal Caucasian women over the age of 65, the authors considered his span trend modest and very predictable (Weyant et al. 1999).

Tezal et al. (2000) evaluated the relationship between systemic bone loss and periodontal disease, controlling for known confounding factors on a study population of 70 postmenopausal Caucasian women, aged 51 to 78 years. BMD was assessed at the lumbar spine and femur using DXA. The periodontal examination included measurements of supragingival plaque, calculus, gingival bleeding, probing depth and CAL loss on all teeth present in the dentition. Alveolar bone loss was determined from intraoral radiographs by the method of Hausmann et al. (1992) from four posterior bitewing and six anterior periapical X-rays. After simultaneously adjusting for age, age at menopause, estrogen supplementation, body mass index, smoking and supragingival plaque, multiple linear regression analyses revealed that BMDs at all femoral regions and spine were correlated with alveolar bone level ($r = -0.20$ to -0.27). This relationship reached

statistical significance for the trochanter ($r = -0.27$), Ward's triangle ($r = -0.26$) and total femur ($r = -0.25$). CAL was also consistently related to spinal ($r = -0.17$) and femoral ($r = -0.10$ to -0.16) BMDs, but with lower partial correlation coefficients that did not reach statistical significance (Tezal et al. 2000).

NHANES III is a cross-sectional study designed to obtain information on the health and nutritional status of the non-institutionalized population of the United States. During the data collection, a sample of 11,655 subjects (5,733 males and 5,922 females) was selected. Probing depth, clinical attachment level, calculus index and bleeding upon probing were recorded for the mesio-facial and facial sites of one maxillary and one mandibular quadrant. BMD of the proximal femur was assessed with DXA. After adjusting for confounders, females with high calculus scores and low BMD had significantly more CAL than females with normal BMD and similar calculus scores ($P < 0.0001$). No association was observed among women with low and intermediate levels of calculus. The greater clinical attachment loss present among women with low BMD was associated with gingival recession (Ronderos et al. 2000).

Von Wowern et al. (2001a) has evaluated in a 10-year longitudinal study, in young patients ($n = 24$; age range 22 to 42 years) with severe periodontitis, whether the mandibular and skeletal BMC or density (BMD) and the bone metabolism differed from normal values and, whether the BMC in the mandible showed any changes at the follow-up visit. Half of the patients were smokers (>7 cigarettes/day). Mandibular and forearm BMC were estimated at the initial visit with DPA, while at the follow-up visit, the above-mentioned BMC estimations were repeated, supplemented by standardized BMD estimations of the lumbar spine and the left femoral neck by DXA. The periodontal examination included registration of number of teeth and periodontal pockets, measured to the nearest mm with a periodontal probe at each 6 sites of each tooth, excluding the third molars. The alveolar bone loss was determined on a full mouth set of 14 periapical radiographs taken for each patient at the start and at the end of the study. The mandibular BMC was significantly below normal BMC. No significant differences were demonstrated between the BMC values measured at the two visits in the mandible and forearms. At the follow-up visit, a significantly larger alveolar bone loss was found, but the periodontal pocket depth was not significantly changed from the initial examination (von Wowern et al. 2001a).

A large cross-sectional study on an older (age range 60–75 years), ethnically diverse population (n = 1084) was conducted in Persson et al. (2002) The mandibular cortical index (MCI) was assigned as appropriate for the cortical area below the mandibular foramen for suggesting osteoporosis bone changes. The extent of alveolar (horizontal) bone height was assessed using a composite periodontal index (0–3) after identifying the frequencies of mesial and distal vertical bone defects >3 mm around remaining teeth along with the number of molars showing an identifiable interradicular marginal radiolucency. In women group only, the subjects with self-reported osteoporosis and a positive MCI showed higher horizontal alveolar bone loss ($P < 0.01$). No differences in prevalence or vertical alveolar bone defects were found.

More recent studies evaluating the interrelationship between periodontitis and osteoporosis are included in Table 7.5 (Phipps et al. 2007; Takaishi et al. 2005; Shen et al. 2004; Yoshihara et al. 2004; Mohammad et al. 2003; Inagaki et al. 2001).

The effects of osteoporosis on both systemic health and oral health need to be well understood. As a healthcare provider, the dentist could serve as a pre-screener of patients with the potential for osteopenia or osteoporosis. Familiarity with the risk factors could help identify these individuals and aid in earlier diagnosis. Proper counseling of the need for prevention and treatment together with referral for further evaluation of their bone status could greatly benefit our patients. An additional tool for pre-screening may lie in the information obtained on our dental radiographs. The usefulness of the alveolar trabecular pattern analysis and mandibular alveolar bone mass for prediction of skeletal BMD was evaluated. An index to assess the alveolar trabecular patterns was developed and a significant correlation was found with skeletal BMD. Evaluation of the coarseness of trabeculation of alveolar bone, as seen on intraoral radiographs, could be a helpful clinical indicator of skeletal BMD and better than densitometric measurements of the alveolar bone. Dense trabeculation is a strong indicator of high skeletal BMD, whereas sparse trabeculation may be used to predict low skeletal BMD. Further studies are being conducted to develop protocols for pre-screening our patients for reduced skeletal BMD on dental radiographs (Geurs 2007).

When defining the relationship between osteoporosis and periodontitis, several issues should be considered. Most published studies support a positive association

between these common diseases; however, almost all studies are cross-sectional in nature, include relatively small sample sizes and have inadequate control of potential confounding factors. Interpretation of publishing findings is complicated by the varying methods to assess both osteoporosis and periodontitis. Additionally, studies use varying definitions of both osteoporosis and periodontal disease when presenting the outcomes of interest. Not all studies rely on some measures of bone density. Many studies rely on clinical observations of events such as bone fracture (Wactawski-Wende 2001).

Techniques used to assess periodontitis include radiographic measurements of alveolar bone height or surrogate measures of alveolar bone loss, such as pocket probing depth and clinical attachment loss (Garcia et al. 2001). To further complicate understanding of the relation between osteoporosis and periodontal disease, the demographic makeup of the population under study (age, gender, race) and control of potential confounding variables (smoking, oral hygiene status) differs markedly across studies. As a result, interferences on the association between osteoporosis and periodontal disease require careful considerations. However, in the limited number of published studies, a positive association between osteoporosis and loss of alveolar crestal height was showed (Wactawski-Wende 2001). Large-scale and long-term studies are needed (Garcia et al. 2001; Reddy 2001).

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Periodontal-Restorative Interactions

9

Alexandrina L. Dumitrescu, Mitsugi Okada, and Koji Inagaki

For many years, dentistry was influenced by a series of paradigms based predominantly on mechanical concepts (Nyman and Lang 1994). Periodontal disease progressively destroys the supporting tissues of the teeth. In later stages of the disease, the breakdown of the periodontium may have progressed to a level where treatment must frequently involve extraction of one or several teeth. In such cases, prosthetic rehabilitation is often needed to restore function and/or aesthetics (Nyman and Lindhe 1976). Over 45 years ago, a claim was made that, "The total periodontal membrane area of the abutment teeth should equal or exceed that of the teeth to be replaced" (Ante 1926). This principle, which means that teeth that have lost a great deal of their supporting structures due to periodontal disease cannot be successfully retained and used as abutments for fixed prosthetic reconstructions, has consequently determined that a number of teeth with reduced periodontal support could no longer serve as abutments for fixed bridgework, but had to be extracted and, hence, replaced. Such a concept easily led to overtreatment (Nyman et al. 1975; Nyman and Lang 1994).

9.1 Prosthetic Rehabilitation of Healthy Dentitions with Reduced Periodontal Support

In a longitudinal study undertaken to evaluate the possibility of rehabilitating patients, by utilizing fixed bridges on a few isolated abutments with severely reduced periodontal tissue support as part of the overall periodontal treatment, Nyman et al. (1975), showed that permanent stability of the bridgework can be obtained. The study consisted of 320 adults, aged 27–69 years, mean age 48.9 years, with advanced periodontal breakdown, often combined with extensive loss of teeth. After periodontal treatment, patients were rehabilitated with fixed bridges. Once in every 3–6 months, the patients were recalled and subjected to dental prophylaxis, and followed up for 2–6 years. The individual mean bone scores of the initial and terminal examination were 6.77 ± 0.17 and 6.76 ± 0.17. Further loss of alveolar bone between the insertion of the bridgework and the final examination (2–6 years) did not occur in any of the patients. A detailed analysis of the bone height measurements revealed that the bone levels remained unaltered in all abutment teeth. The radiographs did not show a widening of the periodontal membrane of any of the abutment teeth, in any case. The mobility of the fixed bridges, immediately after insertion, was classified as degree 0 in 17 cases, degree 1 in seven cases, and degree 2 in two cases. In none of these 20 patients did the mobility change during follow-up (Nyman et al. 1975).

While evaluating the periodontal ligament area of abutment teeth treated for advanced periodontal disease including prosthetic replacement of lost teeth, only five bridges (8%) out of the 60 cases examined were found to be supported by abutment teeth, which fulfilled the demands of periodontal support outlined in Ante's law. In the majority of cases (57% of the total group), the bridge restorations were supported by teeth surrounded by a periodontium which was less than 50% of that of the replaced teeth. In 12 out of the 60 bridges examined, the total ligament area of the abutment teeth was less than 25% (16–24%) of the corresponding area of the pontics. Despite the markedly

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reduced periodontal support, all the bridges examined have functioned for 8–11 years without further loss of attachment around the abutment teeth (Nyman and Ericsson 1982).

For successful treatment of patients with advanced periodontal disease and few available abutments several technical considerations are necessary. The basic principles do not differ from those of patients with many available abutments, but the clinical and technical difficulties are more pronounced (Glantz and Nyman 1997).

9.1.1 Occlusal Considerations

Increasing tooth mobility is one component of the clinical symptoms in advanced stages of destruction of the supporting tissues in the periodontal disease. Even though the inflammatory process is resolved as a result of proper periodontal treatment, hyper-mobility of individual teeth will frequently persist after the completion of treatment (Nyman and Lindhe 1976). However, if the height of the supporting tissues is reduced, while the width of the periodontal ligament is unchanged, the amplitude of root mobility within the remaining periodontium is the same as in a tooth with normal height of the periodontal bone height. Hence, the so-called hyper-mobility of the periodontal healthy tooth with reduced support but normal width of the periodontal ligament should be considered as physiological tooth mobility (Nyman and Lang 1994). When tooth mobility has reached the amplitude where masticatory function is disturbed, or when signs of increasing mobility are present and so, the risk of tooth extraction during chewing develops, a splint must be inserted. Such a splint may be either fixed or removable. A removable splint (e.g., partial denture), however, should not be used in cases with advanced breakdown of the periodontal tissues; the daily removal and insertion of a removable splint will traumatize the abutment teeth and produce a further increase of tooth mobility. Moreover, a partial denture has only a limited splitting effect on nonabutment teeth (Nyman and Lindhe 1976).

As a rule, in patients with pronounced loss of periodontal support, the fixed bridge is to be preferred to the removable partial denture (RPD), because the fixed bridge is more rigid and may provide more favorable distribution of the mastication forces over the remaining periodontium (Nyman and Ericsson 1982; Glantz and Nyman 1997). The only way to preserve this dentition being in the final stages of periodontal disease, when the remaining periodontal tissues can no longer withstand the masticatory forces, is to use the teeth as abutments for a fixed splint of cross-arch design. The objective of such a bridge construction is to create a situation in which the mobility of the entire bridge is either "normal" or at least nonprogressive. A cross-arch design of a fixed bridge significantly reduces the lever effect of the occlusal forces. Hence, the stability of the entire bridge is assured, and the mobility of the individual teeth before bridge installation is no longer a pertinent problem. In teeth with minimal amounts of remaining, healthy periodontium, a unilateral bridge would most likely be luxated and the abutment teeth, extracted as the result of the functional forces of normal magnitude. Since such teeth cannot serve as individual chewing units either, similar effects would also be expected if they were incorporated in a conventional partial, removable denture (Nyman and Lang 1994). If the bridge is given a cross-arch design and the number and distribution of abutment teeth are favorable in relation to the extension of the bridge, in most instances, the bridge will exhibit stability, despite the extensive mobility of the abutment teeth, i.e., it will not exhibit a mobility amplitude exceeding what is regarded as "normal mobility" for teeth (Nyman and Lindhe 1977).

However, since the rate of progression of periodontal breakdown and the loss of teeth often affect the dentition in an irregular fashion, the remaining teeth do not exhibit an increased and/or increasing mobility following periodontal treatment, but have a distribution within the dental arch which is unfavorable from a prosthetic reconstruction point of view. In this case, the bridgework may migrate, tilt, or develop further increased mobility. However, such complications may be avoided even in extreme borderline cases, provided the occlusion is designed to obtain and maintain stability (Nyman and Lindhe 1977). The occlusion should be designed to obtain a balanced load to each site of the fulcrum of bridge mobility. In other words, simultaneous occlusal contacts must be established in the anterior and posterior regions not only in centric occlusion, but also during frontal excursion of the mandible from a retruded to a protruded contact position. This in turn, implies that the palatal surfaces of the crowns in the front region are given a functional anatomy with horizontal shelves providing gliding contacts in the protrusive–retrusive mandibular movement, in the sense that the functional forces establish a balance of the bridge. The pretreatment degree of

overbite of the abutment teeth should be reduced and the overjet, increased (Nyman and Lang 1994).

Similar thoughts should govern the treatment planning in patients with reduced periodontal support around the teeth in the maxillary front region. It is well known that functional forces of normal magnitude may induce protrusive migration of such teeth. Stabilization of the front teeth may not be obtained by merely joining them together, since in cases with advanced loss of the periodontium around the teeth, the entire front segment may tilt in the frontal direction. Stabilization of such front teeth can only be obtained by extending the splint/bridge posteriorly, thereby neutralizing the anterior lever effect of the functional forces (Nyman and Lang 1994).

In a hyper-mobile bridge, where distal abutment teeth are lacking, the balance of the total bridge can be obtained by the use of cantilever pontics. Thus, cantilever pontics can be used to secure, rather than jeopardize, stability. But the presence of cantilever pontics requires good retention of the abutment crowns (Nyman et al. 1975; Nyman and Lindhe 1977). In an attempt to reduce the risk of loss of retention, the abutment should be prepared following a carefully chosen design. As a general principle, the maximum surface area with minimum conicity gives the best resistance to any force acting in a direction other than the direction of insertion of the reconstruction. However, the optimal taper of the preparation for clinical practice is generally agreed to be around 5° inclination from the vertical. Furthermore, if the surface area of the abutment tooth available for retention of a reconstruction is insufficient, retention and stability may be improved by different measures (Hammerle 1994). Full crowns are often indicated in these cases, sometimes combined with horizontal pin retention (Nyman et al. 1975). Laurell et al. (1991), in a retrospective study, evaluated the long-term prognosis of extensive fixed partial dentures, including unilateral or bilateral poly-unit cantilevers in patients with healthy, but reduced periodontal support. It was shown that although there was a slight but insignificant increase in the mean number of deepened periodontal pockets from 1.9 to 2.6, there was no further, radiographically detectable, loss of periodontal support in any of the 34 patients. The mean bone scores for the abutment teeth was maintained throughout the entire observation period (64 ± 11%), although on the average only 30% of the original periodontal ligament remained as indicated by the Periodontal support index, (the ratio between the total remaining periodontal ligament area of abutments and the total maximal periodontal ligament area of the entire extension of the prosthesis, as if all teeth were used as abutments, each with maximal alveolar bone height). This markedly reduced periodontal support was obviously sufficient to withstand long-term operating functional forces. The authors concluded that where distal end abutments are not available as support for extensive fixed prosthodontics, posterior polyunit cantilevers can be successfully incorporated in the fixed partial dentures provided:

1. The jaw relationships permit the establishment of occlusal contacts anteriorly as well as posteriorly with an occlusal morphology guiding the occlusal forces in an apical direction.
2. Lateral movements are anterior-guided with no cantilever contacts.
3. Great care is given to the preparation procedure to secure the retention of the abutment crowns. This might call for preprosthodontic crown-lengthening surgery.
4. The dimension of the metal framework at least mesial and distal to the distal retainer crown should measure a minimum of 5 mm in height and 4 mm in width.

9.1. 2 Provisional Acrylic Bridge

In situations where it is not possible to determine in advance, the development of increasing mobility of a planned bridge, a provisional acrylic bridge should be inserted. In the provisional bridge, changes in mobility can be followed and checked over a prolonged time period, and the occlusal relief can be continuously adjusted until a point is reached, when it is obvious whether or not, stability (i.e., nonincreasing mobility) can be obtained. The occlusal patterns of the acrylic bridge should then be reproduced in the permanent bridge (Nyman and Lindhe 1977).

9.1.3 Selection of Retainers Crowns and Preparation Design

Glantz and Nyman (1997) recommended the veneer crowns in dentitions with few abutments. Concerning the preparation design, if the preparations are given low angles of convergence (<20°), the strength of cement films is generally sufficient to prevent loss of retention, resulting from forces acting in the opposite direction to

the direction of the path of insertion of the bridge. However, the strength of the cement is not sufficient to withstand horizontally directed forces if the preparations and the retainers do not have sufficient height and correct dimensions (Glantz and Nyman 1997).

9.1.4 Vitality of the Abutment Teeth

It has been showed that the root-filled teeth used as abutments for extensive bridgework have a higher tendency to mechanical failure than vital teeth (Nyman and Lindhe 1979).

Randow and Glantz (1986) have analyzed the protective mechanism mediated by mechanoreceptors, with the aim of a better understanding of the biomechanical principles governing abutment teeth function. It has been shown that, on cantilever loading, there is a difference in the biomechanical reactions between vital and nonvital teeth, with the higher tolerated levels being in nonvital teeth. Furthermore, since anesthetization brought vital and non-vital teeth to the same increased level of tolerating loading, the conclusions were, that, for teeth with optimal alveolar bone support the mechanoreceptor function is efficient as lower degrees of bending than those at which the mechanoreceptors of the periodontium of nonvital teeth are brought into action. In this context, it was noted that when the alveolar bone support is markedly reduced, high initial strain levels in the periodontal membranes can probably be reached. In such cases, periodontal mechanoreceptor function may be engaged at lower levels of cantilever loading than those engaging the mechanoreceptor function of vital tooth structures. The observed shifts of the rotation centers (hypomoklion) were dramatic between vital and nonvital teeth. Therefore, the possibility that, mechanoreceptor function in the periodontal membrane is to some extent dependent on the vitality of teeth, cannot be excluded (Randow and Glantz 1986).

However, if the aim is to reduce iatrogenic damage to the dental pulp resulting from the trauma associated with full crown preparation, sharp and suitable instruments should be used with ideal cutting speed, and with abundant cooling to prevent the development of heat. Immediately following preparation, the future abutment tooth needs to be cleaned from its smear layer, dentin chips, saliva, and possibly microorganisms. A three-step cleaning procedure includes separate application of (1) an oxidizing agent such as a weak hydrogen peroxide solution, (2) a solvent such as ethanol, and (3) a surfactant cleanser. Following the application, the tooth must be rinsed with water to remove loosened material and to prevent the different agents from inhibiting the cleansing effect of the others (Hammerle 1994; Glantz and Nyman 1997).

9.1.5 Restorative Margin Placement

In daily practice, overhanging and/or open margins of dental restorations present a frequently observed problem (Schatzle et al. 2001), which may greatly interfere with the maintenance of gingival and periodontal health (Leon 1977) (Figs. 9.1 and 9.2). It is suggested that the shift of the composition of the subgingival microbiota towards an increased proportion of periodontopathic microorganisms will eventually lead to loss of periodontal support. It was documented that subgingival marginal placement of dental restorations results in a significant loss of attachment, which may be detected 1–3 years after the restorative procedures. The fact that the gingival restoration index scores improved concomitantly, suggests that a proportion of the subgingivally placed restoration margins had become located at gingival margin or supragingivally due to recession of the marginal gingiva (Schatzle et al. 2001). Histological studies have shown that every filling margin placed subgingivally

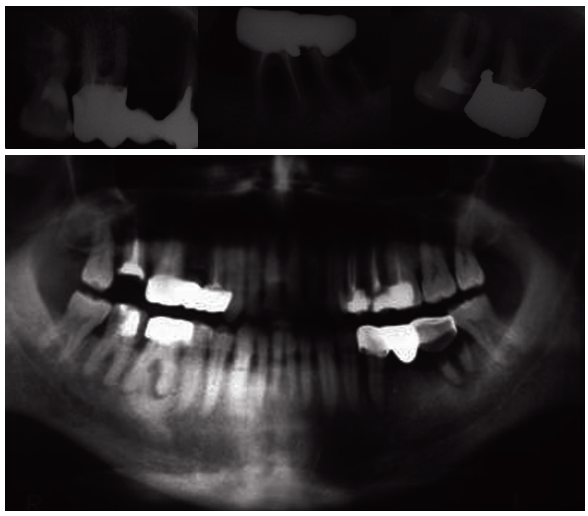

Fig. 9.1 Crown margin overhangs and open margins

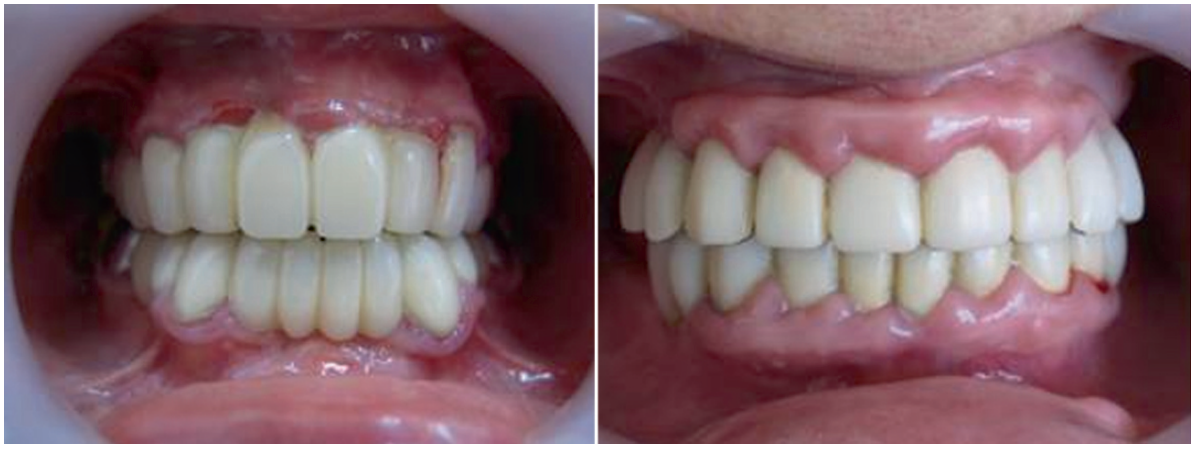

Fig. 9.2 Gingival inflammation due to crown margin overhangs and open margins

represents a plaque retaining factor, even if the marginal adaptation may be clinically acceptable (Waerhaug 1960). Furthermore, the placement of slightly overhanging filling margins was shown to result in a change of the subgingival microbiota adjacent to the subgingival restoration, favoring the colonization of gram-negative, strictly anaerobic rods (Lang et al. 1983).

To reduce the risk of progressive periodontitis, some principles are valuable in providing care (Hammerle 1994). All the crowns have to be designed in such a way as to allow the patient to remove all dento-gingival plaques. This means that the crown margins should be kept supragingival whenever possible and that, the width of the interproximal areas should be adjusted to the size of the instrument used for interproximal tooth cleaning. Furthermore, the buccal and lingual surfaces should be contoured in such a way that it should be possible to reach the dento-gingival junction with the toothbrush (Nyman et al. 1975).

Several studies have shown that subgingivally located restorative margins may adversely affect periodontal health. Bader et al. (1991), on a sample of 831 regularly attending patients, showed that the presence of a crown margin is associated with an increase in pocket depth of 0.28 mm. Another way of expressing this difference is that, approximately 20 of every 100 surfaces would be categorized as 2–3 mm, rather than 0–1 mm, in a physiologically normal range (Bader et al. 1991).

Freilich et al. (1992) reported that both, horizontally under-extended and over-extended retainers had significantly greater GI scores than their matched unrestored teeth, at long-term examination (5 years). The retainers that were un-extended, and those that were both subgingival and overextended also had significantly greater GI scores as their matched un-restored teeth, at short-term examination (6 months). The retainer margin located 2–3 mm apical to the gingival crest exhibited significantly increased mean PD measurements (2.67 ± 0.49 mm) than, either those retainer margin that were located 1 mm apical to the crest (2.26–0.54 mm), or those in the at-crest/supragingival group (1.92 ± 0.57 mm) ($P < 0.05$).

Felton et al. (1991) reported a highly significant correlation between marginal discrepancy (0.16 ± 0.13 mm) and gingival index, and between marginal discrepancies and crevicular fluid volume (49.9 ± 31.1 mm), in a sample of 29 patients, 39–71 years of age, indicating a direct relationship between tooth/restoration marginal discrepancies and periodontal inflammation.

At least four factors appear to adversely affect the degree and extent of the inflammatory changes that are associated with subgingival margin placement. These factors include, failure to maintain proper emerge profile, inability to adequately finish or close subgingival margins, placement of subgingival margins in an area with minimum to no, attached gingiva, and violation of the biologic width. *A poorly fitted and contoured restorative margin is unacceptable in any position, but even a perfectly fitted and contoured margin that extends more than 0.5 mm below the marginal gingiva in a healthy environment will violate the biological width, and will result in marginal inflammation*. The more supragingivally a restorative margin can be placed, the lesser the chance that the margin will contribute to gingival

inflammation. In addition, a supragingival margin can be finished better and can be more easily evaluated for recurrent caries or marginal deteriorations, at periodic, supportive, maintenance, recall visits (Reeves 1991).

However, for esthetic reasons, the patients may prefer crown margin to be placed slightly subgingivally. In addition, in periodontally compromised patients, sometimes the restoration has to be over-contoured in order to replace the lost interdental papilla. This may be tolerated, provided the patient is instructed with proper plaque control measures (Hammerle 1994), as it was demonstrated in an animal model that over-contoured gold crowns placed subgingivally have only slight effects on the microbiological composition in dogs when an intensive oral hygiene regimen was executed (Kohal et al. 2003, 2004).

9.1.6 Crown and Pontic Contours

In a 6 year longitudinal study, Broadbent et al. (2006) showed that site-specific periodontal attachment loss due to dental caries or restorative events occurs in adults in their third and fourth decades of life, suggesting that, in general, dentists should consider inter-proximal caries and dental restorations to be local risk factors for localized periodontal attachment loss. Accordingly, they should take appropriate steps to minimize the occurrence of, and to monitor carefully (and treat where necessary), the inter-proximal sites that have (or require) adjacent restorations.

Oral hygiene may be jeopardized severely by over-contoured reconstructions. Especially, the interdental surfaces may impinge on gingival papillae owing to the lack of provision of the necessary space for the prosthetic material, by the inadequate tooth preparation. The very delicate structures (such as vessels arrangement, supracrestal fibers and epithelial attachment of the col area) of the interdental papillary region are easily severed by restorative procedures or by plaque accumulation as a result of inadequate access with interdental oral hygiene devices. The dental laboratory technicians must therefore be encouraged to respect the interproximal contact area of reconstructions with adequate space for the available cleaning devices (Lang 1995).

Multirooted reconstructed teeth with initial furcation involvement often present problems for adequate oral hygiene practices. Special efforts have to be made to accentuate the outline of the root trunk while preparing the tooth to provide adequate access for cleaning devices in the furcation area. If roots had to be resected or amputated, and the remaining roots are still used as abutments for fixed reconstructions, the areas of the root concavities must be exaggerated in the reconstruction to avoid over-contouring of regions, most susceptible to plaque accumulation. Again, proper oral hygiene instructions have to be given to patients with such morphological circumstances (Lang 1995).

Especially on bridge pontics, the esthetic demands often result in concavities of the pontic body on the apical surface in contact with the mucosa of the edentulous ridge. Such ridge-lap designs inevitably result in mucosal irritation, chronic inflammation, and ulceration of the area. As a clinical consequence, bridge pontics should be constructed with only convex surfaces (egg-shaped). However, if esthetics demands dictate a slightly ridge-lap design, contact with the mucosal surface should be avoided to provide adequate space for cleaning devices. Placement of pontics into extraction sockets is not biological, and must be considered as malpractice, since it leaves permanent irritation and jeopardizes the healing process of an extraction socket (Lang 1995) (Figs. 9.3 and 9.4).

9.1.7 General Considerations

Dentitions with reduced periodontal support offer a number of advantages over intact periodontal conditions in preventing technical failures. The increased distance from the gingival margin to the occlusal surface of the antagonist tooth provides the possibility of a greater surface for retention and allows for increased dimensions of the artificial bridge parts. Supragingivally located crown margins are covered by lips and cheeks and thus kept out of sight, and possible over-contouring distant to the periodontal tissues represents less risk for plaque accumulation. The pulp chambers often become smaller, thus decreasing the risk of devitalizing as a result of tooth preparation. A certain degree of tooth mobility reduces the risk of fracture of the abutments. Finally, following destructive periodontal disease, the loading forces are distributed over a smaller area of the periodontal ligament. Hence, a particular force leads to a higher pressure in periodontitis patients than in individuals with an intact periodontium. Therefore, the periodontal and osseous mechanoreceptors fire at lower functional forces, decreasing the risk of technical failures (Hammerle 1994).

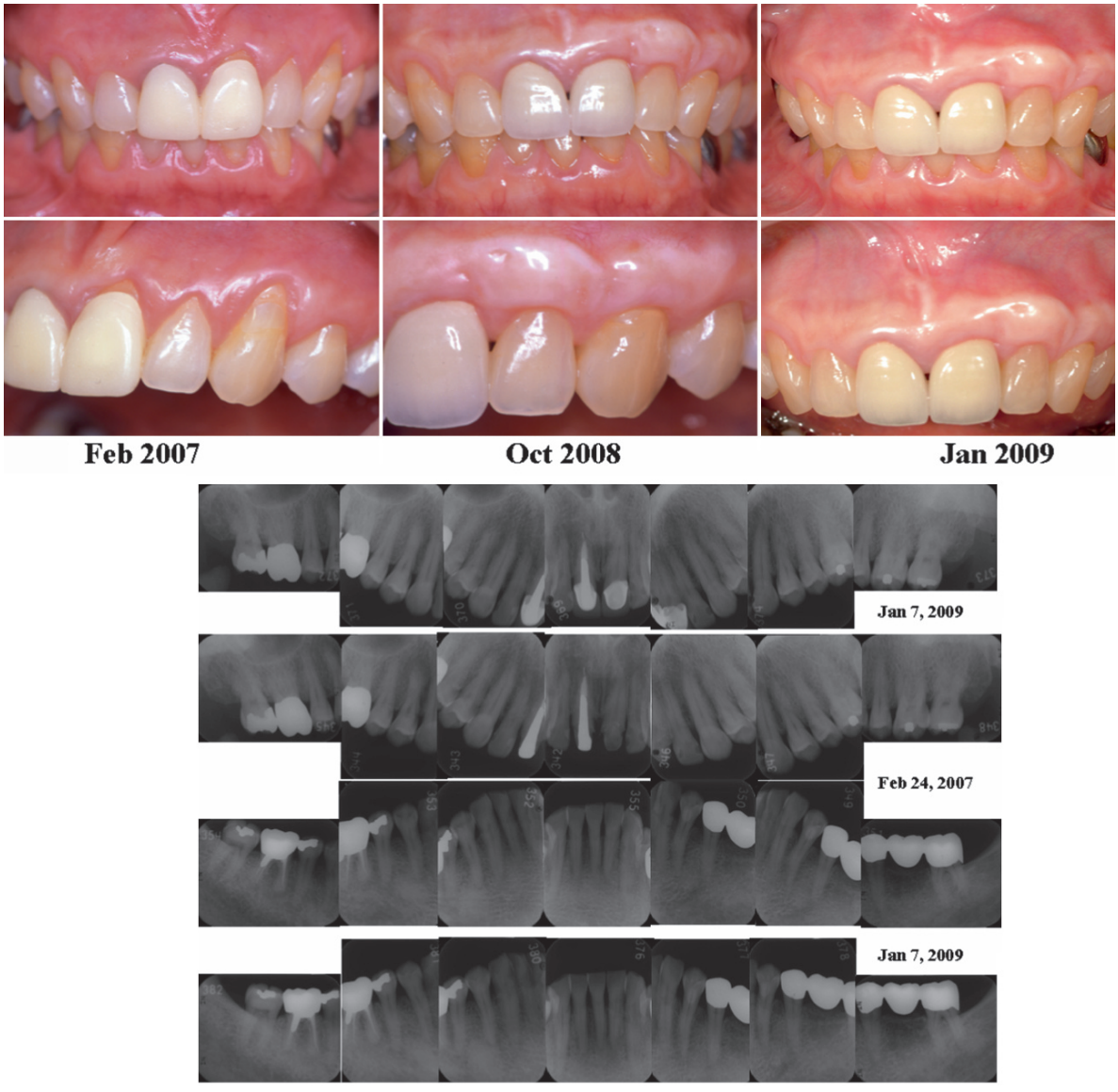

Fig. 9.3 Treated gingival recession on frontal teeth

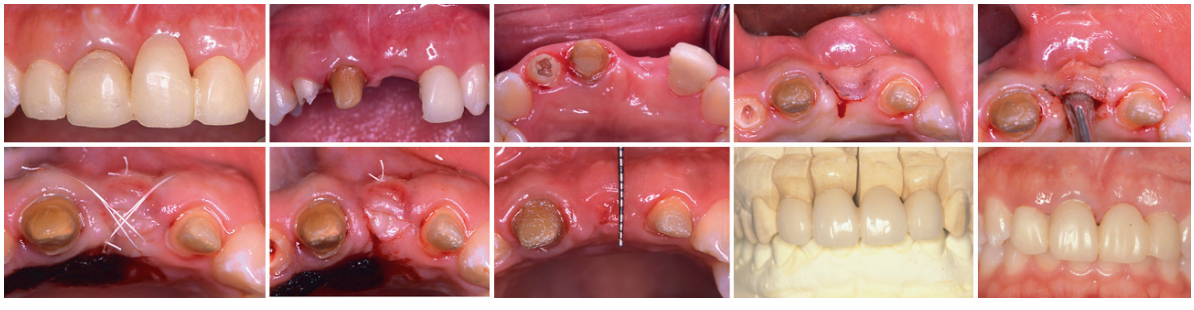

Fig. 9.4 Clinical results of reconstruction of deformed alveolar ridge augmentation (horizontal defect subclass small ≤3 mm) (Seibert 1983; Wang and Al-Shammari 2002) with interpositional graft in a 25 -year- old female patient

Nyman et al. (1975) pointed out several prerequisites that must be fulfilled for the successful treatment of patients with advanced breakdown and who require extensive bridgework. Firstly, the treatment of the periodontal tissues should be carried out in accordance with a detailed plan that is arrived at by careful examination of the periodontium around every single root. Secondly, no treatment of this kind should be given to patients, unwilling or unable to maintain a high standard of plaque control. However, the biological response of the hard and soft tissues to complex therapy and the psychological motivation of the patient are not quantifiable initially. Yuodelis and Faucher (1980) stated that the removal of hopeless teeth, followed by the placement of provisional restorations, is an essential step in managing the mutilated dentition. However, there is continuing debate regarding the appropriate time to extract questionable teeth that have received long-term periodontal care (McCreery and Truelove 1991; This decision is related to patient motivation and personal skill development to perform an extremely high standard of plaque control. (Kawamura et al. 2004). Thirdly, all the technical procedures involved in bridge production (preparation, impression technique, laboratory work etc.) must be properly understood and performed. In cases with advanced periodontal disease, the bridgework is an important part of the overall periodontal treatment (Kawamura et al. 2004) (Fig. 9.5). This bridgework is not a means of treating chronic, destructive inflammatory periodontal disease, but a *sine qua non* for the maintenance of the teeth (Nyman and Lindhe 1976).

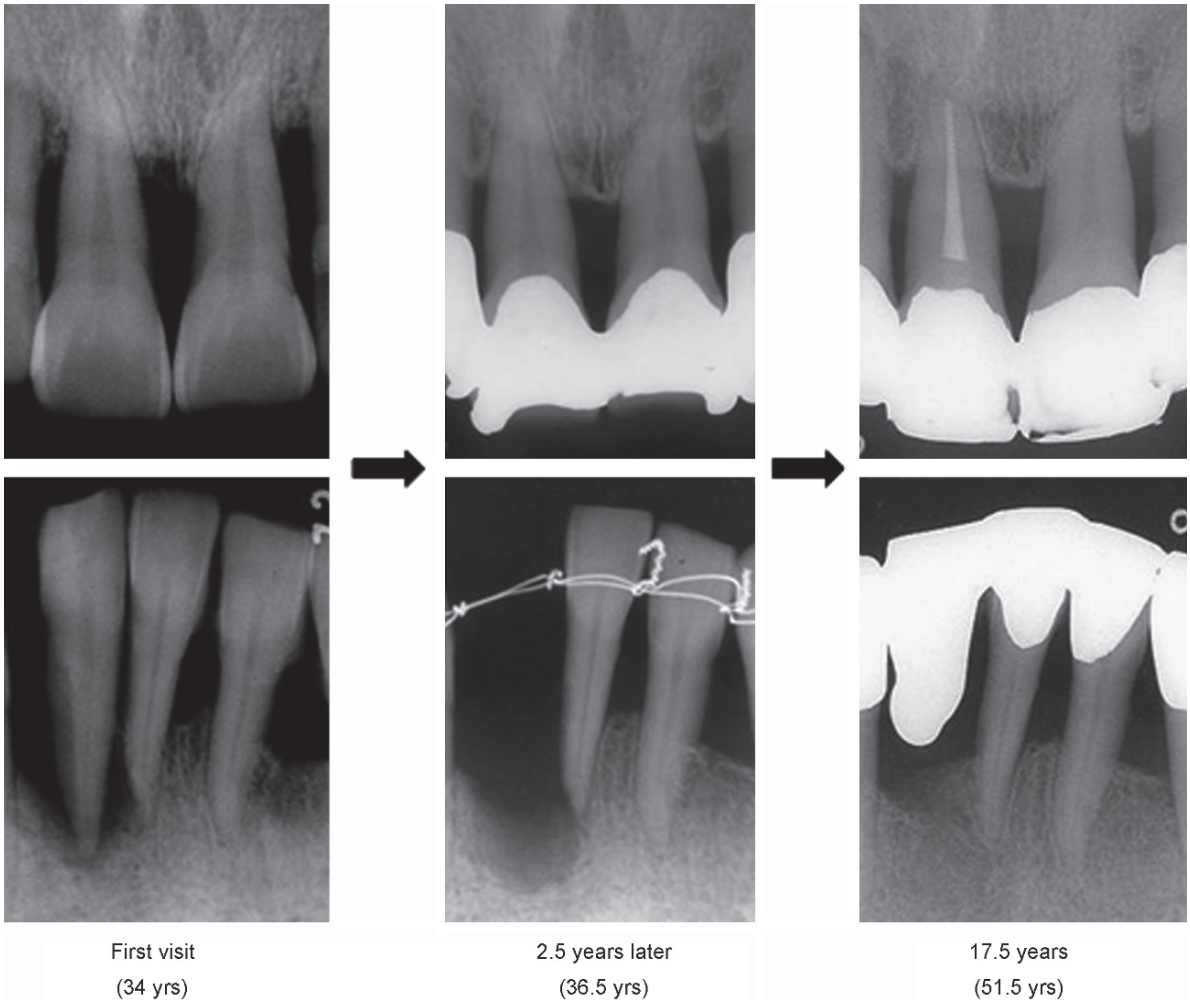

Fig. 9.5 A possibility of provisional restorative treatment. Radiographic evaluation (17.5 years later) revealed that there had been no further bone loss in either of the defect sites or in other regions (Kawamura et al. 2004) (with permission from Wiley-Blackwell Publishing)

Demand for esthetic dental restorations and public concerns about adverse systemic effects from dental metals and alloys have led to the increased use of ceramics in patient care. It is widely agreed the conventional glazed porcelain is the restorative material that least encourages plaque accumulation and allow plaque to be easily removed. However, the periodontal response to the recently marketed ceramics was scarcely investigated in the literature. Gemalmaz and Ergin (2002) found no significant difference in the gingival health status of teeth with all-ceramic crowns that had margins placed above or at the level of the gingival margin. However, in crowns that had subgingival margin finish lines, the percentage of bleeding, on probing around the crowns was significantly higher than that of the contra-lateral controls. For 12 months, Burke et al. (2001) evaluated the clinical performance of dentin-bonded ceramic crowns and reported optimal gingival health (70%) at the labial aspects of the all-ceramic crowns examined, whereas 30% of the examined crowns showed inflammatory changes. However, in a more recent study, Al-Wahadni et al. (2006) reported that in general practice, patients teeth with all- ceramic crowns had poorer periodontal health and more clinically evident plaque than uncrowned teeth.

9.2 The Effect of Removable Partial Dentures on Periodontal Health of Abutment and Nonabutment Teeth

A removable partial denture (RPD) is a common treatment available for the restoration of partially edentulous ridges, where a fixed partial denture is not indicated. Although RPDs serve as excellent means for the replacement of missing teeth, they may pose a serious problem to the patient's remaining teeth (Zlatarić et al. 2002).

Several longitudinal studies indicated that RPDs have been associated with increased gingivitis, periodontitis, and abutment mobility (Zlatarić et al. 2002; Vanzeveren et al. 2002; Kern and Wagner 2001; Li and GT 2006), due to increased plaque accumulation, not only on tooth surfaces in direct contact with the denture, but also on teeth on the opposite arch, and in some cases, even on buccal surfaces of teeth (Zlatarić et al. 2002). The periodontal alterations were also attributable not only to poor oral hygiene but also to transmission of excessive forces to the periodontal structures from occlusal surfaces of the framework of RPDs (Zlatarić et al. 2002; Bergman 1987).

For a long time, it was stated in practically all textbooks in prosthodontics, and taught in most dental schools, that a full complement of teeth is a prerequisite for a healthy masticatory system and satisfactory oral function. In consequence with this opinion,which is considered as a dogma by many, teeth that were lost should be replaced to avoid a number of negative sequelae. For example, when discussing indications for RPD it was stated that: "Many times it is much better to preserve what is left instead of replacing what has been lost." If a patient manages well with a reduced dentition, there is no reason to recommend prosthetic appliances, as replacing missing molars is a common source of iatrogenic periodontal disease, and should therefore be avoided if esthetics and functional stability can be satisfied (Zlatarić et al. 2002).

The term "*shortened dental arches*" (SDA) was first used in 1981 when it was concluded that there is sufficient adaptive capacity in subjects with SDA when at least four occlusal units are left (one unit corresponds to a pair of occluding premolars; a pair of occluding molars corresponds to two units). Gradually, the findings that dental arches comprising the anterior and premolar teeth in general constitute a functional dentition has gradually met increased acceptance. However, the SDA concept is still considered controversial by many clinicians. For example, it has been criticized, because loss of molars is associated with reduced masticatory performance and has been reported to be associated with an increased risk for changes in the temporomandibular joint, and to lead to mandibular displacement and various changes in the body, at any rate in animals (Kanno and Carlsson 2006; Al-Ali et al. 1998), despite the fact that several authors have reported an increased occlusal stability in SDAs (Sarita et al. 2003). The SDA concept does not contradict current occlusion theories and appears to fit well with the problem-solving approach favored in modern dentistry. Advocating the SDA offers some important advantages, one of which may be a decreased emphasis on restorative treatments for the posterior regions of the mouth (Armellini and von Fraunhofer 2004).

9.3 Surgical Lengthening of Clinical Crown

Crown-lengthening surgery is designed to increase clinical crown length for various reasons. The clinical crown is that portion of the tooth that extends

occlusally or incisally from the investing soft tissue, usually the gingiva. Teeth with subgingival caries or shortened by extensive caries, fractures, short clinical crowns with or without esthetic deficiencies, and teeth shortened by incomplete exposure of the anatomic crown, are all candidates for surgical lengthening. Failure to perform a surgery prior to margin placement in these situations often leads to margins placed too near the alveolar crest, thus invading the biologic width space. Therefore, in the early stages of restorative treatment planning, if the clinician believes that the margin of the final restoration will be ≤3 mm from the alveolar bone crest, crown lengthening should be recommended. This can not only be accomplished by surgery but also by orthodontic forced eruption, or a combination of both. Numerous factors may determine if crown lengthening is needed and often, more important, if a particular tooth (or teeth) is indeed a candidate for crown-lengthening surgery. Before proceeding with surgery, the clinician should first consider whether orthodontic extrusion is appropriate. Failure to consider orthodontic extrusion can lead to poor cosmetic outcomes (i.e., gingival recession, particularly in anterior teeth), poorer crown:root ratios, and loss of bone support on adjacent teeth (Padbury et al. 2003; Perez et al. 2007; Pontoriero and Carnevale 2001).

Gargiulo et al. (1961) reported a certain uniformity of the dimension of some components of biologic width:

- Mean depth of the histologic sulcus is 0.69 mm.
- Mean junctional epithelium measure is 0.97 mm (0.71–1.35 mm).
- Mean supra-alveolar connective tissue attachment is 1.07 mm (1.06–1.08 mm).

The total of the attachment is therefore 2.04 mm (1.77–2.43 mm) and is called the biologic width, essential for preservation of periodontal health and removal of irritation that might damage the periodontium (prosthetic restorations, for example). Violation of that space by restorations impinging on the biological width has been associated with gingival inflammation, discomfort, gingival recession, alveolar bone loss, and pocket formation (Dibart et al. 2003).

Surgical methods for crown lengthening include (a) gingivectomy, (b) apically positioned flap surgery, and (c) apically positioned flap surgery with osseous reduction. Gingivectomy and apically positioned flap surgery without osseous reduction are limited because bone removal is often necessary to provide adequate distance from the osseous crest to the anticipated restoration margin, allowing for biologic width. Therefore, apically positioned flap surgery with osseous surgery is the most common technique for crown-lengthening surgery. Apically positioned flap surgery with osseous surgery consists of a reverse bevel incision and subsequent mucoperiosteal flap reflection. Vertical releasing incisions are often made to allow better access and apical positioning of the flap. Initial incisions may be intra-sulcular if gingival width is narrow, or scalloped when gingival width is wide. Generally, an adjacent tooth, on each side of the tooth to be lengthened is included in the surgical procedure to allow for proper contour of the gingiva and the underlying bone. Initial osseous recontouring is completed with the use of rotary handpieces and then with chisels and curettes to achieve the desired reduction while maintaining a scalloped, parabolic bony contour to follow the desired contour of the overlying gingiva. In addition, end cutting burs are currently available that are designed for removing the bone with minimal risk of damaging the root (Padbury et al. 2003) (Fig. 9.6).

9.4 Iatrogenic Dental Restorations

There are several keys to produce dental restorative dentistry that are compatible with periodontal health. The proximal contact type, the restorative margin location, the margin quality, and the restorative material biocompatibility should receive consideration in the treatment planning and implementation of periodontal therapy (Padbury et al. 2003; Hodge 1998).

Overhanging dental restorations have long been viewed as a contributing factor to gingivitis and periodontitis (Padbury et al. 2003).

A number of studies have demonstrated that the prevalence of overhanging amalgam restorations is high. Reported studies have found prevalence rates for overhangs to be between 25 and 87% (Padbury et al. 2003; Pack et al. 1990; Sikri and Sikri 1993; Kells and Linden 1992; Lervik et al. 1984; Gilmour et al. 2008).

It was demonstrated that overhangs not only increase plaque mass but also increase the specific periodontal pathogens in the plaque, with increased proportions of Gram-negative anaerobic rods, in particular black pigmented *bacteroides* (Padbury et al. 2003). Deeper periodontal pockets, increased clinical

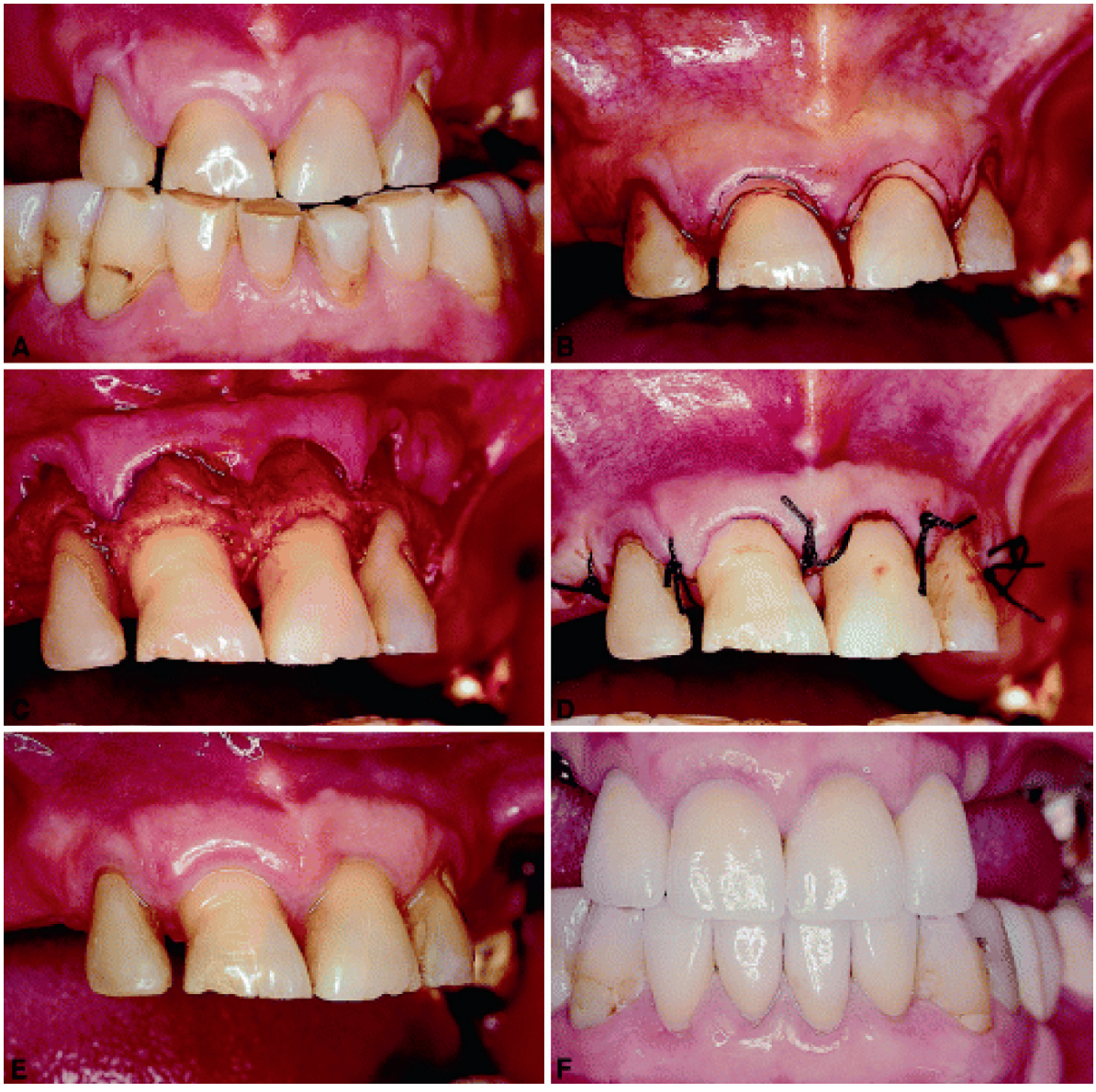

Fig. 9.6 A crown-lengthening procedure, utilizing the principle of apically positioned flap, was performed to facilitate crown placement and aesthetic requirements. (**A**–**D**) Surgical procedure. (**E**) 4 weeks of postoperative healing. (**F**) Final restoration (Wang and Greenwell, 2001) (With permission from Wiley-Blackwell)

attachment loss, gingival inflammation, and loss of alveolar bone, are more likely to be observed at sites where overhanging restorations are present, than on sites without overhanging restorations (Pack et al. 1990; Parsell et al. 1998; Jansson et al. 1994; Highfield and Powell 1978; Jeffcoat and Howell 1980; Than et al. 1982; Hakkarainen and Ainamo 1980; Gilmore and Sheiham 1971).

A further significant complication associated with overhanging restorations is the potential to develop recurrent caries, particularly where oral hygiene measures are inadequate. The use of dental floss is one recommended interdental hygiene product, particularly following restoration of proximal surfaces. Overhangs may lead to difficulty, as fraying of the floss will often occur during its removal. Therefore, any system which minimizes overhang production is of direct benefit to the patient (Gilmour et al. 2008).

Small overhangs can be removed, but larger overhangs can only be remedied by the replacement of the

entire restoration (Gilmour et al. 2008). It was demonstrated that EVA reciprocating motor driven system diamond tip is faster in removing overhangs and led to smoother restorations compared to sonic scalers and curettes, respectively (Padbury et al. 2003; Lim and Ong 1989). Removal of amalgam overhangs has been shown to be associated with an improvement of gingival health (Gilmour et al. 2008; Rodriguez-Ferrer et al. 1980; Roman-Torres et al. 2006).

9.5 Implant Restorations

Dental implant placement is an effective and predictable treatment modality for replacing missing teeth in various types of edentulous patients (Brånemark et al. 1995; Mericske-Stern et al. 1994; Jemt 1986; Buser et al. 2002). The number of patients requiring implant-supported reconstructions has increased substantially in the past few years (Bornstein et al. 2008).

Aglietta et al. (2009) reviewed that the overall estimate of the implant survival after 5 and 10 years, calculated with a standard Poisson regression analysis amounted to 98.5% (95%CI: 97.1–99.3%) and 97.1% (95%CI: 94.3–98.5%). Some researchers hypothesize that implant prognosis in periodontally compromised patients may be less favorable than in periodontally healthy subjects, even in the case of a successful periodontal therapy before implant installation (Kornman et al. 1997; Page et al. 1997). In a review of Karoussis et al. (2007), no statistically significant differences in short-term and long-term implant survival existed between patients with a history of chronic periodontitis and periodontally healthy individuals. Therefore, more studies, uniformly designed, preferably longitudinal, prospective and controlled, would be important.

Despite the high success rate shown by longitudinal studies, failures do occur, even in patients who present appropriate clinical conditions (Montes et al., 2007). According to the review of the study, most failure occurred before loading (88.2%). Failure was more frequent when the implant was installed in the posterior jaw (58.5%). Most implant loses (75%) did not have an apparent clinical use. Identified causes were 17.5% iatrogenic conditions (surgical technique, contamination, and/or occlusal trauma), 3% poor bone quality and quantity, and 1% peri-implantitis. Implant failures that occurred after loading, without accounting for implants lost during the initial healing period were caused by the sequelae of advanced peri-implantitis, implant fracture in an observation period of over 10 years (Brägger et al. 2005; Eliasson et al. 2006).

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Endodontic and Periodontal Interrelationship

10

Alexandrina L. Dumitrescu and Koji Inagaki

The interrelationship of periodontal and endodontic diseases has been a subject of controversy for many years. Investigators have expressed an entire spectrum of opinions on this interrelationship, ranging from statements that the pulp of all periodontally involved teeth be removed to redirect the nutritional supply from the pulp to the periodontium, to statements that the histological condition of the pulp is completely independent of periodontal disease and that the amount of periodontal involvement does not affect the health of the pulp in any way. These hypotheses are obviously incompatible (Czarnecki and Schilder 1979). As long as the pulp maintains vital functions, although inflamed or scarred, it is unlikely to produce irritants that are sufficient to cause pronounced breakdown of the periodontium. Consequently, no benefit will be gained from pulp extirpation as an adjunct or alternative to the treatment of teeth for periodontal disease, unless a retrograde pulpits from the periodontal tissue breakdown is suspected (Beghenholtz and Hasselgren 1997).

10.1 Pathways of Communication Between the Dental Pulp and the Periodontium

There are two forms of possible pathways for the bacteria and their products connecting the two tissues: anatomical and nonphysiological (Zehnder et al. 2002).

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10.1.1 Anatomical Pathways

Direct communication between the pulp and periodontal ligament exists by way of the apical foramen or foramina, the lateral and/or accessory canals and the dentinal tubules (Harrington 1979).

The root canal ramifications were first described some 100 years ago, and have since been subdivided into furcated, collateral, lateral, secondary, accessory, intercanal, and reticular canals, as well as furcation canals. It has been speculated that these channels are created by the interference of persistent blood vessels during the downward growth of the sheath of Hertwig. This hypothesis is underlined by the finding that accessory canals usually contain blood vessels (Zehnder et al. 2002).

The overall incidence of lateral canals is estimated to be somewhere between 30 and 40%, 11.9% for maxillary central incisors, and with a higher preponderance in multirooted teeth, approximately 50–60%, occurring especially in the apical one third of the root (Harrington 1979). Kirkham (1975) evaluated one hundred permanent human teeth, extracted due to periodontal disease, to determine the percentage of teeth in which accessory canals were located within a periodontal pocket. Two percent of the 100 teeth studied had accessory canal located within a periodontal pocket. Seventeen percent of the teeth examined had one accessory canal, 6% had two accessory canals, and no teeth had more than two accessory canals. As groups, mandibular premolars and mandibular molars had the highest percentage of teeth exhibiting accessory canals, and maxillary molars and mandibular incisors had the lowest percentage of teeth with accessory canals (Kirkham 1975).

Clinically, positive identification of the presence of a lateral canal can usually be made only when a discrete lateral lesion associated with a pulpless tooth can

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be identified radiographically, or when some of the root canal filling material is forced into a lateral canal during the condensation procedure. In addition, radiographic identification of a notch on the lateral root surface is suggestive of the presence of an orifice into a lateral canal (Harrington 1979).

In addition to the apical foramina and accessory canals, there is a third possible route for bacteria and their products, the dentinal tubules. Dentinal tubules are formed or, better, left out during tooth development by odontoblasts, which trail their processes as they grow centripetally while secreting the dentin matrix. The extent of these processes in the dentinal tubules of fully formed dentin is a matter of dispute; however, it is most likely that the odontoblastic process does not reach further than 0.5 mm into the dentin. In a mature tooth, each individual dentinal tubule can be regarded as an inverted cone with the smallest dimension at the periphery and the largest dimension at the pulp. The opening of each of these small tunnels facing the periodontal ligament is sealed with cementum. At 3.5 mm distance from the pulp, the mean tubule diameter was found to be 0.8 mm, compared to 2.5 mm at the pulpal wall (Zehnder et al. 2002). Dentinal tubules can be exposed by the congenital absence of cementum in the region of the cementoenamel junction in approximately 5–10% of the population, from traumatic injuries of the root cementum, from root planing procedures and via root resorption (Chen et al. 1997). The root cementum act as an effective barrier between an infected pulp and the periodontal ligament, and when it is damaged, the noxious contents of the infected pulp pass through the dentin and stimulate more aggressive and rapid root resorption (Chen et al. 1997).

The following anatomical entities or pathways have also been mentioned as possible causes of periodontally derived endodontic lesion: lingual grooves, root/tooth fractures, cemental agenesis/hypoplasia, root anomalies, intermediate bifurcation ridges, fibrinous communications, and trauma-induced root resorption (Meng 1999) (Figs. 10.1 and 10.2).

10.1.2 Nonphysiological Pathways

Iatrogenic root canal perforations are serious complications during dental treatment and have a rather poor prognosis. Perforations may be produced by powered rotary instruments during the attempt to gain access to the pulp, or during preparation for a post. Improper manipulation of endodontic instruments can also lead to a perforation of the root. The second group of artificial pathways between periodontal and pulpal tissues are vertical root fractures (VRTs) (Figs. 10.3 and 10.4). VRTs are caused by trauma and have been reported to

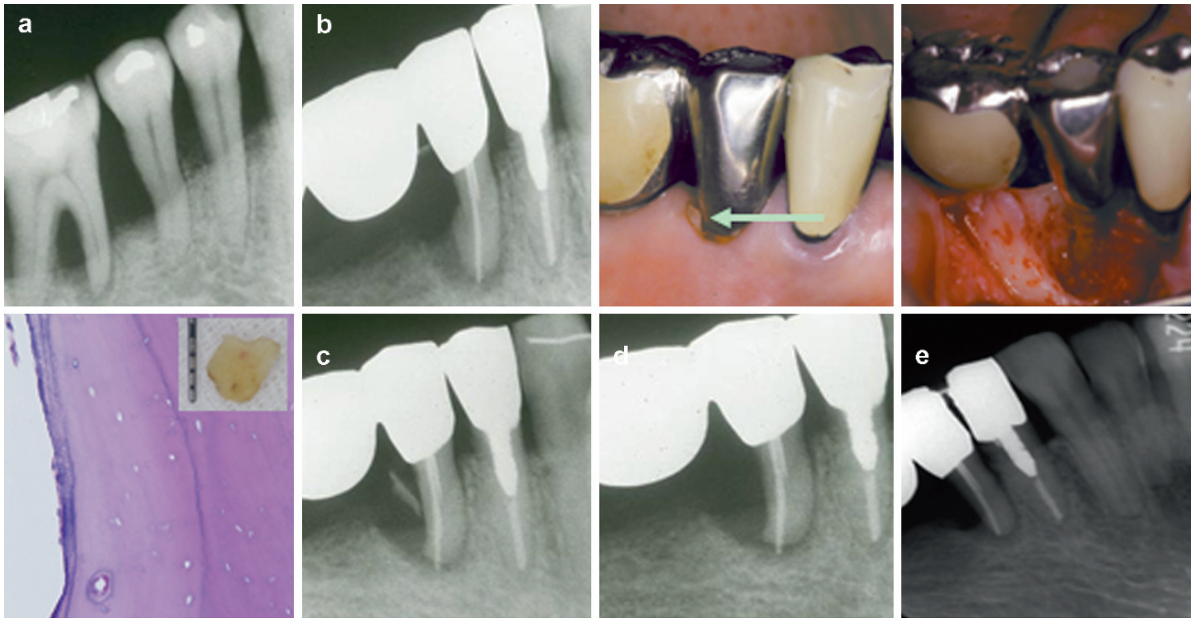

Fig. 10.1 Periodontal findings of a tooth with cemental tear in a 35-year-old female patient (Probing depth 5–6 mm) (**a**) Nov 1982; (**b**) Apr 1998; (**c**) Jan 1999; (**d**) June 1999; (**e**) Mar 2007

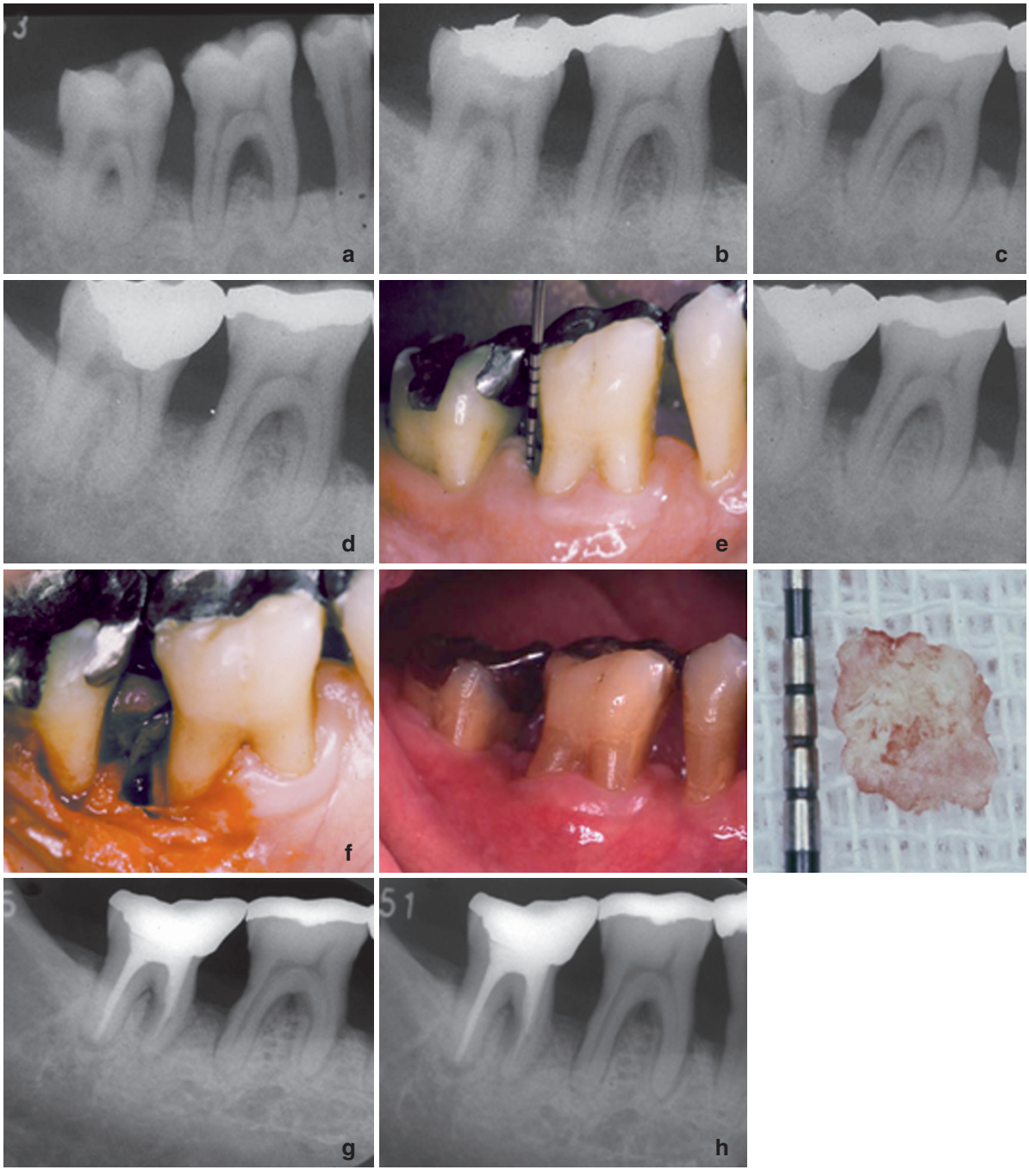

Fig. 10.2 Periodontal findings of a tooth with cemental tear in a 40-year-old male patient (**a**) Apr 1983; (**b**) May 1985; (**c**) Feb 1988; (**d**) Apr 1994; (**e**) Jan 1995 (Probing depth = 5–6 mm); (**f**) Apr 2002; (**g**) Aug 2002; (**h**) Feb 2005 (Probing depth = 3–4 mm)

occur in both vital and nonvital teeth. In vital teeth, vertical fractures can be continuations of coronal fractures in the 'cracked tooth syndrome' or can occur solely on root surfaces. In endodontically treated teeth, the incidence of VRTs is higher in teeth that were filled with lateral condensation technique, as compared to teeth filled with single cone technique. Teeth restored with intracanal posts are more susceptible to fracture than root-filled teeth without posts, and the extension of posts beyond the coronal half of the root canals has a significant negative effect on the incidence of root fractures as compared to shorter posts (Zehnder et al. 2002).

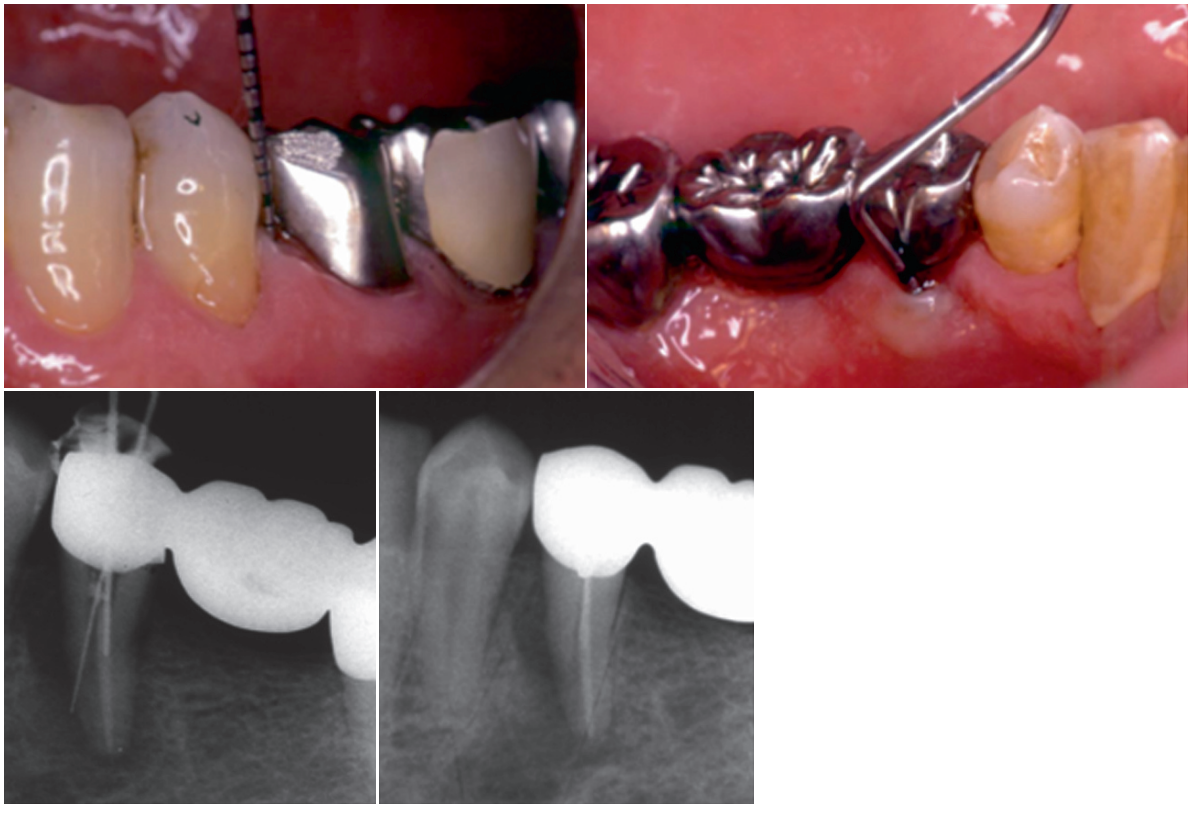

Fig. 10.3 Clinical and radiographical periodontal findings of a tooth extracted for a root fracture in a 65 –year- old male patient

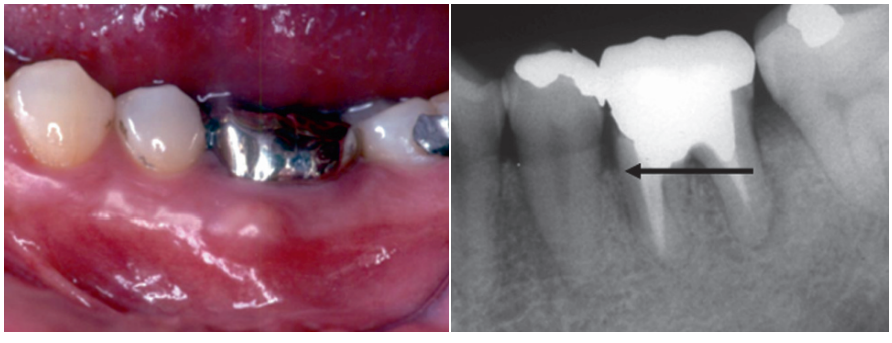

Fig. 10.4 Clinical and radiographical periodontal findings of a tooth extracted for a root fracture in a 65-year-old male patient (Probing depth = 7 mm)

10.2 Effect of Periodontal Disease and Procedures on the Pulp

Over the years, there has been a consistent stream of speculation as to the effect of periodontal disease on the dental pulp. Recent publications have suggested that "periodontal disease" is a "direct cause of pulpal atrophy and necrosis," "periodontal disease" is "more deleterious to the pulp than both caries and restorations combined," and "periodontal disease and periodontal treatments should be regarded as potential causes of pulpitis and pulpal necrosis." Such interpretations have little basis in current scientific facts, but demonstrate the persistence of an often repeated point of view in our literature (Harrington and Steiner 2002). A review of several studies (Bergenholtz and Nyman

1984; Czarnecki and Schilder 1979; Haskell et al. 1980; Jaoui et al. 1995; Kirkham 1975; Langeland et al. 1974; Ross and Thompson 1978; Tagger and Smukler 1977; Torabinejad and Kiger 1985) related to the "periodontal–endodontic" failed to reveal a real evidence of this link (Harrington and Steiner 2002).

It also appears that periodontal treatment, such as for periodontal disease, has a negligible effect on the dental pulp (Bergenholtz and Lindhe 1978; Wong et al. 1989; Ross and Thompson 1978; Jaoui et al. 1995; Bergenholtz and Nyman 1984).

Unless periodontal disease extends all the way to the tooth apex, the weight of evidence in the literature suggests that the dental pulp is capable of surviving significant insults and that the effect of periodontal disease as well as periodontal treatment on the dental pulp is negligible. It also appears that the clinical significance of the relationship between periodontal disease and the dental pulp has been greatly exaggerated in historical and much of the current periodontal-endodontic literature (Harrington and Steiner 2002).

10.3 The Effect of Endodontically Involved Teeth on Periodontal Health and Healing

Experimental and clinical studies have evaluated the effect of necrotic pulpal diseases on the periodontal tissues.

In patients without periodontal disease, no influence of the quality of endodontic treatment could be established on the periodontium (Miyashita et al. 1998). However, in periodontitis-prone patients, the presence of a periapical lesion was found to be associated with a lower marginal bone level (Jansson et al. 1995), deeper pockets (Jansson et al. 1993a), a higher risk of losing periodontal attachment (Jansson et al. 1993b), significantly more frequent horizontal furcation depths ≥3 mm (Jansson and Ehnevid 1998) and a higher rate of progression of periodontal disease (Jansson et al. 1995). It was also revealed that in periodontitis patients, teeth with endodontic treatment had more bone loss as compared with untreated contralaterals (Timmerman and van der Weijden 2006) (Figs. 10.5 and 10.6).

The negative consequences of an endodontic infection on marginal periodontal healing following periodontal therapy also appear to be significant (Ehnevid et al. 1993a, b; Chen et al. 1997).

Three pathways are proposed to account of the development of localized periodontal attachment loss consequent to pulpal disease. This hypothesis accounts for the sudden deterioration of periodontal sites under regular review, the strict localization of alveolar defects, with the normal alveolar bone immediately adjacent, the presence of site-specific bacteria (secondary colonizers of deep pockets), which cannot cause disease when transferred to healthy sites, and the antibody responses directed against them (Hirsch and Clarke 1993). Three pathways are proposed to account for the development of localized periodontal attachment loss consequent to pulpal disease. This hypothesis accounts for the sudden deterioration of periodontal sites under regular review, the strict localization of alveolar defects with normal alveolar bone immediately adjacent, the presence of site-specific bacteria (secondary colonizers of deep pockets) which cannot cause disease when transferred to healthy sites, and the antibody responses directed against them.

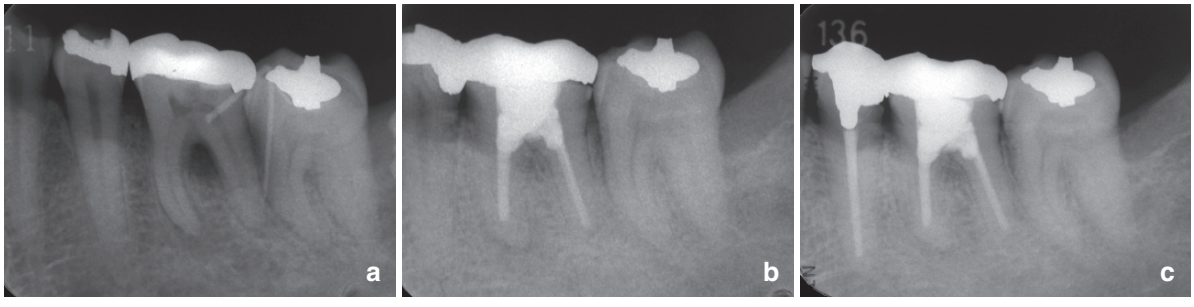

Fig. 10.5 (**a**) Chief complaint of a 47-year-old patient was a gingiva swelling. Fistule was found on the tooth 36. Pulp test by an electric device was nonvital. Probing depth was <3 mm. (**b**, **c**) Periodontal changes after endodontic treatment

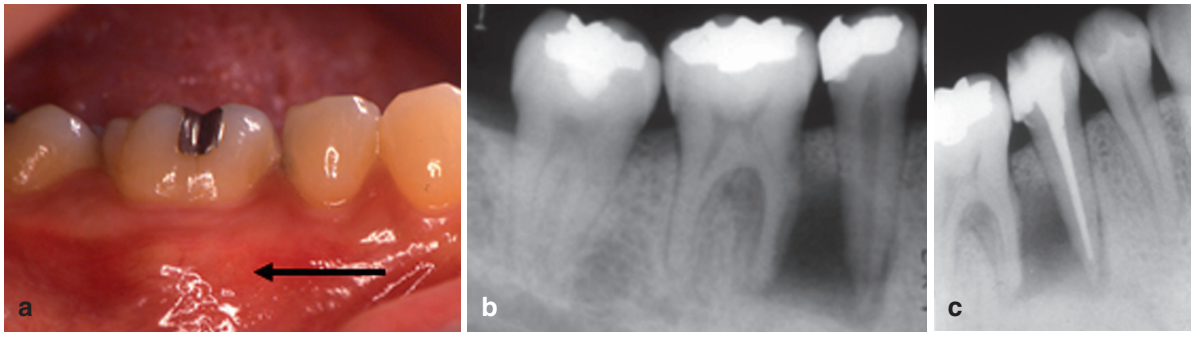

Fig. 10.6 (**a**) Chief complaint of a 26-year-old female patient was a gingival swelling. Trace of central tubercle was found on the occlusal surface of the tooth 35. Pulp test by an electric device was nonvital. (**b**) Initial radiographic aspect. (**c**) Radiographic aspect 2 months later after endodontic treatment

10.4 Impact of the Endodontic Treatment on the Periodontium

Periodontal inflammation can also result from mechanical and chemical irritation initiated by the root canal treatment.

10.4.1 Root Perforations

Root perforations are artificial openings in root walls created by boring, piercing, cutting, or resorption, that result in a communication between the pulp space and periodontal tissues. This may cause secondary periodontal involvement and eventual loss of the tooth. Iatrogenic perforations are often due to inadequate access preparation, misdirection of a bur, over-instrumentation of root canals, or the misuse of a rotary instrument in preparing post or dowel. Diagnostics require a combination of symptomatic findings and clinical observations. Symptoms of perforations may include sudden pain during treatment procedures, taste of irrigation solution leaking either through a cervical perforation or under a leaky crown margin. In addition, the presence of a sinus tract or the appearance of a localized problem such as pocket formation or furcation involvement after apparently adequate endodontic therapy may indicate the existence of a perforation. Diagnostic aids include indirect bleeding assessment, using a paper point, radiography, and an apex locator. For mid root and cervical perforations, nonsurgical approaches including placement of an internal seal are preferable (Beghenholtz and Hasselgren 1997).

In addition to providing a good seal, the material of choice for repair of root perforations must be biocompatible, nontoxic, insoluble in the presence of tissue fluids, and capable of promoting regeneration of the periradicular tissues (Main et al. 2004).

Many materials have been used to repair perforations; they include amalgam, Cavit (SPE America 3 M, Norristown, PA), Super-EBA (HI Bosworth Co, Skokie, IL), glass ionomer, and others (Alhadainy 1994; Main et al. 2004). The success rate of these materials has been variable. For example, it was shown that light cured glass ionomer provides a better seal, followed by Cavit and amalgam (Alhadainy and Himel 1993).

Mineral trioxide aggregate (MTA), composed mainly of tricalcic silicate, tricalcic alluminate, bismuth oxide, is a particular endodontic cement. It is made of hydrophilic fine particles that harden in the presence of dampness or blood. It is biocompatible, radiopaque and it is harder to infiltrate, compared to classic materials for root filling. MTA has been recommended as a repair material for sealing and filling perforations of the pulp chamber and of the root (Casella and Ferlito 2006). Many studies have demonstrated MTA sealing ability (Lee et al. 1993; Nakata et al. 1998; Tsatsas et al. 2005), biocompatibility to the surrounding tissues (Vajrabhaya et al. 2006), and the ability to allow regeneration of these hard tissues (Koh et al. 1997, 1998; Torabinejad et al. 2009).

A search of the literature revealed several case reports (Koh 2000; Yildirim and Dalci 2006; Jacobovitz and de Lima 2008; Menezes et al. 2005; Hsien et al. 2003; Hembrough et al. 2003), case series (Pace et al. 2008; Joffe 2002), short-term (Arens and Torabinejad

1996; Schwartz et al. 1999) and long-term studies (Main et al. 2004; Silveira et al. 2008; Oliveira et al. 2008; Ibarrola et al. 2008) that evaluated the clinical efficacy of MTA (ProRoot MTA, Dentsply Maillefer, Ballaigues, Switzerland) as a perforation repair material.

10.4.2 Vertical Root Fractures

Vertical root fracture (VRF) is defined as a longitudinal fracture confined to the root that usually begins on the internal canal wall and extends outward to the root surface. The fracture may initiate at the apex or midroot and occurs primarily in a facial-lingual plane. This type of fracture is different from the cracked tooth, which is an incomplete fracture that runs mesio-distal, is centered occlusally, involving one or more marginal ridges, and extends from the occlusal surface toward the cervical, and eventually down the root. It also differs from the split tooth, in which there is a complete fracture due to the long -term extension of an incomplete fracture (crack tooth) or sudden occurrence (Yang et al. 1995).

Posterior teeth seem to be more susceptible to vertical root fractures (VRFs). It has been shown that VRTs are statistically more prevalent in mandibular molars and maxillary premolars (Cohen et al. 2006). Differences in VRFs with respect to tooth distribution were demonstrated in endodontically vs. nonendodontically treated teeth. It has been revealed, in a Chinese population, in which of all the VRTs, 40% occurred in nonendodontically treated teeth. In comparison with those in endodontically treated teeth, VRTs in nonendodontically treated teeth tended to occur in patients with a higher mean age (55 vs. 51 years) and were more frequent in male patients (78 vs. 58%). VRTs occurred in nonendodontically treated teeth, more often in molars (84 vs. 53%), less often in premolars (16 vs. 33%), and seldom in anteriors (1 tooth vs. 27 teeth) (Chen et al. 1997). This may be related to the heavier masticatory force associated with first molars, to thin or flat roots in first molars, or to the habitual use of the first molars in the chewing of hard food (Chen et al. 1997).

The cause of VRFs mainly is iatrogenic, resulting from dental treatment excesses (for example, excessive canal shaping, excessive pressure during compaction of gutta-percha, excessive width and length of a post space in relation to the tooth's anatomy and morphology, or excessive pressure during placement of the dowel). Trauma is the most likely cause of VRFs in vital teeth, typically occurring from physical trauma, clenching or bruxism, or occurring in teeth undergoing apexification. Early diagnosis of a VRF usually begins with the gathering of a comprehensive dental history, listening well to the patient, asking many questions and encouraging the patient to recall when the symptoms first occurred (Cohen et al. 2003; Fuss et al. 2001).

The diagnosis of a VRF can be difficult. The determination is often made based on the constellation of associated signs and symptoms, yielding a prediction rather than a definitive diagnosis. VRF often presents no specific signs and symptoms, and it is therefore difficult for dentists to make a definite diagnosis of the condition. The most common clinical symptoms of VRF are the presence of dull pain, swelling, and sinus tract, with a deep localized probing defect. Radiographs may show a widening of the periodontal ligament and osseous resorptive defects (Chan et al. 1999). Although a VRF may be observed directly on a radiograph, the X-ray beam must be in the same plane as the fracture to be observed, and because this is not likely most of the time, the radiograph may not be of much help in diagnosing these fractures (Cohen et al. 2006).

Because signs and symptoms can appear years after the completion of the operative procedures in the root, coronal restorations would not interfere with the correct clinical diagnosis of VRTs. Frequent recalls are recommended to diagnose VRTs early, especially in susceptible teeth, such as premolars and mesial roots of mandibular molars (Fuss et al. 2001).

VRTs that involve the gingival sulcus/pocket area usually have a hopeless prognosis due to continuous bacterial invasion of the fracture space from the oral environment. Single rooted teeth will have to be extracted. In multirooted teeth, a treatment alternative is hemisection and extraction of the fractured root (Beghenholtz and Hasselgren 1997). In a retrospective study, which analyzed all endodontically treated permanent teeth that were extracted in a multidisciplinary clinic (n = 547), VRT represented the reasons for extraction in 8.8% and were more prevalent in restored than in unrestored teeth (Zadik et al. 2008). Fuss et al. (1999) studied 147 extracted teeth. The major reasons for extraction were restorative (43.5%) and endodontic (21.1%), followed by VRTs (10.9%).

10.5 Clinical Diagnostic Procedures

The diagnosis of periodontal lesions associated with pulpal diseases may be relatively simple if a patient has been monitored over a period of time and records (e.g., radiographs) are available. The diagnosis becomes more difficult when a complete history is unavailable. A growing periapical area with secondary formation of a deep periodontal pocket may be similar, in clinical and radiographic appearance, to a longstanding periodontal lesion that has progressed to the root apex. The radiographic image of bone resorption, including the apical and furcal or marginal regions, may confuse rather than aid in making a diagnosis. However, if radiographs taken during the progression of bone resorption reveal it to be extending from the apex to the crest, the apical region can be positively identified as the origin of the infection. In general, it is easier to determine the origin of the lesion when a vital pulp test is obtained, because the test results usually will rule out an endodontic etiology. However, pulp tests may not always be reliable. This consideration is particularly relevant when challenges to pulpal status arise from periodontal diseases. Partial necrosis of a pulp, especially in a multirooted tooth, may be the result. This may allow for positive responses to pulp testing suggesting vitality, despite the existence of a combined lesion. It has been suggested that when doubt exists about a pulp's status, a test cavity can be made. However, this may not always provide a more exact determination of pulpal status once partial pulpal necrosis has occurred (Meng 1999).

A nonvital or endodontically treated tooth associated with a combined lesion presents a greater diagnostic problem. In this situation, pulpal necrosis is frequently associated with inflammatory involvement of the periodontal tissue. The location of these pulpal lesions is most often at the apex of the tooth, but may also occur at any site where lateral and furcal canals exit into the periodontium (Meng 1999).

Determination of pulp vitality is essential for a *differential diagnosis* and for the selection of primary measures for the treatment of inflammatory lesions in the marginal and apical periodontium. Deep restorations, dental trauma, endodontic treatment, previous pulp capping, and pulp vitality testing are the factors to be considered when assessing the need for endodontic treatment as a part of overall periodontal therapy. Location and extent of pockets, probing depth, and furcation invasions are also essential for the differential diagnosis. If the pulp reacts clinically normal, but a periodontal pocket can be located, then periodontal tissues should be suspected as the origin of either the acute or chronic inflammatory process. On the other hand, when the pulp is found to test nonvital, the inflammatory process which passes through a lateral canal or the apical foramen will cause a lesion of endodontic origin. When pulpitis is clinically recognized in a tooth with preexisting periodontal disease, the pulpitis may be secondary to the periodontal disease, particularly in the absence of any other obvious cause of pulpitis. It is important to note the presence of subgingival calculus deposits and the degree and location of inflammation, in determining the primary source of the disease (Meng 1999).

10.6 External Root Resorptions

Root resorption has traditionally been divided into external (root surface) resorption or internal (root canal) resorption. As the classification indicates, the origin of the resorptive process is assumed to be either the root surface or the root canal walls (Andreasen and Andreasen 1992). External resorption is a process that leads to an irreversible loss of cementum, dentin and bone. It takes place in both vital and pulpless teeth, and the identification is mostly made during routine radiographic or clinical examination as the majority of cases are asymptomatic (Bergmans et al. 2002).

Classification plays an important role in the process of diagnosis and treatment planning by the clinician (Heithersay 2007). Andreasen (1985) has, over the past 40 years, made a unique contribution to the understanding of tooth resorption following dental trauma and his original classification remains the most widely accepted. Three types of external root resorption have been distinguished: surface resorption, replacement resorption (ankylosis) and inflammatory resorption. However, Andreasen's original classification does not include other resorptive processes which have been identified over the past two decades. Of these, a third type of internal resorption namely, transient apical internal surface resorption, should also be added, along with other types of hyperplastic tooth resorption. These hyperplastic resorptions labeled, invasive coronal, invasive cervical, or invasive radicular resorption – do not fall into any of the original categories but may follow dental trauma and other potential predisposing factors (Heithersay 2007) (Figs. 10.7 and 10.8).

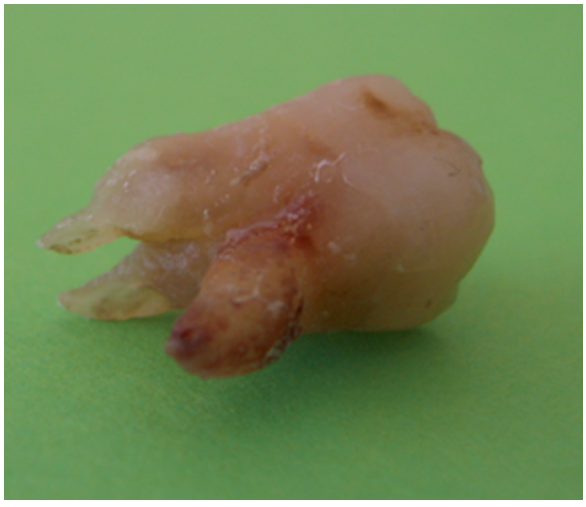

Fig. 10.7 Radiograph showing external root resorption

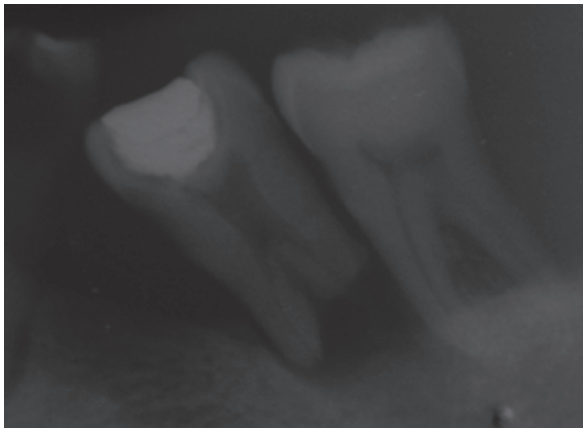

Fig. 10.8 Radiograph showing external apical pulpal infection root resorption

An alternative classification of tooth resorption was recently proposed by Lindskog et al. (2006), as cited by Heithersay (2007). This classification subdivides resorptions into three broad groups: (1) trauma induced tooth resorption; (2) infection induced tooth resorption; and (3) hyperplastic invasive tooth resorptions. There are some rare tooth resorptions of unknown cause that do not fit into any of the above categories and they are usually labeled "idiopathic." The advantage of this classification is that it makes a simple and clear distinction between each category, and as such provides important clues to the clinical management. For example, infection induced tooth resorption, as with any infective process, requires elimination of the invading micro-organisms as an integral component of clinical management (Heithersay 2007) (Fig. 10.9).

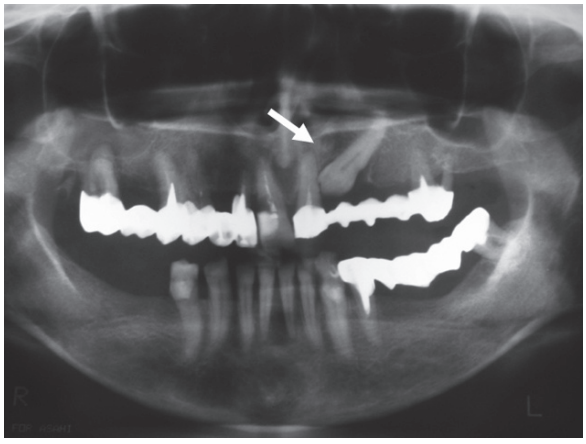

Fig. 10.9 Radiographic view demonstrates impacted tooth pressure root resorption (*arrow*) of central incisor by an impacted canine

Consequently, a lack of uniformity in nomenclature is still present, thus confusing the dental practitioners (Bergmans et al. 2002) (Table 10.1).

10.6.1 Andreasen's Original Classification of External Root Resorption (1985)

10.6.1.1 Surface Resorptions

Surface resorption was defined as a minor, self-limiting resorption process without any inflammatory cells in the neighboring periodontal tissue. The mechanism behind this type of resorption has not been specifically investigated but it seems that it is caused by combination of damage of the cementoblasts in a limited area and necrosis of the adjacent periodontal tissues. However, before repair takes place, injured cells and intercellular material must be removed by macrophages originating from well-vascularized tissue recruited from adjacent intact areas of the periodontal ligament. This process will expose the root surface and thereby hydroxyapatite, together with a still unsettled relationship between ingrowth vessels and superficial resorption, which is soon repaired with new cementum deposited from cells, presumably invading the injury zone from the adjacent intact tissue (Andreasen and Andreasen 1992; Hammarstrom and Lindskog 1992). This type of resorption may also be called "root resorption associated with minor necrosis" or "necrosis

Table 10.1 A summary of the groups and sub-groups of dental resorptions

Andreasen's original classification of tooth resorption (1985)		
Internal	Inflammatory	
	Replacement	
External	Surface resorptions	
	Replacement resorptions	
	External inflammatory resorption	Peripheral inflammatory root resorptions (PIRR)
		External inflammatory root resorptions (EIRR)
Lindskog et al. s classification of external root resorption (2006)		
Trauma induced tooth resorption	Surface resorption	
	Transient apical internal resorption	
	Pressure resorption	
	Orthodontic resorption	
	Replacement resorption	
Infection induced tooth resorption	Internal inflammatory root resorption (apical and intraradicular)	
	External inflammatory root resorption	
	Combined internal-external lesions	
Hyperplastic invasive tooth resorption	Internal replacement (invasive) resorption	
	Invasive coronal resorption	
	Invasive cervical resorption (class 1–4)	
	Invasive radicular resorption	

associated with root resorption" (Hammarstrom and Lindskog 1992).

These resorptions may be caused by localized injury to the root surface and the periodontium in conjunction with external trauma (Beghenholtz and Hasselgren 1997). The same type of resorption seems to be occurring during orthodontic treatment when the so called hyaline zone is resorbed (Hammarstrom and Lindskog 1992).

10.6.1.2 Replacement Resorptions

In severe traumatic injuries (intrusive luxation or avulsion with extended dry time), injury to the root surface may be so large that healing with cementum is not possible, and the bone may come in contact with the root surface without an intermediate attachment apparatus. This phenomenon is termed as "dento-alveolar ankylosis" (replacement resorption). Normally, the bone is resorbed and formed physiologically in a remodeling process without any specific stimulation with organic tissues protecting the dentin. Osteoclasts are in direct contact with the mineralized dentin in the exposed root surface after severe trauma to the root surface. Therefore, resorption can occur without any further stimulation and the bone is laid down instead of the dentin. The process may be reversed if less than 20% of the root surface is involved. Because there is no stimulation factor and the process proceeds as a result of the direct bone attachment to dentin, the term "ankylotic resorption" is adequate. Clinically, ankylotic teeth lack the physiological mobility of normal teeth. This is one diagnostic sign for ankylotic resorption. In addition, these teeth usually have a special metallic percussion sound and if the process continues, they are in infra-occlusion. Radiographically, resorption lacunae are filled with bone, and the periodontal ligament space is missing. No radiolucent areas are observed, and at some stage, the whole root may be replaced by the bone (Fuss et al. 2003).

10.6.1.3 External Inflammatory Resorption

Inflammatory root resorption is histologically characterized by the resorption of cementum and dentin associated with inflammatory cells in the neighboring periodontal tissue. Clinically, there are two main forms of external resorption associated with inflammation in

the periodontal tissues: peripheral inflammatory root resorptions (PIRR) and external inflammatory root resorptions (EIRR) (Gold and Hasselgren 1992).

Peripheral Inflammatory Root Resorptions (PIRR)

Over the years, this type of external resorption has been given many names: asymmetric internal, progressive intradental, peripheral cervical, cervical external, supraosseous extracanal invasive, subosseous or, more common, invasive cervical resorptions. However, even if the location is often cervical, this is not always the case. The location is related to the level of the marginal tissues and the pocket depth (Gold and Hasselgren 1992). PIRR presents a special type of pathological tooth condition that could be classified in the group of inflammatory resorptions. In recent years, several etiologic factors have been advocated and some morphological descriptions were made. Nevertheless, prediction and prevention are still impossible and an exact diagnosis and treatment is often far from easy, depending on the severity and localization of the defect (Bergmans et al. 2002).

Clinically, cervical external resorption is associated with the inflammation of the periodontal tissues and does not have any pulpal involvement. The pulp remains protected by a thin layer of predentin until late in the process and it has been postulated that bacteria in the sulcus sustain the inflammatory response in the periodontium. This feature differentiates cervical external resorption from another type of inflammatory resorption called external inflammatory resorption, which is continued by necrotic pulp tissues and infected root canal content (Bergmans et al. 2002; Andreasen 1985).

Cervical external resorption occurs immediately below the epithelial attachment of the tooth. As a result, it must be noticed that the location is not always cervical but related to the level of the marginal tissues and the pocket depth. Unless proper treatment is initiated, this type of resorption continues and a large irreversible loss of tooth structure may appear by time (Bergmans et al. 2002).

Although the precise pathogenic mechanisms and the natural history of external cervical resorption are only loosely identified, a number of possible contributory factors have been implicated. These include trauma, orthodontic tooth movement, dento-alveolar surgery, and periodontal disease and its treatment. In the endodontic literature, intra-coronal bleaching is the most commonly cited etiological factor (Patel et al. 2002; Vossoughi and Takei 2007; Plotino et al. 2008). The proposed theory of pathogenesis involves predisposing root conditions and perpetuating bacterial factors. It is hypothesized that an initial physical injury to the root surface or the presence of natural cementum defects may predispose resorption by altered host tissue modified by a bacterially driven stimulus. The microorganisms tip the balance from a potentially reversible physiological resorptive process to a progressive pathological one (Patel et al. 2002).

External Inflammatory Root Resorptions (EIRR)

The commonly called external inflammatory resorption, is triggered by the destruction of cementoblasts and cementoid, and continued by necrotic, and probably also infected, pulp tissue (Gold and Hasselgren 1992).

10.6.2 Lindskog et al.'s Classification of Tooth Resorption (2006)

10.6.2.1 Trauma Induced Tooth Resorption

In this category, using a broader interpretation of the term "trauma," resorptions may have resulted from, pressure from unerupted or erupting teeth or some neoplasms, biomechanical forces involved in orthodontics, mechanical trauma (luxation and avulsion injuries) and surgical, thermal or chemical trauma. In all trauma induced (non-infective) tooth resorption, some damage to the cementum/cementoid-periodontal membrane complex has occurred which stimulates clastic activity. Trauma induced tooth resorption may be subdivided into: (1) surface resorption; (2) transient apical internal resorption; (3) pressure resorption; (4) orthodontic resorption; and (5) replacement resorption (Heithersay 2007).

10.6.2.2 Infection Induced Tooth Resorption

The response of the dento-alveolar apparatus to infection is characterized by inflammation, which may result

in tooth resorption. This may be a consequence of infective endodontic pathosis alone or superimposed on trauma induced resorption. These infection induced resorptions, which are generally termed inflammatory root resorptions, may occur as internal resorptions, external resorptions or combined internal-external lesions. *Internal inflammatory resorptions* may be classified according to location as: (1) apical and (2) intraradicular. *External inflammatory root resorption* occurs when infection is superimposed on a traumatic injury – usually following replantation of an avulsed tooth or a luxation injury. Nevertheless, it can also be induced in some cases of endodontic pathosis. *Communicating internal-external inflammatory resorption* is created where resorption has extended from an internal inflammatory resorption to involve the external surface. This can be recognized radiographically by a radiolucency within the tooth structure, extending to the exterior surface and the surrounding bone (Heithersay 2007).

10.6.2.3 Hyperplastic Invasive Tooth Resorptions

The third group of dental resorptions is insidious in nature and generally present complex therapeutic challenges. In these cases, resorbing tissue invades the hard tissues of the tooth in a destructive, and apparently uncontrolled fashion, akin to the nature of some fibroosseous lesions such as fibrous dysplasia. An important distinguishing factor for this third group of resorption is that, unlike the first two types of resorption, simple elimination of the cause of the lesion is ineffective in arresting their progress. Hyperplastic resorptions may be subdivided into internal replacement (invasive) resorption, invasive coronal resorption, invasive cervical resorption and invasive radicular resorption (Heithersay 2007).

A clinical classification has been developed by Heithersay (2004) for the invasive cervical resorption, both for research purposes and also to provide a clinical guide in the assessment of cases of invasive cervical resorption. *Class 1* Denotes a small invasive resorptive lesion near the cervical area with shallow penetration into dentine. *Class 2* Denotes a well-defined invasive resorptive lesion that has penetrated close to the coronal pulp chamber, but shows little or no extension into the radicular dentine. *Class 3* Denotes a deeper invasion of dentine by resorbing tissue, not only involving the coronal dentine but also extending into the coronal third of the root. *Class 4* Denotes a large invasive resorptive process that has extended beyond the coronal third of the root (Heithersay 2004).

10.7 Root Dentin Hypersensitivity

Dentine hypersensitivity has been defined as a short and sharp, painful response to an external stimulus applied to exposed dentine (Chabanski and Gillam 1997; Holland et al. 1997). The term "dentin hypersensitivity" has probably been used most widely in the literature to describe this common and painful dental condition. Several other terms, such as dentine sensitivity, pulpal sensitivity, tooth sensitivity, cervical sensitivity, tooth hypersensitivity, have also been used (von Troil et al. 2002; Holland et al. 1997). A clear differentiation should be established between dentin hypersensitivity and root sensitivity. The latter term is used to describe the sensitivity associated with periodontal disease and therapy (Sanz and Addy 2002).

The prevalence of root sensitivity in the adult population varies considerably (von Troil et al. 2002; Rees and Addy 2004). The figures for self-reported root sensitivity range between 9 and 52%, and are higher than those determined by clinical testing, usually performed by air or mechanical stimuli. The clinically determined figures range between 14 and 18%. The prevalence figures also seem to depend on the patient source. In some studies, the subjects were recruited among persons attending general practices, or were randomly selected (von Troil et al. 2002).

It is well established that following initial cause related therapy, root sensitivity is common, especially if treatment involved surgical procedures. The intensity of root sensitivity increases during the few weeks following nonsurgical periodontal therapy and then decreases (Sanz and Addy 2002). Reports on root sensitivity during supportive periodontal therapy vary from 15 to 98% and are often associated with root surface exposure and gingival recession (Chabanski et al. 1997; Karadottir et al. 2002; Taani and Awartani 2002). The very high prevalence of root sensitivity reported by Chabanski et al. (1997) was based on patients previously treated for periodontitis (Renvert and Persson 2004). When different surgical periodontal therapies were compared, it was revealed that postoperative

dentin hypersensitivity were associated significantly with age, type of therapy and higher scores on Corah's Dental Anxiety Scale. All surgical procedures (modified Widman flap, flap with osseous resection and gingivectomy), produced significantly more dentin hypersensitivity than did nonsurgical therapy (scaling and root planning) (Canakçi and Canakçi 2007).

Chronic trauma from tooth-brushing, acid erosion from the environment, gastric regurgitation or dietary substances, anatomical factors, gingival recession caused by periodontitis or periodontal surgery are some of the factors that have been implicated in etiology of dentin hypersensitivity (von in etiology of dentin hypersensitivity Troil et al. 2002; Dowell et al. 1985, Addy 1990; Chabanski and Gillam 1997; Walters 2005).

The physiopathological mechanisms behind dentine hypersensitivity are still poorly understood. It is suggested that the occurrence of sensitivity on denuded root surfaces following periodontal therapy may be a condition distinct from dentine hypersensitivity occurring after hydrodynamic stimulation because of bacterial penetration into dentinal tubules. Open dentinal tubules are the prime factors that determine the occurrence of root sensitivity as well as dentine sensitivity (Vaitkeviciene et al. 2006; Walters 2005).

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Occlusal Considerations in Pathogenesis of Periodontal Disease

11

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Periodontal disease is a multifactorial disease that affects only a limited number of people within a population. Our current understanding of periodontal disease is that it occurs in these susceptible people in the presence of multiple risk factors, such as bacterial plaque and smoking. Periodontal disease does not appear to be due to a single cause such as a specific bacterial species, but rather to be the result of multiple risk factors. This disease model is relevant to many chronic inflammatory diseases. Just like smoking, which does not cause periodontal disease but is a significant risk factor in the progression of periodontal disease, occlusal discrepancies also do not cause periodontal disease but may be a significant risk factor in their progression. Removing the risk factor of occlusal discrepancies through selective grinding and/or occlusal appliances during periodontal therapy has been shown to produce significant changes in the progression of the disease and improve the results of treatment of the inflammatory component of the disease (Harrel et al. 2006).

11.1 Trauma from Occlusion and Periodontal Disease

The International Workshop for the Classification of Periodontal Diseases and Conditions in 1999 (Ishikawa et al. 1999) evaluated the available materials relating to the effects of occlusion on the periodontium and the role played by occlusion in periodontal disease.

The following working definitions for the types of occlusal trauma were developed by the group:

Occlusal trauma. Injury resulting in tissue changes within the attachment apparatus as a result of occlusal force(s).

Primary occlusal trauma. Injury resulting in tissue changes from excessive occlusal forces applied to a tooth or teeth with normal support. It occurs in the presence of: (1) normal bone levels, (2) normal attachment levels, and (3) excessive occlusal force(s).

Secondary occlusal trauma. Injury resulting in tissue changes from normal or excessive occlusal forces applied to a tooth or teeth with reduced support. It occurs in the presence of: (1) bone loss (BL), (2) attachment loss, and (3) "normal"/excessive occlusal force(s).

Considerable energy has been directed in trying to determine the answers to these questions, because of the possibility that trauma from occlusion *might* contribute to the pathogenesis of periodontal disease. Research studies designed to examine the effects of occlusion fall into three categories (Davies et al. 2001):

- Human cadaver investigations.
- Animal studies.
- Human clinical studies.

11.1.1 Human Cadaver Investigations

Glickman and coworkers reported evidence of an altered pathway of destruction in studies utilizing human autopsy material and concluded that excessive occlusal forces in the presence of plaque-associated

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inflammation caused a change in the alignment of the periodontal ligaments, allowing an *altered pathway of inflammation/destruction*, resulting in vertical bony defects. Because there were two separate pathologic processes working together to cause bone loss, the process was termed a *"co-destructive" effect*. Summary of Glickman and coworkers indicated that excessive occlusal forces (trauma from occlusion) were a co-destructive force in the presence of gingival inflammation and could lead to vertical osseous defects. Based on these observations, the use of occlusal adjustment was advocated as part of the treatment for existing periodontal disease. Because no evidence existed that excessive occlusal forces initiated periodontal disease, occlusal adjustment to prevent periodontitis was not advocated (Hallmon and Harrel 2004).

Waerhaug (1979) evaluated a large number of human autopsy specimens to determine the relationship of subgingival plaque to the morphology of osseous defects and any association with the presence or absence of excessive occlusal forces. He found that the "*plaque front*" (i.e., the apical border of the subgingival plaque) was always in very close approximation to the epithelial attachment level and always followed the morphology of the bony defect. In addition, the relationship of the plaque level between adjacent teeth (either at the same or different apicocoronal levels) was associated with either horizontal or vertical interproximal BL. He also observed that excessive occlusal forces bore no relationship with the underlying bony defect, and that vertical defects were found equally around traumatized and nontraumatized teeth. Waerhaug concluded that BL was always associated with the downgrowth of plaque, and that there was no relationship between excessive occlusal forces and vertical BL (Hallmon and Harrel 2004).

The use of human autopsy material to study the effect of occlusal forces has the inherent problem that rarely if ever is there a true understanding of the patient's occlusal relationship that existed in life. Some knowledge can be obtained by studying the wear patterns on the teeth, but there is no assurance that the teeth actually occluded in the assumed manner or that the wear facets represent current and active occlusal trauma. Therefore, any conclusion or observations concerning the role of occlusal forces on the progression of periodontal disease, based on autopsy material has to be questioned (Hallmon and Harrel 2004).

11.1.2 Animal Studies

The most significant animal studies were performed in the 1970s by two research groups, one at Eastman Dental Center in Rochester, N.Y. (Kantor et al. 1976; Perrier and Polson 1982; Philstrom et al. 1986; Polson 1974; Polson and Zander 1983; Polson et al. 1974, 1976a, b, 1979) and the other at the University of Gothenburg in Sweden, (Ericsson and Lindhe 1982, 1977, 1984; Lindhe and Ericsson 1976, 1982; Lindhe and Svanberg 1974; Nyman et al. 1982, 1978; Svanberg 1974; Svanberg and Lindhe 1973, 1974) and they often are referred to as the American and the Scandinavian occlusal studies, respectively. Both evaluated the effect of occlusal trauma and gingival inflammation in animals. The American group used repeated applications of orthodontic-like forces on the teeth of squirrel monkeys, and the Scandinavian group used occlusal forces similar to those of a "high" restoration in beagle dogs. Both groups evaluated the effects of these traumatic occlusal forces in animals: those in which good oral hygiene was maintained with little gingival inflammation, and those in which a soft diet allowed the buildup of plaque and subsequent inflammation (Harrel 2003).

The *periodontal attachment level was* one of the three types (Davies et al. 2001):

- A normal healthy periodontal support.
- A healthy periodontal support but a reduced bone height. This is the experimental model equivalent of a postperiodontal therapy level.
- An active plaque-induced periodontitis.

The *type of force* that was applied to the animal tooth was (Davies et al. 2001):

- Either a jiggling force produced by multidirectional displacement of a tooth in alternating buccolingual or mesiodistal directions. This is usually created in the animal by the provision of a supraoccluding onlay.
- Or an orthodontic force created by a spring, which is a unilateral force that results in the deflection of the tooth away from the force.

The results of these studies were very similar despite the differences in the animal model and the manner of applying excessive occlusal forces. Excessive occlusal forces in the absence of plaque were found to cause loss of bone density and mobility of the tooth, but no evidence was found that excessive occlusal forces alone would cause attachment loss. When excessive occlusal forces were removed, it was found that the loss of bone density was reversible. In the presence of plaque, inflammation of the gingiva and periodontal supporting structures were noted, and in the presence of excessive occlusal forces and plaque together, there was an indication that more bone density was lost in both animal models. In the beagle dog model, there was evidence of attachment loss when both, plaque and excessive occlusal forces were present. This was not found in the squirrel monkey model (Harrel 2003).

These two series of studies were exhaustive in their evaluation of the relationship of occlusal forces and plaque in an animal model. Both studies concluded that there was no evidence that excessive occlusal forces alone caused loss of attachment. The studies by Lindhe showed that in special circumstances there may be attachment loss when both plaque and excessive occlusal forces were present. Both studies agreed that the control of plaque and gingival inflammation would stop the progress of periodontal disease in the presence or absence of excessive occlusal forces. These studies helped to establish that bacterial plaque is the initiating factor and the main cause for the progression of periodontal disease (Harrel 2003).

11.1.3 Human Clinical Studies

There is a paucity of studies evaluating the effect of occlusion in humans. This is due, in part, to ethical difficulties related to the nontreatment of diagnosed periodontal disease. In order to perform a controlled clinical trial, the gold standard of clinical research, it is necessary to compare treatment methods. However, in evaluating the combined effects of excessive occlusal forces and periodontal disease, it would be necessary to treat one group of patients, and leave the other group untreated. This approach would be ethically unacceptable due to the known deleterious effects of the nontreatment of periodontal disease. The World Workshop in Periodontics stated "prospective studies on the effect of occlusal forces on the progression of periodontitis are not ethically acceptable in humans." As a result, most occlusal studies in humans have been descriptive and/or retrospective in nature (Harrel 2003).

As Hallmon and Harrel (2004) reviewed, patients with occlusal discrepancies have less severe periodontal destruction than patients without occlusal discrepancies (Jin and Cao 1992; Pihlstrom et al. 1971; Shefter and McFall 1984). However, it was also revealed that patients who received occlusal adjustment as part of their periodontal therapy had greater attachment gain, than patients who did not receive occlusal adjustment (Burgett et al. 1992; Fleszar et al. 1980).

A series of retrospective reports on private practice patients (Harrel and Nunn 2001a, b; Nunn and Harrel 2001) provides strong evidence of an association between untreated occlusal discrepancies and the progression of periodontal disease, and shows that occlusal treatment significantly reduces the progression of periodontal disease over time, and can be an important adjunct therapy in the comprehensive treatment of periodontal disease.

Patients who had complete periodontal examination records, including occlusal analysis, that were recorded at least 1 year apart were divided into a group that had none of the recommended treatment (untreated n = 30), those who had only non-surgical treatment (partially treated n = 18), and a control group that had completed all recommended treatment (surgically treated n = 41). The data for each tooth of each patient, including occlusal status, were placed in a database and analyzed using the generalized estimating equations method.

Teeth with initial occlusal discrepancies were found to have significantly deeper initial probing depths ($P < 0.0001$), significantly worse prognoses ($P < 0.0001$), and significantly worse mobility than teeth without initial occlusal discrepancies. In addition, this association between initial occlusal discrepancies and initial periodontal condition was also found to hold good for various subsets considered, including posterior teeth only, and patients with good oral hygiene only (Nunn and Harrel 2001).

Worsening in overall clinical condition, as measured by worsening in prognosis, indicated that teeth with no initial occlusal discrepancies and teeth with

treated initial occlusal discrepancies were only about 60% as likely to worsen in overall clinical condition over time, compared to teeth with untreated occlusal discrepancies. Teeth with untreated occlusal discrepancies were also shown to have a significantly greater increase in probing depth per year, than either teeth without initial occlusal discrepancies or teeth with treated initial occlusal discrepancies ($P < 0.001$). In addition, teeth with untreated occlusal discrepancies had a significant increase in probing depth per year ($P < 0.001$), whereas teeth without initial occlusal discrepancies and teeth with treated initial occlusal discrepancies had no significant increase in probing depth per year ($P > 0.05$) (Harrel and Nunn 2001a)

Recently, Ishigaki et al. (2006) indicated that the chewing movements which deviated from the normal chewing movements increased the mobility of specific types of teeth, while Bernhardt et al. (2006) investigated the potential associations between dynamic occlusal interferences and signs of periodontal disease in posterior teeth, based on dental and medical measurements obtained from a 2,980 population-based sample (20–79 years of age) in the cross-sectional epidemiological study entitled, "study of health in pomerania" (SHIP). The presence of nonworking side contacts alone was significantly related to probing depth ($P < 0.0001$) and attachment loss ($P = 0.001$). The presence of nonworking side contacts and working side contacts on the same tooth was significantly related to increased probing depth ($P = 0.004$) but not the attachment level. The effect magnitude was a mean increase of 0.13 mm for probing depth and 0.14 mm in attachment loss. Known risk factors for periodontal disease that also showed significant associations with probing depth and attachment loss included male gender, age, smoking, education, and plaque score. Other factors significantly related to probing depth and/or attachment loss were tilted teeth, restored occlusal surfaces vs sound surfaces, elongated teeth, and tooth type (molar versus premolar).

11.2 Tooth Mobility/Fremitus

In cases of reduced periodontal attachment, teeth often show increased mobility (Schulz et al. 2000). Conventional methods for measuring tooth mobility are based on the application of a force to the crown of the tooth to assess the degree of tooth movement in the horizontal and vertical directions. Pathological mobility is defined as horizontal or vertical displacement of the tooth beyond its physiological boundaries. Normal physiological movement is thought to vary between 10 and 150 µm and would not be detectable on clinical examination.

Clinically detectable mobility indicates some change in the periodontal tissues (i.e., it is pathological) and the cause of the mobility needs to be diagnosed (Davies et al. 2001).

Tooth mobility can be recorded using *Miller's Index* (Davies et al. 2001):

1. Up to 1 mm of movement in a horizontal direction.
2. Greater than 1 mm of movement in a horizontal direction.
3. Excessive horizontal and vertical movements.

11.2.1 Manual Evaluation

Manual evaluation of mobility is best carried out clinically using the handles of two instruments to move the teeth buccally and lingually (Davies et al. 2001).

11.2.2 Fremitus

Fremitus is the movement of a tooth or teeth subjected to *functional* occlusal forces. This can be assessed by palpating the buccal aspect of several teeth as the patient taps up and down (Davies et al. 2001). Fremitus is graded as:

1. Movement felt but not visible.
2. Just visible perceptive movement.
3. Distinct visible movement (Crawford and Trevor Burke 2003).

11.2.3 Periodontometers

A periodontometer was a research tool used in the 1950 and 1960s to standardise the measurement of even minor tooth displacement (Mühlemann periodontometer). Till

date, this instrument has been used in a few clinical studies and has limited practical use (Davies et al. 2001).

11.2.4 Periotest®

Periotest (Siemens AG, Benssheim, Germany) is designed to evaluate mobility of tooth by detecting the damping capacity of periodontal ligament at the first time. Periotest is composed of a handpiece with a built-in metal slug that is advanced to the teeth using an electromagnet. Periotest measures the time required for the tapping head to make contact with a tooth using an accelerometer. The software in the instrument relates the contact time to tooth mobility (Oh et al. 2009). Several authors suggest that the Periotest could be an effective tool in assessing the state of the periodontium of traumatised teeth (Andresen et al. 2003), and for determining implant stability in clinics (Oh et al. 2009).

11.3 Effects of Occlusal Hypofunction on the Periodontium

Loss of occlusal function has been reported to induce atrophic changes in the periodontal ligament such as narrowing of the periodontal space, disorientation of collagen fibers (Kaneko et al. 2001; Afanador et al. 2005), reducing bone mineral density (Kunii et al. 2008) and significantly suppression of alveolar and jaw bone formation (Shimomoto et al. 2007). Decrease in proteoglycans, chondroitin sulfate, decorin, and heparan sulfate expression were evident in the periodontal ligament due to the loss of occlusal function. These results suggest that chondroitin sulfate, decorin and heparan sulfate are closely related to occlusal function, and play important roles in tissue homeostasis or tissue remodeling (Kaneko et al. 2001; Esashika et al. 2003).

Disorientation of the collagen fibers, proliferation of the connective tissue fibroblasts, and enlargement of epithelial intercellular gaps were observed in gingival tissue of rat molars with experimental occlusal hypofunction (Ishida et al. 2008; Johnson 1989; Deporter et al. 1982).

It was also revealed that occlusal stimuli induce cell proliferation of periodontal ligament cells by increasing IGF-1 and IGF-1 receptor expression (Termsuknirandorn et al. 2008).

11.4 Correlations Between Periodontal Disease and Biting Abilities

Biting ability, including biting force and occlusal contact area, is a useful objective measure of masticatory function. Biting ability is affected by the following factors: age, gender, number of teeth present, types of prosthesis, salivary flow, and temporomandibular joint disorder (Takeuchi and Yamamoto 2008). Mechanoreceptors situated in the periodontal ligament provide detailed information about intensive and spatial aspects of tooth loads, which support the neural control of masticatory forces. It was hypothesized that a reduced periodontal ligament due to periodontitis, and, thus, an altered mechanoreceptive innervation of the teeth, would affect masticatory behavior when subjects used incisors to hold and split food (Johansson et al. 2006). Several studies investigated the relation between periodontal condition and biting ability using strain gauge or pressure-sensitive sheets.

Kleinfelder and Ludwigt (2002) showed that reduced periodontal tissue support does not seem to limit bite force with maximal strength in natural dentitions. Bite force was assessed at 4 mm mouth opening in the premolar region without and following splinting of the posterior teeth and transduced using a strain-gauge (full-bridge circuit).

An epidemiological study using pressure-sensitive sheets in a Chinese adult population revealed that the mean clinical attachment level (CAL) showed a significantly negative correlation with biting force and occlusal contact area (Morita et al. 2003). No significant correlation was observed between the mean probing depth, percentage of pocket ≥4 mm, bleeding index and any of the biting abilities.

Similar results were reported by Alkan et al. (2006a) and Johansson et al. (2006) who showed that the biting ability of the subjects with healthy periodontia was

significantly greater than that of chronic periodontitis patients with reduced periodontal tissue support.

Monitoring the occlusal change using pressure-sensitive sheets has been reported to be useful for evaluating the treatment prognosis in periodontal surgery and biting ability in patients who had entered the maintenance phase of periodontal treatment (Alkan et al. 2006b; Takeuchi and Yamamoto 2008). The authors suggested that changes in bite force, bite pressure and the number of occlusal contact areas were not affected by periodontal surgery. However, mean mobility values and bite force were correlated. In patients with chronic periodontitis during the maintenance phase of periodontal treatment, multiple stepwise regression analysis showed that the total biting force and the occlusal contact area were positively associated with the number of teeth present, and negatively associated with mean CAL and mean probing pocket depth.

11.5 Pathologic Tooth Drifting or Migration

Pathologic tooth migration (PTM) is a common complication of moderate to severe periodontitis, and is often the motivation for patients to seek periodontal therapy (Fig. 11.1). Prevalence of PTM among periodontal patients has been reported to range from 30.03 to 55.8% (Brunsvold 2005).

The etiology of PTM appears to be multifactorial. Periodontal BL appears to be a major factor in the etiology of PTM (Table 11.1). Occlusal factors connected to the etiology of PTM include posterior bite collapse from loss of posterior teeth, Class II malocclusion, occlusal interferences, the anterior component of force, protrusive functional patterns of mastication, bruxism, and shortened dental arches. Soft tissue forces of the tongue, cheeks, and lips are known to cause tooth movement, and in some situations can cause PTM. Also considered important in the etiology of PTM is the pressure produced from inflammatory tissues within periodontal pockets. Because extrusion is a common form of PTM, clinical observations support the theory that eruption forces sometimes play a role in the etiology of PTM. Habits that have been associated with PTM include lip and tongue habits, fingernail biting, thumb sucking, pipe smoking, bruxism, and playing wind instruments (Brunsvold 2005). Classification of the pathology of tooth drifting was proposed by Inagaki (1999) (Fig. 11.2; Table 11.2).

Treatment of severe PTM often involves orthodontic therapy that is preceded by nonsurgical and surgical periodontal therapy and prosthodontic treatment. This interdisciplinary approach is very effective, but impractical for many adult dental patients. However, when PTM is in the initial stages and is localized, the

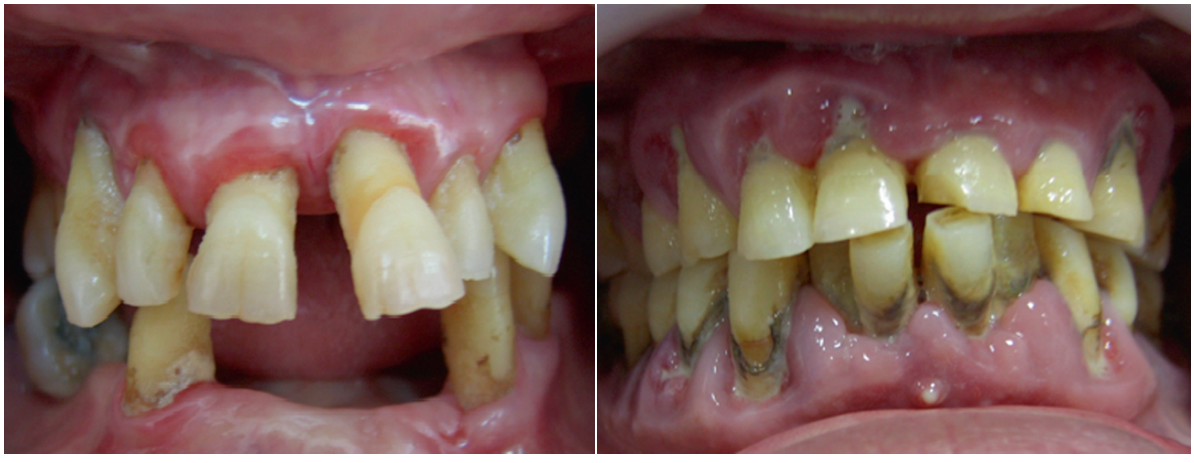

Fig. 11.1 Pathologic tooth migration (PTM)

Table 11.1 Summary of clinical studies evaluating destruction of periodontal tissues and PTM

Author, year	Study population	Periodontal evaluation	Findings
Martinez-Canut et al. 1997	852 periodontal patients (36.7% male, 63.3% female) whose ages ranged from 19 to 72 years (mean 42.5 ± 9.9)	Bone loss (BL), tooth loss, gingival inflammation according to gingival index	PTM was statistically associated with BL ($P < 0.001$), tooth loss ($P < 0.001$) and gingival inflammation ($P < 0.001$). The odds ratio indicated that PTM probability increased between 2.95 and 7.97 times as BL increased
Towfighi et al. 1997	343 patients with moderate to severe periodontitis	Clinical attachment level (CAL)	The mean CAL of migrated teeth (4.79 ± 0.28 mm) was significantly greater ($P < 0.0001$) than control teeth (3.21 ± 0.18 mm)
Costa et al. 2004	32 patients of both sexes (mean age: 46.0 ± 11.6 years) diagnosed with generalized chronic periodontitis and selected on the basis of the presence of pathologic migration in one or more anterior teeth	CAL and percentage of radiographic BL	However, greater severity of BL and CAL were observed in teeth with this type of migration (59.44% and 8.42 mm, respectively), and in teeth with facial flaring (45.17% of BL and 6.07 mm of CAL)

Table 11.2 Clinical diagnosis of PTM (Inagaki 1999)

Degrees of PTM	Clinical signs
Degree 1	A unilateral labial drifting and extrusion in the maxillary and mandibular anterior teeth creating diastema. It is weakened by loss of periodontal support and posterior deflective contact
Degree 2	Both maxillary and mandibular anterior incisors drift labially and extrude, creating facial flaring in the maxilla and crowding in the mandible
Degree 3	Reduction in periodontal support leads to further migration of the teeth and mutilation of the occlusion. The maxillary and mandibular anterior incisors continue to drift and extrude, creating severe facial flaring with crowding in the maxilla and extreme crowding in the mandible
Degree 4	Missing anterior teeth with advanced teeth migration. Occlusal disharmonies created by pathologic migration of teeth traumatize the supporting tissues of the periodontium and aggravate the destruction caused by the inflammation

treatment may be greatly simplified for the patient. Correction of PTM can be divided into four categories: (1) Extraction and replacement of migrated teeth when migration is very severe; (2) spontaneous correction of the early stages of PTM following periodontal therapy; (3) limited or adjunctive orthodontic therapy; and (4) conventional orthodontic treatment (Brunsvold 2005).

Gaumet et al. (1999) has evaluated the frequency of spontaneous repositioning of pathologically migrated teeth after routine periodontal therapy, and the relation between the severity of migration and the degree of repositioning following treatment. It was revealed that only after scaling and root planing, 48.5% of all the sites exhibited some degree of repositioning with 36.4% of all the sites closing completely. After surgery (6 months after baseline observations), 69.7% of all the sites exhibited some degree of repositioning with 51.5% of all the sites closing completely. When only small to moderate diastemata were considered (<1 mm), 77.8% of the sites closed completely (Figs. 11.3–11.5).

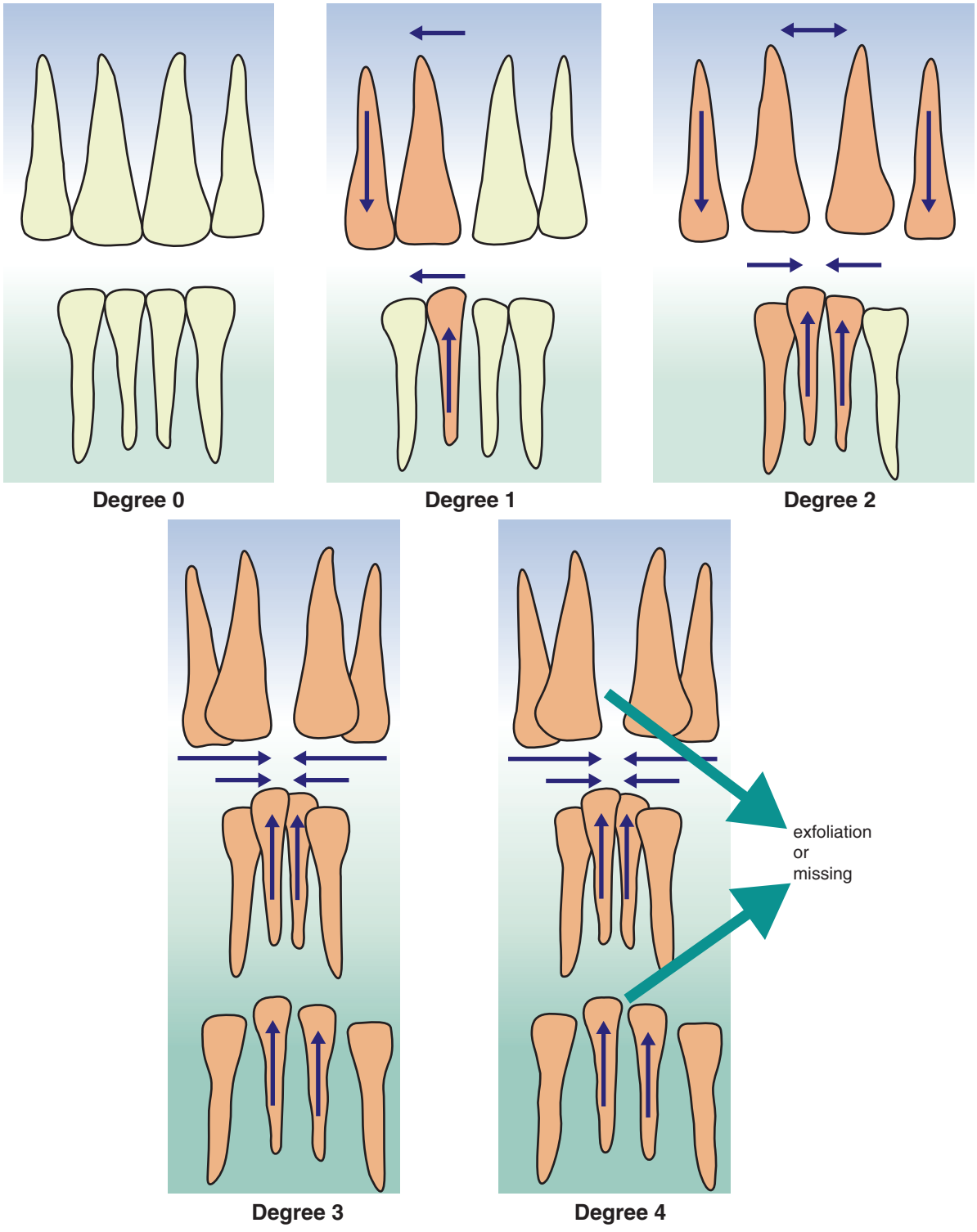

Fig. 11.2 Clinical diagnosis of PTM (Inagaki 1999)

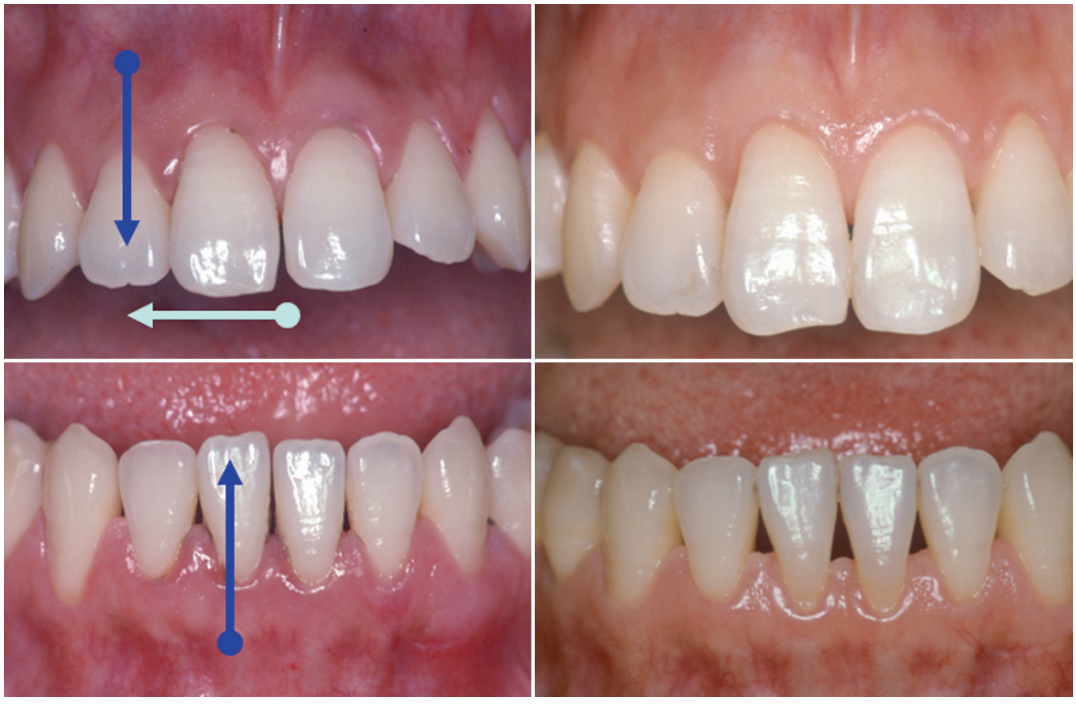

Fig. 11.3 Spontaneous correction of the degree 1 stage of PTM following periodontal therapy

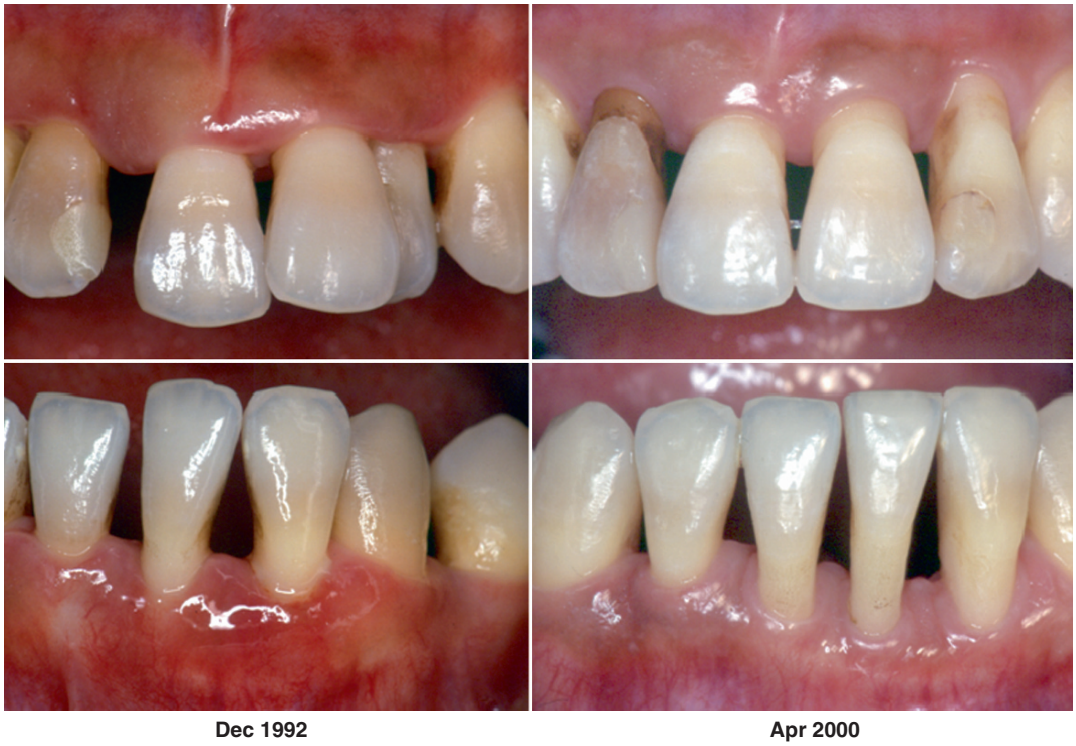

Fig. 11.4 Orthodontic correction of the degree 2 stage of PTM following periodontal therapy

Fig. 11.5 Orthodontic correction of the degree 4 stage of PTM following periodontal therapy

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Orthodontics and Periodontics

12

Alexandrina L. Dumitrescu and Koji Inagaki

The possibility of saving and rehabilitating a deteriorating dentition not only depends on the number of remaining teeth and their periodontal status, and the degree of destruction, but also largely on the morphology of the alveolar process and the position of the teeth within the alveolar process (Melsen and Agerbaek 1994).

The need of orthodontics as an integrated part of therapy is often related to the migration of teeth. Once the balance between forces acting on the teeth and the constraints of the periodontal tissues has been disturbed by either extraction of the teeth and/or reduction of the periodontium, an unstable situation arises. The migration may then lead to a change in function, which further aggravates the situation. At the same time, the migration, as well as the loss of teeth per se leads to a change in the alveolar process with respect to morphology and height (Melsen and Agerbaek 1994).

The question of whether orthodontic tooth movement may have deleterious effects on the periodontal tissues has been evaluated in a number of clinical and experimental studies. The results show that if periodontal health and proper oral hygiene standards are maintained during the phase of orthodontic therapy, no injury, or only clinically insignificant injury to the supporting tissues will occur. However, if the oral hygiene is less effective and periodontal inflammation is present during orthodontic treatment, the studies have indicated an increased risk of adverse effects on the periodontium. This is important to be remembered, if orthodontic tooth movements should be performed in areas with infrabony defects or in areas with reduced bone height (Thilander 1996).

12.1 Periodontal Tissue Response to Orthodontic Movement of Teeth with Periodontal Tissue Breakdown

Orthodontic forces per se are unlikely to convert gingivitis into a destructive periodontitis, but poorly executed orthodontic therapy in patients with periodontitis can easily lead to further periodontal breakdown. The combination of inflammation with occlusal trauma or dental movement will produce a rapid destruction of the support apparatus. The loss of alveolar bone in periodontitis patients results in an apical displacement of the center of resistance of the involved teeth, making bodily movement (translation) very difficult. The subsequent effect is that the teeth become prone to tipping. In addition to these mechanical difficulties, the formation of a hyalinized zone adjacent to a periodontally compromised tooth can be deleterious, since regeneration of the PDL does not occur in the presence of a bacterial infection, resulting in extensive loss of alveolar bone. Thus, in case of a deep periodontal infection, teeth should be moved only after proper periodontal therapy has been performed, and deep infection has been eliminated (Cardaropoli and Gaveglio 2007). This implies a great emphasis on oral hygiene instruction, appliance construction, and periodic checkups throughout the treatment. The orthodontic appliance has to be properly designed. It must provide stable anchorage without causing tissue irritation, and must be aesthetically acceptable. For physiologic reasons, bonded plastic or ceramic brackets are preferred in the most visible regions, generally for the six maxillary anterior teeth,

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whereas stainless steel, or gold-coated attachment are most commonly used elsewhere in the mouth. To counteract the tendency of orthodontic appliances to increase the accumulation of plaque on the teeth, attempts should be made to keep these appliances and mechanisms simple, and to avoid hooks, elastomeric rings and excess flash around the bracket base. The use of steel ligatures is recommended on all brackets and bonds are preferable to bands (Zachrisson 1997).

In an animal model, Ericsson et al. (1977) demonstrated that it was possible to shift a supragingivally located plaque into a subgingival position by orthodontic tooth movement. The mesio-apical movement of the plaque infected teeth resulted in the formation of infrabony defects characterized by the presence of a pocket epithelium, a large supra- and infrabony inflammatory cell infiltrate, and an angular widening of the marginal periodontal ligament (angular bony defect). In four out of five test dogs, the movement of plaque infected tooth resulted in an apical shift of the connective tissue attachment. In the control regions, where the teeth were maintained free from plaque during the experimental period, similar tooth movements did not result in the formation of infrabony defects. Hence, despite the fact that the dento-gingival epithelium of the control, tooth regions became interposed between the alveolar bone and the root surface, it maintained the character of a junctional epithelium.

From a clinical point of view, in many patients with a periodontally involved dentition, the migration of the anterior teeth leads to spacing and eruption, resulting in serious functional and esthetic problems. In these cases, orthodontics can be a reliable therapeutic treatment, because it does not result in a decrease of the marginal bone level, provided gingival inflammation is controlled (Fig. 12.1) (Cardaropoli and Gaveglio 2007). Boyd et al. (1989) showed that tooth movement in adults, with normal periodontal tissues, and with reduced but healthy periodontium, does not result in further significant periodontal breakdown or tooth loss.

Proper and timely combination of orthodontic and periodontal treatment has been shown to improve reduced periodontal conditions, suggesting the possibility of a long-term success. Best results are obtained when orthodontic movements are performed with light forces, and the line of action of the force is passing close to the center of resistance. The central point for the effectiveness of such treatments is in the ability of the procedure used to remove the subgingival plaque and calculus from the root surface. Following these guidelines, orthodontic treatment is no longer contraindicated when a major marginal bone loss has occurred due to periodontal disease. The treatment of patients with severe periodontal disease is now being performed with interdisciplinary teamwork between the orthodontist and the periodontist, to improve the possibilities of saving and restoring a deteriorated dentition (Cardaropoli and Gaveglio 2007; Ong and Wang 2002; Ghezzi et al. 2008).

Artun and Urbye (1988) evaluated the loss of periodontal bone support in 24 patients with advanced loss of marginal periodontium, orthodontically treated for pathologic tooth migration in one jaw. Treatment was limited to realignment of the front teeth in one arch, and tasted for an average of only 7 months. There appeared to be no association between initial bone loss and loss of periodontal bone support before or after orthodontic treatment (Artun and Urbye 1988).

It may be speculated that oral hygiene cooperation is critical to minimise further loss of periodontal bone support during orthodontic realignment of pathologically migrated teeth (Artun and Urbye 1988). Machen (1990) indicated that if the efforts of the dentist in improving oral hygiene care are unsuccessful the treatment should be terminated. Termination, if properly handled, will be more easily defended than permitting the condition to worsen. However, if proper procedures are followed, termination of care will be rarely needed (Machen 1990).

12.2 Periodontal Tissue Response to Orthodontic Movement of Teeth into Infrabony Defects

The effect of bodily movement of teeth into infrabony defect has been evaluated in animal models, in dogs (Wennström et al. 1993), monkeys (Polson et al. 1984; Geraci et al. 1990) and recently in rats (Nemcovsky et al. 2007).

Wennström et al. (1993) evaluated the effect of orthodontic tooth movement on the level of connective tissue attachment in sites with infrabony defects, i.e., angular bony defects with the plaque-associated inflammatory lesion and the dentogingival epithelium extending apical to the alveolar bone. In four beagle dogs, one premolar was moved away from the angular bony defect, and one premolar was moved into and through the angular bony defect. The applied force was 30–50 gm, and the active orthodontic tooth movement was continued

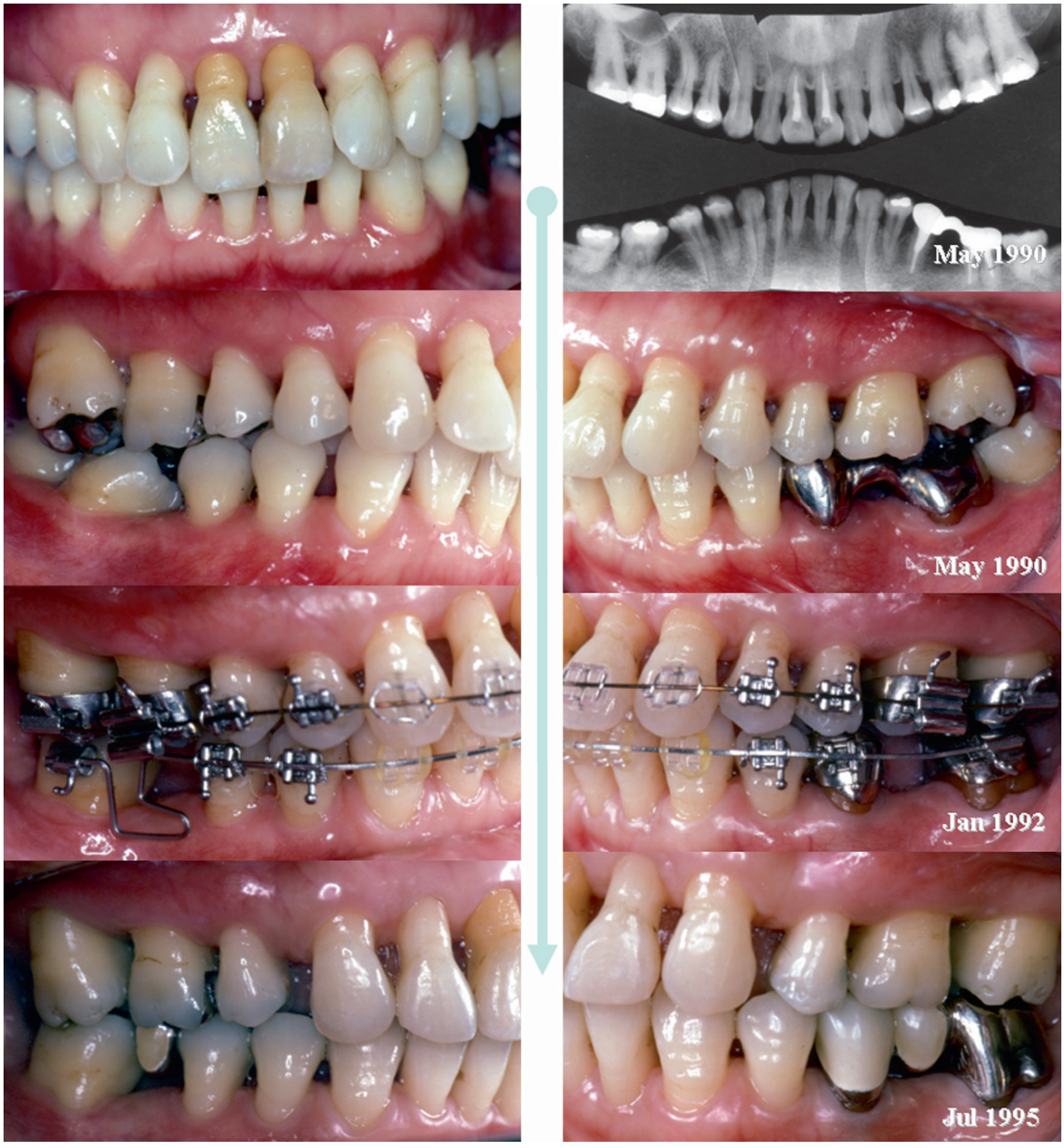

Fig. 12.1 Orthodontic treatment in a 40-year-old female patients with advanced bone loss and physiologic tooth migration after missing right first molar

for 5–6 months until a bodily tooth displacement corresponding to at least half of the mesiodistal width of the tooth had been obtained. At the termination of the active phase of orthodontic treatment, the appliance was used to retain the teeth in their new position for a period of 2 months. The results showed that orthodontic therapy involving bodily movement of teeth with inflamed, infrabony defects may enhance the rate of loss of the connective tissue attachment.

Polson et al. (1984) performed an experimental study in monkeys, where periodontal treatment was performed prior to the orthodontic tooth movement, and the animals were subjected to plaque control measures during the entire course of the experiment. It was

reported that even if substantial alterations of the osseous morphology occurred after such tooth movement in intrabony pockets, these alterations were not accompanied by any changes in connective tissue attachment levels.

In a rat model, it was suggested that a favourable orthodontic tooth movement on restraining epithelial apical down-growth and a decrease in pocket depth at the orthodontically treated side. Orthodontic tooth movement could not completely avoid formation of a long epithelial attachment along the planed root surface. Therefore, periodontal regenerative surgery prior to orthodontic tooth movement is indicated. Orthodontic tooth movement shortly after periodontal surgery produced no side-effects on periodontal soft tissue healing (Nemcovsky et al. 2007).

Clinical studies that have examined the effect of orthodontic movement in periodontal patients with infrabony defects also abound in the literature. A combined therapy, made of open flap surgery and orthodontic intrusion, resulted in the realignment of the treated teeth with radiological bone fill, gain in clinical attachment level and probing pocket depth reduction, confirming the possibility of achieving a final healthy periodontium (Cardaropoli and Gaveglio 2007; Corrente et al. 2003). It has been suggested that teeth can be successfully moved and intruded into bone defects previously augmented with bovine bone substitute and fibrin glue. During the orthodontic treatment, this combined augmentation material was able to be replaced by bone-like hard tissue. At the end of the therapy, an improvement in esthetics and periodontal health status was registered (Re et al. 2002a). Similar results were obtained after orthodontically moving the migrated teeth into infrabony defects augmented with a biomaterial: collagen bovine bone mineral. Reduction of probing pocket depths to physiologic values, clinical attachment levels gain, and radiologic defect resolution were obtained (Cardaropoli et al. 2006).

In conclusion, since orthodontic movement of teeth into inflamed infrabony defects may create a high risk for additional additional periodontal destruction, and because infrabony defects are frequently found at teeth that have been tipped and/or elongated as a result of periodontal disease, it is clinically essential that periodontal treatment with the elimination of the plaque-induced lesions is performed prior to orthodontic therapy. It is equally important that excellent oral hygiene is maintained throughout the course of the orthodontic treatment in patients with infrabony defects resulting from periodontal disease (Zachrisson 1997).

12.3 Periodontal Tissue Response to Orthodontic Movement of Teeth into Edentulous Areas with Reduced Bone Height

In patients with partially edentulous dentition, because of congenital absence, or the extraction of teeth, orthodontic treatment has to be performed often. By positioning the teeth toward, or into, the edentulous area, improved esthetic and functional results may be gained. In many of these individuals there is a reduced alveolar bone height (Thilander 1996).

Orthodontic forces, induced for bodily tooth movement, will result in different reactions in the periodontal tissues on the pressure and the tension side. Bone resorption occurs on the pressure side as a consequence of trauma-induced reactions within the periodontal ligament tissue, while on the tension side, a continuous bone apposition will be seen in a maintained width of the periodontal ligament (Lindskog-Stokland et al. 1993).

In a beagle dog model, Lindskog-Stokland et al. (1993) have shown that a tooth with normal periodontal support can be orthodontically moved into an edentulous area with reduced bone height with maintained height of the supporting apparatus, i.e., maintained connective tissue attachment level and in all essentials, maintained alveolar bone support. The newly established periodontal ligament exhibited a normal width both on the pressure and on the tension side of the displaced teeth. With regard to bone support, certain differences were noted between the tension and pressure side of the orthodontically moved teeth. On the tension side, i.e., at the mesial aspect of the root, both the original height and width of the supporting bone were fully maintained. On the pressure side, on the other hand, supporting alveolar bone was also present extending far coronal to the surrounding, experimentally created bone level, but not reaching the complete height and not the same width as the original supporting bone.

Hence it could be anticipated that, as long as orthodontic tooth movement is performed within the

genetically determined boundaries of the jaw, a tooth moved into a neighbouring edentulous area with markedly reduced bone height, will maintain the original height of the supporting apparatus, i.e., its connective tissue attachment level and its alveolar bone height (Lindskog-Stokland et al. 1993).

Although it was shown that if light forces are used and excellent oral hygiene is maintained, the results are encouraging, Zachrisson (1997) suggested that it is not wise to stretch the indications from tooth movement into constricted areas too far. Marked gingival invaginations are sometimes seen in such areas, and computer tomography analysis and human histological findings indicate that buccal or lingual bone dehiscences may occur, not being revealed by conventional radiography (Zachrisson 1997).

12.4 Periodontal Tissue Response to Orthodontic Movement of Teeth Through Cortical Bone

It is however well known that orthodontic tooth movement may also lead to displacement of the tooth outside the existing alveolar process, leading to resorption of the cortical bone with a dehiscence as a result (Melsen and Agerbaek 1994; Shiloah et al. 1987; Sarikaya et al. 2002; Naaman et al. 2004 Melsen and Allais 2005;).

After performing a labially orthodontic tooth movement in five monkeys, Steiner et al. (1981) reported an important loss of marginal bone and connective tissue attachment as well as gingival recession. Similar results were obtained by Wennström et al. (1987) who demonstrated that orthodontic therapy involving bodily movement of incisors and premolars may result in the recession of the gingival margin and loss of connective tissue attachment. The undesired side effects occurred in areas with gingivitis and in situations when the tooth was moved through the envelope of the alveolar process but appeared to be unrelated to the width of the zone of keratinized gingiva. In fact, the attachment loss noted was similar for teeth moved into keratinized gingiva and alveolar mucosa. It has been postulated that gingival recessions are frequently found at tooth surfaces, which are also associated with alveolar bone dehiscences. In other words, root dehiscences may establish an environment which, for one reason or the other, is also conducive for the loss of gingival tissue. If this assumption is correct, it would imply, with respect to orthodontic therapy, that as long as the tooth movement occurs exclusively within the alveolar bone, no apical shift of the gingival margin is likely to take place. If, however, the orthodontically-treated tooth is moved out of the osseous envelope of the alveolar process and a permanent dehiscence becomes established, there is a risk that recession of the gingiva may result. To some extent, this hypothesis was supported, demonstrating that gingival recession with concomitant attachment loss can occur when a tooth is moved, so that at least one of its surface breaks through the surface of the alveolar process (Wennström et al. 1987).

However, it was shown that when the teeth are moved back to their original position, bone tissue apposition will take place in the area of previous dehiscence. Furthermore, these tooth movements are not necessarily accompanied by loss of connective tissue attachment (Karring et al. 1982; Thilander et al. 1983).

The results of these studies indicated that the soft tissue, facial to a produced bone dehiscence, contains a bone matrix with the capacity to remineralize, following repositioning the tooth into the alveolar process. It may thus be speculated that genetic factors controlling the dimensions of the alveolar process may be the reason for the lack of remineralization of the bone matrix buccal to a tooth, which has been moved buccally out of the alveolar housing on the buccal side of the dental arch (Thilander 1996).

The clinical implications of these observations are encouraging. Bone dehiscences, which may occur due to uncontrolled expansion of teeth through the cortical plate may be repaired when the teeth are brought back or relapsed, towards a proper position within the alveolar process even if this occurs several months later (Zachrisson 1997) (Figs. 12.2–12.4).

12.5 Effects of Orthodontic Treatment on Periodontal Health

The effects of fixed orthodontic appliances on marginal periodontal tissue were analyzed predominantly by means of clinical and microbiological studies. Microbiological studies showed that, shortly after the insertion of bands, a qualitative and quantitative change occurs in the subgingival microbiological ecological system:

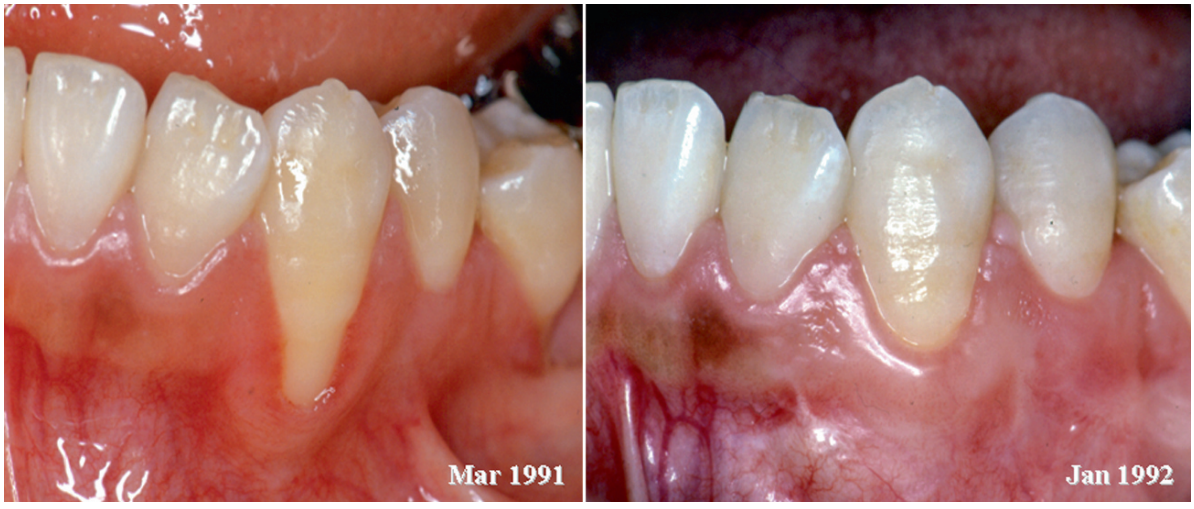

Fig. 12.2 Periodontal plastic surgery on tooth # 33 with Class I Miller recession was performed before orthodontic treatment (14-year-old girl patient)

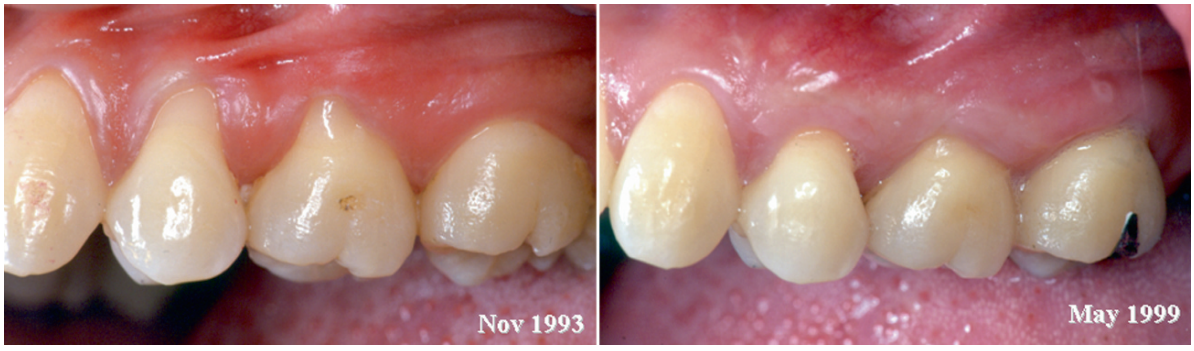

Fig. 12.3 Periodontal plastic surgery on tooth # 24 and # 26 with Class II Miller recession was performed after orthodontic treatment (22-year-old female patient)

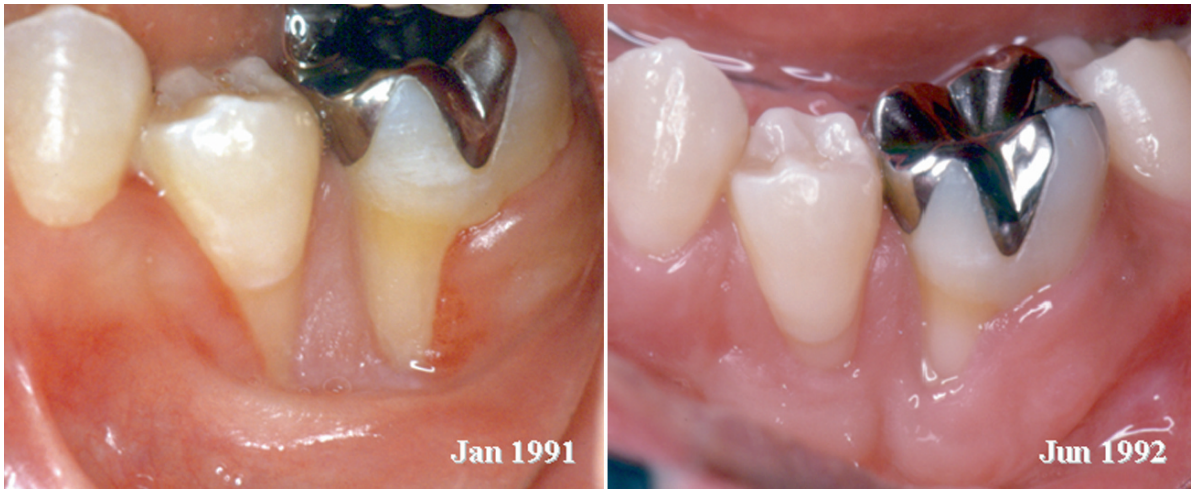

Fig. 12.4 Periodontal plastic surgery on tooth # 35 and # 36 with Class III Miller recession was performed before orthodontic treatment (14-year-old girl patient)

decreasing number of gram-positive cocci, whose presence is characteristic of a physiologically healthy sulcus, and an increasing number of periodontopathogenic microorganisms (spirochetes, fusiform bacteria, rods and gram-negative species: *Actinobacillus, Bacteroides, Prevotella*). Furthermore, the overall quantity of subgingival microbiological flora increased. The clinical outcome is often chronic gingivitis with inflammatory hypertrophy and pseudopockets (Diedrich et al. 2001).

Various reasons for these periodontal reactions were discussed: (1) subgingival plaque accumulation, (2) mechanical irritation due to subgingival band extension, and (3) cytotoxic effects of cement and/or band material (Diedrich et al. 2001).

A recent systematic review performed by Bollen (2008) identified the absence of reliable evidence describing the positive effects of orthodontic treatment on periodontal health. The existing evidence suggests that orthodontic therapy results in small detrimental effects to the periodontium. Weak evidence from one randomized study and 11 nonrandomized studies suggested that orthodontic therapy was associated with 0.03 mm of gingival recession (95% CI: 0.01–0.04), 0.13 mm of alveolar bone loss (95% CI: 0.07–0.20) and 0.23 mm of increased pocket depth (95% CI: 0.15–0.30) when compared with no treatment. The effects of orthodontic therapy on gingivitis and attachment loss were inconsistent across studies (Bollen 2008). It was suggested that the discrepancies between the studies might be due to the more complex etiology of gingival recession, in which orthodontic treatment is the only one factor in its development among others – e.g., periodontal phenotype (Slutzkey and Levin 2008) (Table 12.1).

More recently, Slutzkey and Levin (2008) revealed that in a group of 303 young patients (18–22 years old), prevalence, extent, and severity of recession were correlated with past orthodontic treatment: 27.4% reported orthodontic treatment 1–10 years before examination (average, 4.76 years). Of these, 22.9% showed gingival recession compared with 11.4% with no past orthodontic treatment ($P < 0.001$). A correlation was found between severity and extent of recession to past orthodontic treatment: 8.4% who reported past orthodontic treatment showed recessions of 3 mm or more, compared with only 0.9% with no past orthodontic treatment ($P = 0.007$). Also, 14.5% who reported past orthodontic treatment had three or more teeth with gingival recession compared with only 2.7% with no past orthodontic treatment ($P < 0.01$) (Slutzkey and Levin 2008).

A few histological investigations based either on animal findings (Ericsson et al. 1977) or on human gingival biopsies (Zachrisson 1972; Diedrich et al. 2001) revealed, after the insertion of bands, that the interdental gingiva of all the teeth presented the histological pattern of an established gingival lesion: leukocyte infiltration and inflammatory exudation in the area of the transseptal fibers, damage of the connective tissue attachment close to the cementoenamel junction on the mesial surface and proliferation of the pocket epithelium towards the apex, meaning progression from established gingivitis to an initial periodontal lesion (Diedrich et al. 2001).

12.6 Periodontal Tissue Reaction to Orthodontic Extrusion

The clinical crown lengthening procedure is a common technique with the following objectives: (1) exposure of sound tooth structure for the placement of restoration margins; (2) increase of clinical crown dimensions thereby improving retentive qualities; and (3) providing maintenance of "biologic width" between the new alveolar bone crest and the apical margin of the new restoration (Kozlovsky et al. 1988).

When a root is orthodontically erupted, the attachment apparatus and gingiva may follow to a further coronal position at the adjacent teeth. These periodontal changes represent a significant disadvantage, because, in order to restore the gingival and alveolar contour to the level of the adjacent teeth and to expose the required tooth structure, periodontal surgery follows the orthodontic procedure. To prevent the necessity for the surgical phase of crown-lengthening procedure after forced tooth eruption, a new technique of crown lengthening procedure, which combines controlled eruptive tooth movement with repeated circumferential intrasulcular incisions was proposed. Root-planing to the level of the bony crest should be carried out immediately after resection of the gingival and periodontal fibres. This technique overcomes the disadvantage of preventing coronal displacement of the gingiva and the attachment apparatus. An additional advantage is that the technique allows the direct inspection of extruding sound tooth structure (Kozlovsky et al. 1988).

The reaction of periodontal tissues after orthodontic tooth extrusion has been investigated in several animal

Table 12.1 Relationship between periodontal health and orthodontic treatment

Authors	Type of study	Population	Malocclusion severity	Orthodontic intervention	Periodontitis evaluation	Conclusion
Feliu 1982	Cross-sectional	158 participants (11–15 years of age)	NR	NR	Gingivitis and plaque indices	The patients who had received orthodontic treatment displayed superior oral hygiene to those dental patients who had not received orthodontic treatment
Ogaard 1988	Cross-sectional	98 individuals 19-year-olds	"Different forms of malocclusion"/NR	Fixed	Interdental alveolar bone level on bite-wing radiographs	Individuals subjected to orthodontic treatment, on an average, experience a small loss of marginal bone support, compared with untreated persons
Davies et al. 1991	Cohort 3 years	417 children with significant occlusal variations	Severe anterior crowding, crossbite or spacing, overjet, overbite	Fixed and/or maxillary removable (26%)	Plaque indices, bleeding indices	The children who had received orthodontic treatment had a greater reduction in the plaque and gingivitis scores, but this appeared to be more related to behavioral factors than to improved tooth alignment
Bondemark 1998	Cohort 5 years	Two groups of 20 adolescents, one treated and one untreated	Class II/no or minor	Fixed: In the upper arch with magnets and superelastic coils succeeded by straight-wire appliances in both arches	Interdental alveolar bone level on bite-wing radiographs	The treated group exhibited a statistically significant, larger increase of cementoenamel junction and the alveolar bone crest distance at the mesial surfaces of the first and second maxillary molars, than did the untreated group
Paolantonio et al. 1999	RCT	24 young systemically and periodontally healthy subjects	Malaligned and crowded teeth in the anterior sextants of both dental arches	Fixed: edgewise and passive archwire	Presence of plaque, gingival bleeding on probing and probing depth	The results showed that, during the period with orthodontic appliances, the presence of plaque scores and the gingival bleeding on probing scores were increased significantly
Thomson 2002	Cohort 14 years	452 Study members of the New Zealand cohort study	Definite, and severe or handicapping	Fixed and/or removable	Gingival recession, Attachment loss	There were no significant differences in caries experience, periodontal disease occurrence, or tooth loss between those who had and had not been treated
Allais and Melsen 2003	Cross-sectional	300 adult patients	Class I and II/malocclusion	Fixed: lower incisors, labial movement	Gingival recession	No significant difference (0.14 mm) between members of a pair
Janson et al. 2003	Cross-sectional	30 controls and 86 cases	Class II division 1	Fixed: simplified standard edgewise technique, the edgewise straight-wire system or bioefficient therapy	Heights of the alveolar bone crests	All treated groups had larger, statistically significant CEJ-AC distances than the untreated group primarily at the extraction areas
Levin et al. 2008	Cross-sectional	92 consecutive subjects who arrived for routine dental examination	NR	Fixed: with postorthodontic fixed retainers made of wire and composite resin bonded to the lingual/palatal tooth aspect	Plaque and gingival indices, gingival recession, probing depth, and bleeding on probing	Orthodontic treatment and fixed retainers were associated with an increased incidence of gingival recession, increased plaque retention, and increased bleeding on probing

studies (Berglundh et al. 1991; Schwimer et al. 1990; van Venrooy and Yukna 1985; Kajiyama et al. 1993).

A technique for orthodontic extrusion combined with resection of the supracrestal attachment fibers (fiberotomy) was recently proposed as an adjunct to certain restorative procedures by Berglundh et al. (1991). Using a beagle dog model, it was demonstrated that orthodontic extrusion combined with supracrestal fiberotomy resulted in a coronal displacement of the tooth and was associated with pronounced recession of the gingival margin and extensive loss of connective tissue attachment. The degree of gingival recession and the amount of loss of connective tissue attachment were, however less extensive than the amount of tooth extrusion. Thus, repeated fiberotomy obviously failed entirely to prevent coronal migration of the attachment apparatus (Berglundh et al. 1991).

To assure an aesthetic gingival marginal position following rapid extrusion with fiberotomy therapy, a gingival correction procedure may be necessary (Bach et al. 2004). After proper dentogingival relationships have been cultivated, the new tooth position should be stabilized to allow osseous remodeling. After 6–8 weeks stabilization, a provisional restoration is indicated to assure satisfactory esthetics and functional stability prior to permanent restoration (Schwimer et al. 1990).

Van Venrooy and Yukna (1985) evaluated in beagle dog, the orthodontic extrusion of teeth with advanced periodontal disease, showing that positive clinical and histological results can be achieved. The orthodontic tooth extrusion did not determine an apparent change in the level or shape of the alveolar bone crest around the extruded teeth. Rather, they appeared to have been avulsed with a broad apical periodontal ligament shadow and 80–90% of the root length coronal to the osseous crest. After 3 weeks of stabilization, extruded teeth had less cementum, but there was clear radiographic evidence of newly calcified bone, approximately 1.5–2 mm occlusal to the former bone crest (van Venrooy and Yukna 1985).

Kajiyama et al. (1993) has studied the accompanying movement of the gingivae after vertical tooth movements in monkeys. The findings demonstrated that neither clinical nor histologic problems were encountered in the gingival tissues if the teeth were extruded properly: (1) The gingiva moved in the same direction in which the teeth were extruded. The free gingiva moved about 90% and the attached gingiva moved about 80% as far as the teeth were extruded. (2) The width of the attached gingiva on the labial surface increased as the teeth were extruded. (3) The sulcus depth decreased about 20% of the distance that the teeth were extruded; the clinical crown height was increased to about 20%. (4) The mucogingival junction before the experiment, was positioned the same after the experiment. (5) There was no gingival migration, gingival pocket formation, or inflammation on the labial surface.

In-vivo data related to the accompanying gingival tissue movement after orthodontic extrusion of mandibular incisors were evaluated by Pikdoken et al. (2009). The widths of the attached gingiva and the keratinized gingiva, and the clinical crown length increased significantly after treatment. The position of the gingival margin and the mucogingival junction moved in the same direction as the teeth by 80 and 52.5%, respectively (Fig. 12.5). Clinically, increased gingival width can lead to a gummy smile in patients with a low lip line. Gingival corrective procedures cannot be performed in such cases due to the risk of root exposure.

It was demonstrated that tooth extrusion without fiberotomy might be effective to improve one- and two-wall wide isolated vertical infrabony defects (Iino et al. 2008; Ingber, 1974), and that orthodontic extrusive force can enhance the regenerative potential of guided tissue regeneration, to eliminate an intrabony defect and augment a ridge deformity (Ogihara and Marks 2006).

12.7 Periodontal Tissue Reaction to Orthodontic Intrusion

A common problem in adult patients suffering from periodontal disease is the migration, elongation and spacing of incisors. Disjunction of the equilibrium between the available periodontal support and the forces acting on the teeth may result in positional changes. This frequently leads to trauma from occlusion, a situation that might enhance destruction of the periodontium if plaque-associated inflammatory lesions of the gingiva are present. Anterior teeth are especially prone to elongation since they are not protected by occlusal forces and have no anteroposterior contact inhibiting migration (Melsen et al. 1989).

In cases of pathologic migration and extrusion, intrusive movement has been recommended to realign

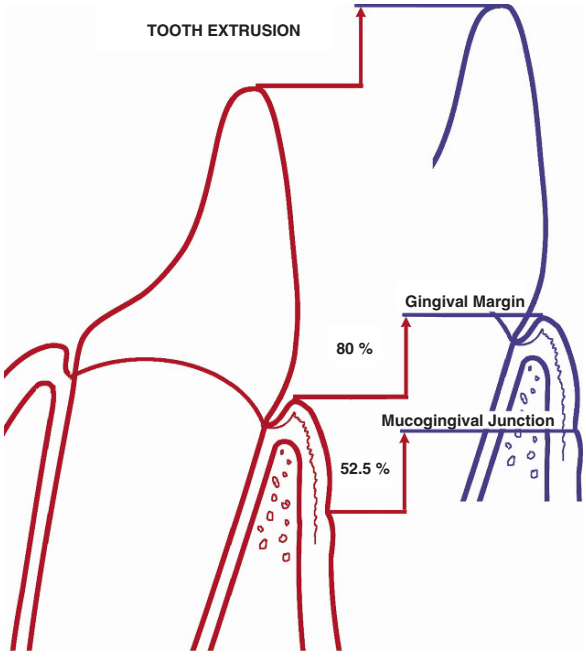

Fig. 12.5 Displacement rates of the gingival margin and the mucogingival junction compared with the amount of actual extrusion (Reprinted from Pikdoken et al. 2009, Copyright 2009, with permission from Elsevier)

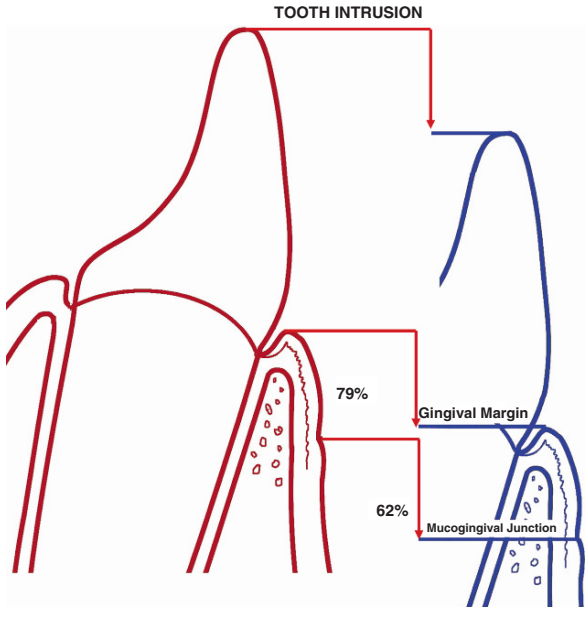

Fig. 12.6 Illustration of the displacement rates of gingival margin and mucogingival junction compared with the amount of actual intrusion. The gingival margin and the mucogingival junction moved apically in the same direction along with the tooth by 79 and 62%, respectively, of the total amount of dental intrusion (Reprinted from Erkan et al. 2007, Copyright 2009, with permission from Elsevier).

the teeth and improve clinical crown lengths and marginal bone levels in periodontally compromised patients (Melsen et al. 1989; Cardaropoli et al. 2001; Re et al. 2002b, 2004; Ferreira Mdo and Ferreira Rdo 2007; Erkan et al. 2007). The orthodontic intrusion does not lead to significant changes in the width of the attached and keratinized gingivae, when adequate plaque control is maintained. The gingival margin and the mucogingival junction moved in the same direction as the teeth by 79 and 62%, respectively (Erkan et al. 2007) (Fig. 12.6).

Animal studies on the reaction of healthy periodontal tissues to intrusive loads have been conducted (Michaeli et al. 1985; Melsen 1986; Murakami et al. 1989; Kanzaki et al. 2007; da Silva et al. 2008). It was shown that the continuous application of relatively low orthodontic loads during intrusion led to minor decreases in the height of healthy alveolar bone. According to the findings from histologic sections, the sulcus base and the mucogingival junction move in the apical direction along with the tooth by 60%, after orthodontic intrusion of maxillary incisors. Additionally, clinical crown length decreases by 40% of the orthodontic intrusion, the gingival sulcus deepens and the junctional epithelium constantly seats on the cementoenamel junction after orthodontic intrusion (Erkan et al. 2007; Murakami et al. 1989). However, the mechanism of alveolar bone crest remodeling during segmental molar intrusion is poorly understood. Kanzaki et al. (2007) hypothesized that bone resorption in the alveolar crest is caused by the pressure of the alveolar crest fibers from tooth intrusion. It was shown that the amount of intrusion was greater and the amount of alveolar bone resorption was smaller, if supracrestal fiberotomy was performed in dogs experiment (Kanzaki et al. 2007).

Bone is not necessarily the only hard tissue resorbed under orthodontic tooth movement. Root resorption involving cementum and dentin can be an unfavourable sequel to orthodontic procedures (Dermaut and De Munck 1986; Levander and Malmgren 1988; McFadden et al. 1989). The amount of resorption increases markedly with the duration of the force, and to a lesser extent, with the magnitude of the appliance activation (Harry and Sims 1982).

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