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REVIEW ARTICLE

Rapid implant therapies: immediate implant placement and immediate restoration

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During the last decade, the effectiveness of implant therapy has greatly improved, and the demands of dental esthetics in implant dentistry have become an important issue. The traditional two-stage implant protocol with delayed restoration has a treatment duration of 1–2 years, in which patients had to wear a removable appliance and experienced significant discomfort during the recovery period. Nowadays, immediate implant placement into an extraction site followed by immediate restoration of a dental implant can shorten the dental rehabilitation time and preserve patients' esthetic appearance at all stages of treatment. However, these treatment protocols always pose a great challenge to clinicians, especially when treating patients with preexisting soft and hard tissue deficiencies. The aim of this report is to present various treatment modalities to provide immediate tissue reconstruction and implant restoration following tooth extraction. With appropriate patient selection and careful clinical planning, these treatment strategies can lessen the number of surgeries required, condense treatment times, reduce discomfort to the patient, and accelerate the restoration process.

Introduction

Tooth replacement with a dental implant has proven to be a reliable and effective method of restoring edentulous dentition. Traditional dental implant placement protocols required preparation of the surgical site to establish intimate contact of the implant with the alveolar bone.[1] After tooth extraction, a 6-month healing period was recommended to allow bone to fill in the extraction socket before implant placement. In addition, a subsequent healing period of 3–6 months after fixture placement was indicated. Consequently, it usually took 1–2 years from the start of treatment to the completion of the restoration for most implant patients.[2] This protocol could leave patients without teeth or with an uncomfortable temporary prosthesis for a long time during implant therapy. In recent decades, implant treatment protocols have been challenged, and new approaches aim to shorten the overall treatment period as follows: (1) immediate implant placement in extraction sockets; (2) immediate restoration following implant placement; (3) immediate implant restoration in extraction sockets; and (4) immediate implant restoration and immediate tissue reconstruction.

The aims of this article are to describe the various options available for rapid implant therapies and expand the clinical considerations, limitations and outcomes of different treatment modalities.

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Meanwhile, we also introduce an integrated treatment approach for immediate restoration in patients with a narrow edentulous ridge.

Immediate implant placement in extraction sockets

The progressive involution of the alveolar bone begins following tooth extraction, and it is usually accompanied by reductions in both the quality and quantity of hard tissue. It was shown that major changes in an extraction site occur in the first 3–12 months after tooth extraction, and an estimated 50% decrease in buccolingual width was demonstrated.[3] Placing implants immediately after tooth extraction can eliminate the waiting period for socket healing and may reduce the bone resorption that normally occurs following the loss of a tooth.[4]

Although several longitudinal studies have shown that immediate implant placement after tooth extraction has a high clinical success rate exceeding 90%,[5–8] some clinical considerations must be addressed. Immediate implantation may be contraindicated in the presence of acute periapical or periodontal lesions.[9,10] The width of the peri-implant gap has a significant impact on the amount of bone-to-implant contact.[11,12] Localized bony defects surrounding implants may influence their primary stability and make it difficult to achieve an ideal prosthesis. To enhance the primary stability, implants installed immediately should be stabilized using the surrounding socket wall and bone beyond the original root apex.[13] It was shown that when the horizontal width of a peri-implant defect was <2 mm, the defect had the capacity to spontaneously heal and produce new bone formation when immediate implant placement was performed.[14–16] However, the gap between the implant and the socket wall can also be occupied by soft tissue.[14]

Recent studies indicated that immediate implant placement cannot completely preserve the entire bony wall surrounding an implant.[17,18] Less bone filling and a greater reduction in the vertical bone height of the buccal plate were noted with larger peri-implant gaps.[19] To enhance peri-implant bone healing and achieve an esthetic final outcome, the use of barrier membranes and/or different graft materials to fill in residual peri-implant defects has been widely documented.[20–22] Barrier membranes may prevent connective tissue and epithelium from invading the gap between the implant and the surrounding bone walls, thereby favoring bone regeneration.[23,24] Grafting materials can, moreover, act as a space maintainer and promote bone formation.[22,25,26] Many studies have shown the successful use of various graft materials, including autogenous grafts,[8,27] freeze-dried bone allografts,[6,28] xenografts[22,26] and synthetic bone grafts,[29,30] in the reconstruction of peri-implant defects in cases of immediate implant placement. However, few researchers have compared clinical outcomes among different graft materials for immediate implant placement. Recently, Hassan et al.[31] demonstrated less marginal bone loss with an autogenous bone graft than with a synthetic bone graft in immediate implant placement treatment. Additional controlled studies are needed to verify the effectiveness of these grafting materials. While the use of ePTFE non-absorbable membranes for immediate implant surgery showed a better space-making effect and encouraged more bone filling,[27,32] many surgeons have experienced high percentages (39–67%) of premature membrane exposure.[6,21,33,34] The exposed membranes can become contaminated by microorganisms,[35,36] which increases the risk of infection, and hinders bone regeneration of the defects.[37,38] Therefore, delayed-type immediate implant placement has been proposed to obtain better flap management for wound closure at extraction sites.[39] According to the delayed-type protocols, implants are placed several weeks after tooth extraction to allow soft tissue healing.[40,41] Delayed-type immediate implant placement exhibited a lower incidence of soft tissue dehiscence during guided bone regeneration compared with immediately placed implants.[42]

Immediate restoration following implant placement

One of the paradigms for successful implant therapy is a non-loading period of 3–6 months following fixture installation to achieve osseointegration. This waiting period is inconvenient for patients because of the delay in final restoration. Recently, techniques in which implants are placed with provisional restoration on the day of surgery have been developed.[43–45] With an immediate restoration protocol, patients require no additional surgery for implant uncovering procedures, and thus benefit from not having to wear removable or bonded provisional restorations during the treatment period.

Immediate restoration refers to immediate loading, in which prosthetic loading occurs within the first few days of implant placement. Such implants can remain unloaded during the initial healing period, especially in patients with a compromised bone condition.[46,47] Although many reports used the words "immediate loading" to describe immediate provisional prosthesis placement on the day of implant installation, the implants in most of those studies were not subjected to direct functional loading,

since the provisional restorations were carefully relieved of both centric and excursive occlusal contacts. It is, therefore, improper to describe these implants as being immediately loaded. A more accurate description for this circumstance would be "immediately restored", which is adopted in this article. Advocating an immediate restoration strategy requires adequate bone volume and a soft tissue contour to achieve primary stability of the implant and an optimal esthetic outcome of the implant prosthesis. Although the immediate restoration of implants can be highly successful,[48–50] such implants may fail in areas where the bone is soft and in patients with problems of wound healing. Patients with diabetes or habits of heavy smoking or bruxism need to be strictly screened.[51]

Recently, immediate restorations have been expanded and applied to the restoration of single missing teeth in the maxillary anterior region. These immediately restored prostheses can act as a scaffold to support the adjacent mucosa and papillae, thus facilitating the creation and maintenance of the soft tissue profile around implants. These immediate restoration cases with unsplinted implants had survival rates ranging from 80% to 100%. In those studies, some of the implants were placed in healed ridges,[52–54] while others were placed in immediate extraction sites.[55–57]

Immediate implant restoration in extraction sockets

To expedite the overall implant treatment course after tooth extraction, more recent efforts have focused on the feasibility of tooth replacement using immediate provisional implant restorations which are placed in extraction sites. Many early studies on immediate loading of restorations mostly dealt with the edentulous mandible, where the bone density is favorable, and it is possible to perform cross-arch splinting to minimize micromovement of the implant during the healing period.[46,58] Recently, some preliminary studies reported that such immediate restorations can also be applied to single-tooth replacement[56,57] and short-span partial edentulous rehabilitation.[58–60]

Obviously, in some patients, significant time can be saved and multiple clinic visits avoided by simultaneously extracting a tooth, placing an implant, and restoring the prosthesis. However, it has been demonstrated that immediately restored implants placed in fresh extraction sites carry a higher risk of failure.[55,58,61] Chaushu et al.[55] studied a group of 26 immediately restored single-tooth implants and found that three of 17 (17.6%) implants placed in extraction sockets failed, while all implants placed in healed ridges survived. They indicated that immediate restoration of single-tooth implants placed in fresh extraction sites carry an approximate risk of failure of 20%. Clinical investigations by Malo et al.[61] and Degidi and Piattelli[58] did not specifically report survival rates after implantation into extraction sockets versus healed ridges; yet, they noted that all implant failures occurred in cases of immediate implantation into extraction sockets. It has also been demonstrated that roughened-surface implants have higher implant survival rates than those of machined-surface implants.[62,63] To optimize results of such implants, the use of rough-surface implants is advised, and immediate restoration should only be performed in cases with primary stability. In these patients, the potential for implant micromovement is minimized by avoiding any centric and eccentric contacts.[54,58,59] In addition, a soft diet and extra oral hygiene care are recommended.[53,56]

Immediately restored implants placed in extraction sockets use a non-submerged protocol. Another adverse event associated with such an implant installation is undesirable recession of the peri-implant soft tissue. During the healing period of immediate implants placed in extraction sites, the buccal crestal bone undergoes remodeling and resorption, which may result in the buccal plate having insufficient height and/or thickness,[17,18] with subsequent soft tissue recession.[26,64] Chen et al.[26] placed 30 immediate transmucosal implants in maxillary anterior extraction sites, and 33.3% of the implants exhibited recession of the mucosa after 6 months. This problem is especially important for patients with a thin biotype. Since the buccal bony plate underneath the thin gingival tissue is also generally thin, it is prone to resorption following tooth extraction and implant surgical procedures. For a more favorable esthetic outcome, tissue reconstruction should always be considered in restoration of immediate extraction cases.[5,65]

Immediate implant restoration and immediate tissue reconstruction

Immediate restoration during implant placement is always a challenge for clinicians, because patients requesting implant treatment frequently present with an insufficient bony height and/or thickness following tooth removal. Numerous procedures have been devised to compensate for the narrow ridge of implant recipient sites.[66,67] A simultaneous or staged approach of implant installation with guided bone regeneration has extensively been used to create new bone.[2,68,69] When implant placement is combined with guided bone regeneration, two-stage implant surgery is highly recommended.

Although reports have shown clinical success in regenerating peri-implant defects when a non-submerged approach is used in conjunction with bone grafts or barrier membranes,[26,70,71] results are thought to be strongly dependent on the technical skills of the surgeon. For immediate implant restoration cases with a compromised ridge, a novel technique combining immediate temporization with simultaneous tissue reconstruction using ridge expansion and soft tissue augmentation to restore preexisting soft and hard tissue deficiencies was developed.[72] The major procedures consist of expanding the ridge during implant site preparation, harvesting a connective tissue graft for rebuilding the soft tissue contour in the edentulous area, and providing an immediate prosthesis. With ridge expansion, a set of tapered osteotome hand instruments is used to expand the narrow ridge and simultaneously create a site for implant installation. The use of an osteotome for implant site preparation can conserve all the remaining bone by pushing bone in front of the osteotome rather than removing valuable bone, which occurs when conventional drilling is performed. The osteotome technique expands the ridge in a gradual and controlled fashion until an accurate shape is attained. This shape widens the ridge in a gentle manner, such that a barrier membrane and bone graft may not be needed. By using the osteotome ridge-expansion technique, ridge augmentation and implant placement can simultaneously be performed. Therefore, immediate implant restoration can potentially be attained. This all-in-one integrated treatment approach (ridge widening, soft tissue augmentation, and provisional prosthesis delivery) simplifies the overall implant procedure, and can be adopted for patients with a narrow recipient ridge who request immediate implant restoration.

Conclusion

With careful patient selection and deliberate treatment planning, different strategies of immediate implant placement and/or immediate restoration have shown promising results in providing reconstruction of the dentoalveolar complex in a one-stage approach. These protocols benefit patients by reducing surgical procedures and the healing period, decreasing the time when they are toothless, and increasing patient acceptance of dental rehabilitation following tooth extraction.

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ORIGINAL ARTICLE

Concomitant upregulation of matrix metalloproteinase-2 in lesions and circulating plasma of oral lichen planus

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Background/purpose: Oral lichen planus (OLP) is a chronic inflammatory disorder characterized by a T cell-mediated immune response against epithelial cells. Matrix metalloproteinases (MMPs) are an important group of zinc enzymes and are thought to play an important role in the degradation of components of the extracellular matrix. The aim of this study was to assess the expression of MMP-2 and MMP-9 in both tissue specimens and circulating plasma.
Materials and methods: Twelve cases of OLP were collected. In addition, six cases with chronic inflammation and six normal subjects were also recruited. Oral tissue specimens were collected for measurement of MMPs by immunohistochemistry, and sera prepared from peripheral blood were used for gelatin zymography to determine MMP levels in plasma.
Results: The level of MMP-2 in patients with OLP was significantly higher than that in the other two groups, both in tissues and sera ($P<0.0001$). However, there was no difference in the expression of MMP-9 among these groups.
Conclusion: MMP-2 overexpression in OLP is consistent with its upregulation in peripheral serum. This result also indicates that MMP-2 might play a role in the pathogenesis of OLP.

Introduction

Oral lichen planus (OLP) is a chronic inflammatory mucosal disease of unknown origin which affects approximately 4% of the adult population, and more frequently affects middle-aged and elderly women.[1,2] Lesions are usually bilateral and mainly affect the buccal mucosa, gingiva, and lateral side of the tongue.[3] Clinically, OLP has a distinct morphology with two typical forms: atrophic-erosive lesions with or without concomitant reticular lesions, and reticular and/or plaque lesions.[4] A diagnosis of OLP is based on the presence of an epithelial-connective tissue interface band of infiltrating T lymphocytes, and the lesion is considered to be an autoimmune disorder mediated by T cells.[5]

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Usually, OLP has a chronic progression, and most cases exhibit inflammation. The levels of some molecules involved in mediating inflammatory processes were reported to be increased in this disease.[6] Among the effects of the inflammatory mediators, the induction of matrix metalloproteinases (MMPs) has been demonstrated.[7] MMPs are a large family of proteolytic enzymes, which are involved in degrading many different components of the extracellular matrix. They are associated with normal tissue remodeling, embryonic development, wound healing, angiogenesis, bone resorption, and inflammation.[8]

Histopathologically, a dense subepithelial band-like infiltration of lymphocytes and increased numbers of intraepithelial lymphocytes are found in OLP, with changes and disruption of the epithelial basement membrane.[9] Basement membrane damage, basal cell liquefaction, and tissue remodeling associated with OLP require degradation of the surrounding extracellular matrix. In the MMP family, gelatinase (MMP-2 and MMP-9) cleaves denatured collagens (gelatins) and type IV collagen, the major component of basement membranes.[10] Previous studies using immunohistochemistry showed that MMP-2 and MMP-3 were mainly found in OLP epithelium, while MMP-9 staining was found in the inflammatory infiltrate.[11]

To the best of our knowledge, no one has elucidated the circulating plasma expression of MMPs in OLP. There is no available information on the interrelationships of MMP-2 and MMP-9 expression in oral lesional sites and circulating plasma. Therefore, our aim was to explore the expression of MMP-2 and MMP-9 in both oral tissue specimens and sera from peripheral blood of OLP patients using immunohistochemistry and gelatin zymography.

Materials and methods

Patient selection

Formalin-fixed, paraffin-embedded OLP specimens were obtained from 12 consecutive patients (9 females and 3 males) at the Department of Oral and Maxillofacial Surgery, Buddhist Dalin Tzu Chi General Hospital, Chiayi, Taiwan. Patients with a history of any systemic disease were excluded from the study. Their ages ranged from 28 to 83 years (mean, 61.08±15.6 years). The diagnosis of each lesion was confirmed by a histologic examination of hematoxylin and eosin-stained sections. The pathologic diagnosis of OLP was established as described by Krutchkoff et al.[12] The types of OLP were subclassified into two clinical forms: reticular and/or plaque lesions (6 cases) and erosive lesions (6 cases). Normal controls (clinically normal oral mucosa) were obtained from six healthy volunteers (mean age, 32.66±10.2 years; range, 21–58 years). Disease controls consisted of six specimens of oral chronic ulcer (ulceration persisting for more than 1 month) (mean age, 66.66±4.3 years; range, 52–70 years). In addition, peripheral blood from eight OLP patients (four cases from each subgroup), four cases of chronic ulcer, and four cases of normal oral mucosa were collected at the same time. Written consent was obtained from all patients before taking specimens. The study protocol was approved by the Research Ethics Committee of our institution.

Immunohistochemistry

Immunohistochemical staining was performed using a peroxidase-labeled streptavidin-biotin technique.[8] Briefly, sections of 5 μm were deparaffinized and then heated in an autoclave for 40 minutes to retrieve the antigenicity before blocking with endogenous peroxidase. Following treatment with 3% hydrogen peroxide in methanol for 10 minutes to quench endogenous peroxidase activity, sections were incubated with goat polyclonal immunoglobulin G (IgG) anti-MMP-2 (sc-8835; 1:20 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-MMP-9 antibodies (sc-6840; 1:20 dilution; Santa Cruz Biotechnology), or leukocyte common antigen, a specific antibody for lymphocytes (Dako, Carpinteria, CA, USA; 1:50 dilution), for 30 minutes. After washing in 10 mmol/L Tris-buffered saline, sections were treated with donkey anti-goat IgG-HRP (sc-2020; 1:100 dilution; Santa Cruz Biotechnology) for 30 minutes. Final products were visualized by a diaminobenzidine (DAB) substrate kit (Liquid DAB+ Substrate Chromogen System, code K3467; Dako) for 5 minutes. Sections were then counterstained with hematoxylin, mounted with mounting medium, and examined by light microscopy. To demonstrate the specificity of staining, negative controls were included, in which the primary antibody was replaced with phosphate-buffered saline. A reddish-brown substrate in the cytoplasm indicated positive staining for MMP-2/MMP-9.

Each specimen was graded at 200× magnification as follows: +++, 50% to approximately 100%; ++, 25% to approximately 50%; and +, <25% of the proportion of positively stained cells in tissue sections.

Gelatin zymography

Serum samples were analyzed for MMP-2/MMP-9 with gelatin zymography as previously described.[13] Peripheral blood was first collected then centrifuged at 400*g* for 10 minutes at room temperature. The plasma and buffy coat layers were then separated.

An additional centrifugation at 1000*g* for 10 minutes was performed on the plasma fraction to remove remaining cellular debris and the plasma was then stored at −80ºC. For each serum sample, 10 μg of total protein was loaded onto precast sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) containing 0.1% gelatin. After electrophoresis, the gels were washed in 2.5% Triton X-100 twice for 30 minutes to remove all sodium dodecyl sulfate. The gels were then incubated in 50 mmol/L TRIS (pH 7.5), 5 mmol/L calcium chloride, and 1 mmol/L zinc chloride at 37ºC overnight. The gelatin cleavage rate from the photographed gels was analyzed with a densitometer (AlphaImager 2000; Alpha Innotech, San Leandro, CA, USA).

Statistical analysis

The JMP Statistical Discovery Software version 5.1.2 (SAS Institute, Cary, NC, USA) was used to analyze the data. Differences in immunohistochemical expression between groups were analyzed using Fisher's exact test. Gelatin zymography data were analyzed by one-way ANOVA. $P<0.05$ was considered to be statistically significant.

Results

Histologic examination of all OLP specimens showed typical findings of this disease, including stratified epithelium, inflammatory subepithelial T lymphocyte infiltration and, occasionally, a sawtooth-like appearance of the epithelium. The infiltrate consisted mainly of leucocytes, which were labeled with leukocyte common antigen (Fig. 1).

Immunohistochemistry results (Table 1) are shown in Fig. 2 for MMP-2 and Fig. 3 for MMP-9. Cells positively stained for MMP-2 were located at epithelial keratinocytes and fibroblasts, and lymphocytes of the subepithelial inflammatory infiltrate. MMP-9-positive cells were located at epithelial keratinocytes and inflammatory infiltrate lymphocytes. There was no difference in MMP-2 expression between the reticular and erosive types of OLP. When all OLP patients were included as one group, the level of MMP-2 in OLP subjects was significantly higher than that in the other two groups ($P<0.0001$). However, the percentage of positive staining for MMP-9 did not significantly differ among the groups ($P=0.5302$).

MMP-2 and MMP-9 activities in sera were assayed by gelatin zymography. The presence of MMP-2 and MMP-9 was indicated as bands of 72 and 92 kDa, respectively, as shown in Fig. 4. The quantitative measurements by the AlphaImager 2000 densitometer are shown in Fig. 5. The density of MMP-2 was significantly elevated in the two OLP subgroups, compared with those of the chronic ulcer group and normal control groups ($P<0.0001$). The levels of MMP-9 in sera, however, did not significantly differ between groups ($P=0.1417$).

Discussion

The first study investigating the relation of lichen planus and MMPs was reported by Giannelli et al.[14]

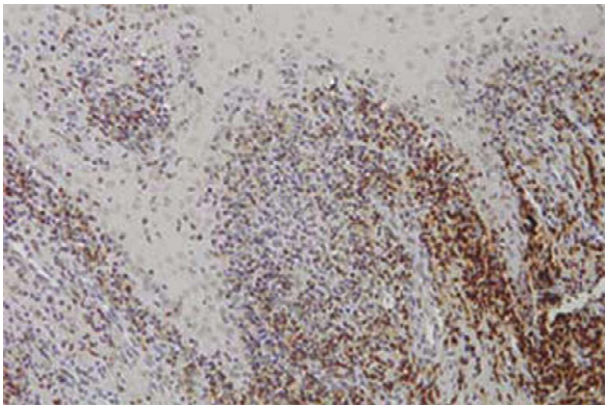

Fig. 1 Leukocytes in an inflamed subepithelial band of an oral lichen planus lesion labeled by leukocyte common antigen using a peroxidase-labeled streptavidin-biotin technique (original magnification ×200).

Table 1. Expression of matrix metalloproteinase (MMP)-2/MMP-9 in oral lichen planus (OLP) and control groups

Study group	MMP-2 expression*			P	MMP-9 expression*			P
	+	++	+++		+	++	+++	
EOLP	0	0	6		2	3	1	
OLP	0	0	6		0	5	1	
Chronic inflammation	0	5	1		3	2	1	
NOM	6	0	0	<0.0001†	1	3	2	0.5302†

*<25% (+), 26–50% (++), 51–100% (+++) of the proportion of positively stained cells in tissue sections; †Fisher's exact test comparing the EOLP and OLP groups combined, the chronic inflammation group and the NOM group. EOLP = erosive oral lichen planus; OLP = reticular and/or plaque oral lichen planus; NOM = normal oral mucosa.

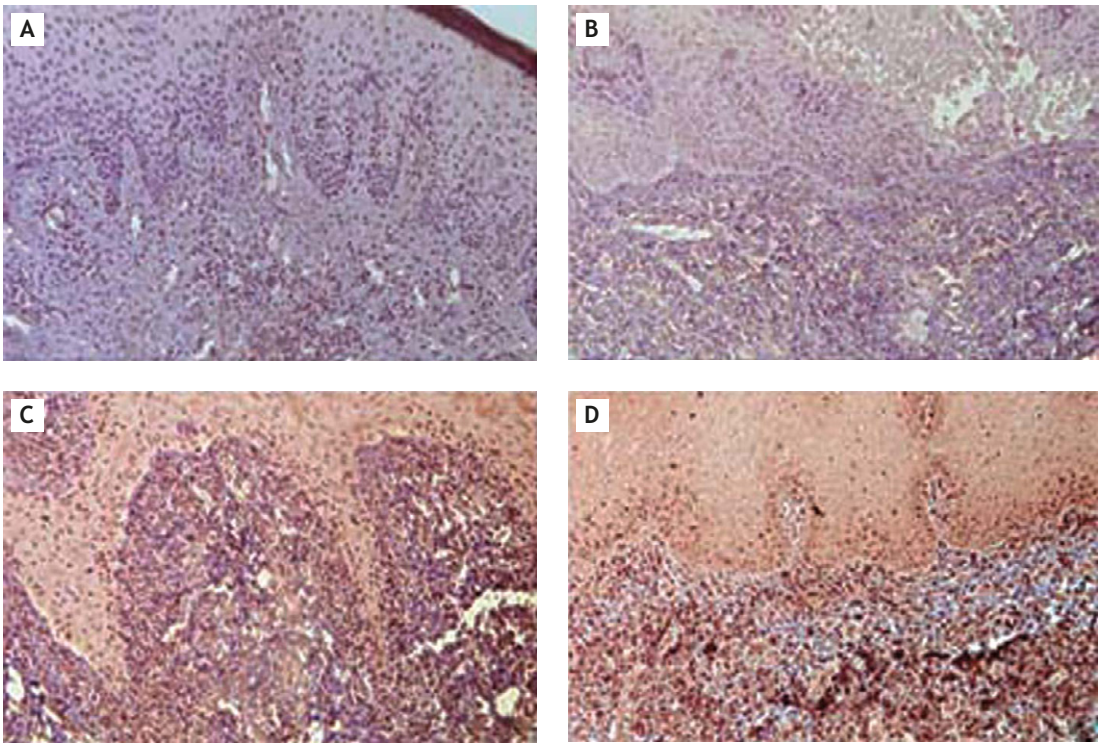

Fig. 2 Immunohistochemical reactivity for matrix metalloproteinase (MMP)-2 (original magnification ×200). (A) The degree of immunoreactivity to MMP-2 in normal oral mucosa was <25%. (B) The degree of staining in chronic inflammation was 25–50%. The staining pattern of MMP-2 was similar (>50%) among the (C) reticular and/or plaque lesions and (D) erosive lesions.

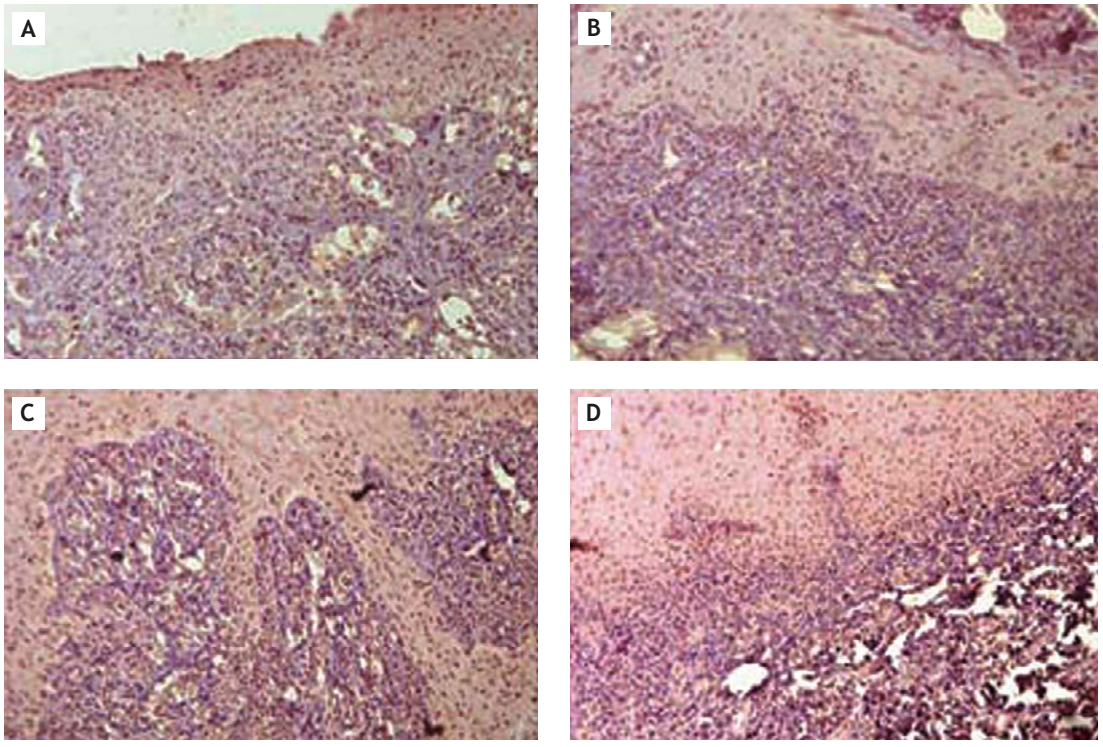

Fig. 3 Immunohistochemical reactivity for matrix metalloproteinase (MMP)-9 (original magnification ×200). (A) Expression of MMP-9 in normal oral mucosa. (B) Expression of MMP-9 in chronic inflammation. (C) Expression of MMP-9 in reticular oral lichen planus (OLP) (D) Expression of MMP-9 in erosive OLP. The intensity of immunostaining of MMP-9 varied among the four groups, and most of the positively stained cells were located at the subepithelial infiltrating lymphocytes.

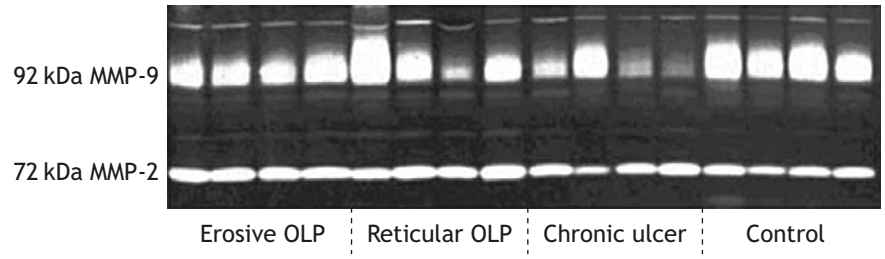

Fig. 4 Gelatin zymogram of matrix metalloproteinase (MMP). Gelatinolytic activity and molecular weight positions of MMP-2 (72 kDa) and MMP-9 (92 kDa) activities are indicated. OLP = oral lichen planus.

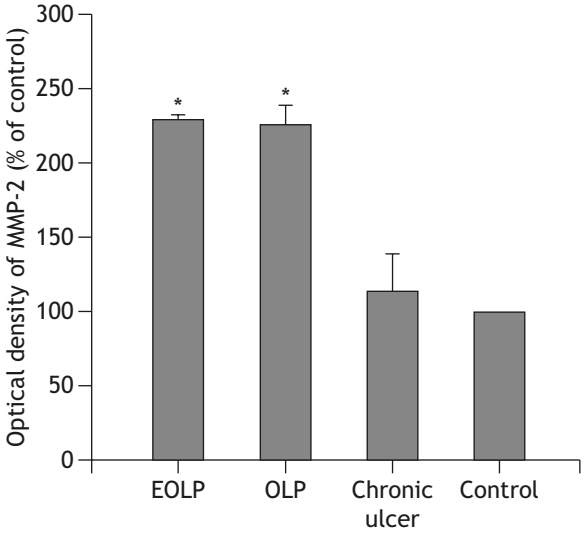

Fig. 5 Optical density values of matrix metalloproteinase (MMP)-2 were calculated from their gelatinolytic activities using an AlphaImager 2000 densitometer. The density of MMP-2 was significantly elevated in the two oral lichen planus (OLP) subgroups, compared with those of the chronic ulcer and control groups. *Significantly differs from the control value, at $P<0.05$. EOLP = erosive oral lichen planus.

They reported increased MMP-2 expression in acute stages of lichen planus and suggested that an altered balance between MMP-2 and tissue inhibitors of metalloproteinase-2 may play a role in the destruction of basement membrane. Zhou et al.[11] also reported MMP-2 expression in OLP epithelium. They suggested that MMPs may act synergistically to degrade the epithelial basement membrane in OLP. Here, we first demonstrated that MMP-2 overexpression in OLP lesions was due to MMP-2 upregulation in circulating plasma. Extracellular proteolysis is generally required for tissue injury in autoimmune and inflammatory diseases, and the plasminogen/plasmin system and MMPs cooperate in such processes.[15] Thus, basement membrane degradation during OLP progression may be mediated by MMPs.

A previous study showed that elevated expression of inflammatory cytokines, such as interleukin-1α and tumor necrosis factor (TNF)-α, was found in OLP.[16] Other studies also showed that MMP synthesis is triggered by tumor necrosis factor-α and interleukin-1α.[17,18] Our results indicated that MMP-2 was upregulated in both oral specimens and circulating plasma of OLP patients. MMP-2 overexpression in OLP may be related to inflammatory cytokines. The phenomenon of MMP-2 upregulation in circulation also implies that OLP might be a systemic disorder.

Kim et al.[19] reported that under upregulation by bone morphogenetic protein (BMP)-4, both MMP-1 and MMP-3 expression in OLP may induce epithelial cells acantholysis and lead to erosive changes. We found that the MMP-2 expression pattern in OLP did not significantly differ between reticular and plaque lesions, and erosive lesions. Thus, the expression of MMP-1 and MMP-3, but not MMP-2 or MMP-9, may be important in determining the phenotype of OLP.

In our clinical experience, some OLP cases were diagnosed by their clinical characterization but failed to match the histopathologic diagnostic criteria. Their pathologic diagnoses were often described as a "chronic ulcer" or "chronic inflammation". Our study revealed that the MMP-2 level in OLP was significantly higher than that in a chronic ulcer. This finding might provide a diagnostic tool for the differential diagnosis between OLP and chronic ulceration, regardless of whether immunohistochemistry or peripheral serum analysis is used.

In conclusion, we have demonstrated that MMP-2 overexpression in OLP lesions was consistent with MMP-2 upregulation in sera. The MMP-2 level of OLP subjects was significantly higher than that of patients with chronic ulcers and normal controls, not only in lesional sites but also in the circulation. Determination of the MMP-2 level in oral specimens and peripheral blood can be used as a tool for the differential diagnosis between OLP and chronic ulcers. This result also implies that upregulation of MMP-2 might play an important role in the pathogenesis of OLP.

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ORIGINAL ARTICLE

Upregulation of lysyl oxidase expression in cyclosporin A-induced gingival overgrowth

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Background/purpose: Lysyl oxidase (LOX) is involved in the initial steps of converting soluble monomers of collagen and elastin into insoluble fibers in the extracellular matrix. LOX was found to be upregulated in some fibrotic diseases. However, little is known about the correlation between LOX and cyclosporin A (CsA)-induced gingival overgrowth. The aim of this study was to compare LOX expression in normal healthy gingival tissues and CsA-induced gingival overgrowth specimens.
Materials and methods: Fifteen CsA-induced gingival overgrowth specimens and five normal gingival tissues were examined by immunohistochemistry. Three oral submucous fibrosis specimens were used as positive controls. In addition, one section from each CsA-induced gingival overgrowth specimen was stained with hematoxylin and eosin to evaluate the magnitude of inflammation at the histologic level. Differences in LOX expression between tissues with low and high levels of inflammation were subsequently analyzed using Fisher's exact test.
Results: LOX staining in gingival tissue was stronger in the CsA-induced gingival overgrowth group than in the normal gingival group ($P<0.05$). LOX staining was detected in the epithelium, connective tissue, inflammatory infiltrates, and endothelium. The LOX signal was mainly expressed in inflammatory cells (100%), followed by endothelial cells (93.3%), fibroblasts (80%) and epithelial cells (60%). In addition, LOX expression was significantly higher in CsA-induced gingival overgrowth specimens with higher levels of inflammatory infiltrates ($P=0.017$).
Conclusion: LOX expression was significantly upregulated in CsA-induced gingival overgrowth specimens. In addition, the expression of LOX increased with the grade of inflammation in CsA-induced gingival overgrowth.

Introduction

Gingival overgrowth is a common side effect of the chronic use of the immunosuppressive drug, cyclosporin A (CsA). The incidence of CsA-induced gingival overgrowth varies from 8% to 85% among studies, depending on the criteria used.[1–3] CsA-induced gingival overgrowth is characterized by thickening of the gingival epithelium as well as a marked increase in the extracellular matrix (ECM)

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of the gingival connective tissue.[4] Etiologic factors causing and underlying gingival overgrowth have been reviewed, and it was determined that local, systemic and genetic factors may contribute to its development and progression.[5,6] Recently, our studies showed that upregulation of plasminogen activator inhibitor-1[7] and cystatin C[8] may contribute to ECM accumulation in CsA-induced gingival overgrowth. However, the exact mechanism whereby CsA induces gingival overgrowth remains largely obscure.

Lysyl oxidase (LOX) is a secreted, copper-dependent oxidase that deaminates the 3-amino group of lysines in collagen and elastin. The resulting aldehydes condense to form cross-linkages between collagen and elastin monomers.[9,10] LOX catalyzes the oxidative deamination of lysine residues in elastin and collagens as an initial step in their extracellular assembly into insoluble fibers.[11] This has the effect of converting soluble monomers of collagen and elastin into insoluble fibers in the ECM.[12] Upregulation of LOX expression and increased LOX activity have been seen in a variety of fibrotic diseases such as liver fibrosis,[9] scleroderma,[13] renal fibrosis,[14] and oral submucous fibrosis.[15,16]

Previously, LOX protein expression was detected in phenytoin-induced gingival overgrowth tissues.[17] The findings suggest that LOX may play an important role in the pathogenesis of CsA-induced gingival overgrowth. On the basis of these observations, the present work was undertaken to identify the *in situ* localization of LOX expression in normal gingival tissues and CsA-induced gingival overgrowth specimens.

Materials and methods

Tissue collection

Normal gingival tissue samples were obtained from five healthy individuals undergoing routine surgical crown lengthening with little, if any, evidence of inflammation and who were not receiving any systemic medication. Fifteen hyperplasic gingival biopsy specimens were obtained from 10 renal transplant patients receiving CsA therapy. These patients had been taking CsA for more than 1 year, and the dose had been adjusted to maintain stable serum levels of about 200 ng/mL. No sign of graft rejection was detected in these renal transplant patients. The samples were obtained during surgical removal of diseased gingival tissue as part of their routine clinical management, which also included intensive plaque control. Institutional Review Board permission at Chung Shan Medical University Hospital was obtained for the use of discarded human tissue.

Immunohistochemistry

The surgically removed gingival tissues were fixed with 10% buffered formalin overnight, and the specimens were then dehydrated in an ascending series of graded alcohol and embedded in paraffin. Five-micrometer sections were stained with the monoclonal anti-LOX antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:100 dilution) using a standard avidin-biotin-peroxidase complex method.[7,8] Diaminobenzidine (Zymed, South San Francisco, CA, USA) was then used as the substrate for localizing the antibody binding. Three biopsy specimens of oral submucous fibrosis were used as positive controls.[16] Negative controls included serial sections from which either the primary or secondary antibodies were excluded. The preparations were counterstained with hematoxylin, mounted with Permount (Merck, Darmstadt, Germany), and examined by light microscopy.

One section from each CsA-induced gingival overgrowth specimen was stained with hematoxylin and eosin to evaluate the magnitude of inflammation at the histologic level. Each specimen was graded at 200× magnification as low (<50% inflammatory cells per field) or high grade (>50% inflammatory cells per field). Grading of each specimen was based on the average inflammatory condition in three consecutive microscopic fields, beginning from the epithelial-connective tissue border and proceeding gradually deeper into the lamina propria.

After immunohistochemical processing for LOX expression, sections graded as low were represented by <50% of positively stained cells, while sections graded as high exhibited >50% positively stained cells on three sections per tissue at 400× magnification. The same fields were used to grade inflammation and LOX staining.

Statistical analysis

Three replicates of each experiment were performed for each test. All assays were repeated three times to ensure reproducibility. Fisher's exact test was used to test for differences in LOX between normal healthy gingival tissues and CsA-induced gingival overgrowth specimens.

Results

Fig. 1 shows gingival tissue obtained from the normal gingival group with faint LOX expression, which was almost totally limited to the epithelium and endothelium. In the CsA-induced gingival overgrowth group, intensive LOX expression was mainly observed in the cytoplasm of fibroblasts, epithelial cells and

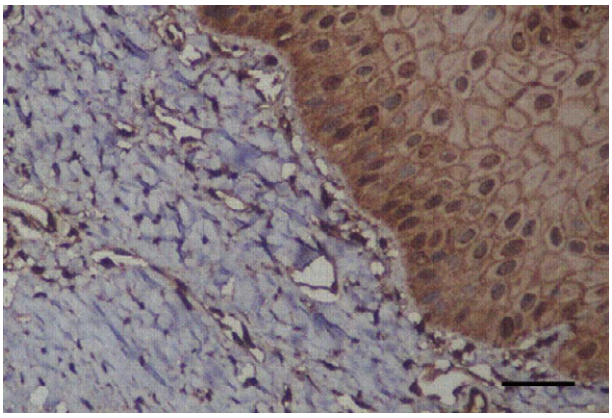

Fig. 1 Very faint immunoreactivity of lysyl oxidase was observed in normal human gingival tissues, and it was almost totally limited to the epithelium and endothelium (original magnification ×400). The bar represents 20 μm.

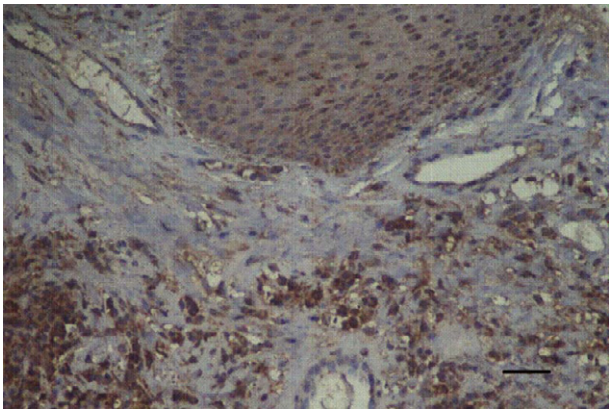

Fig. 2 Strong immunostaining for lysyl oxidase was noted in cyclosporin A-induced gingival overgrowth specimens. Lysyl oxidase was evident as an intense reddish-brown color in the cytoplasm of fibroblasts, epithelial cells, endothelial cells and inflammatory cells (original magnification ×200). The bar represents 20 μm.

inflammatory cells (Fig. 2). LOX staining of gingival tissue was stronger in the CsA-induced gingival overgrowth group than in the normal gingival group ($P<0.05$). The rank order of cells positively stained for LOX was found to be as follows: inflammatory cells (100%) > endothelial cells (93.3%) > fibroblasts (80%) > epithelial cells (60%).

LOX expression levels in CsA-induced gingival overgrowth specimens with either low or high levels of inflammation are given in Table 1. Differences in LOX expression between tissues with low and high levels of inflammation were subsequently analyzed using Fisher's exact test. Significantly greater LOX expression was noted in CsA-induced gingival overgrowth tissues with high levels of inflammation ($P=0.017$).

Table 1. Results of lysyl oxidase (LOX) expression and the grade of inflammation in cyclosporin A (CsA)-induced gingival overgrowth tissues

	Level of inflammation	
	High	Low
LOX expression*		
Low	1	4
High	8	2

*Significantly greater LOX expression was noted in CsA-induced gingival overgrowth tissues with high levels of inflammation compared with tissues with low levels of inflammatory cell infiltrates by Fisher's exact test ($P=0.017$).

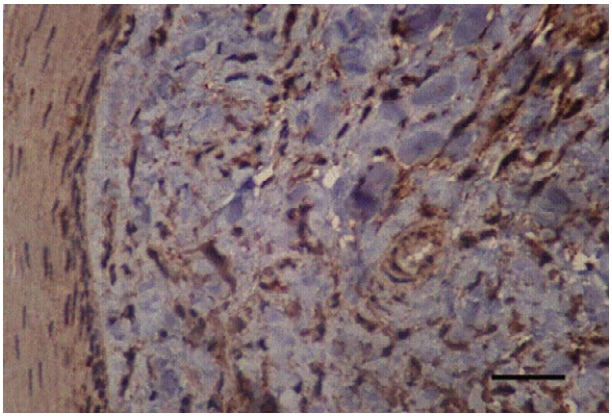

Fig. 3 Photomicrograph showing staining by a peroxidase-labeled streptavidin-biotin technique for lysyl oxidase in an oral submucosal fibrosis specimen which served as the positive control (original magnification ×400). The bar represents 20 μm.

Oral submucosal fibrosis specimens were used as positive controls. As shown in Fig. 3, LOX staining was detected in fibroblasts and endothelial cells.

Discussion

Besides the fact that the increase in the ECM is not well understood in CsA-induced gingival overgrowth, there is no evidence for expression of LOX in this type of lesion. In oral fibrotic disorders, the expression of LOX was found to be significantly upregulated in oral submucosal fibrosis[15,16] and phenytoin-induced gingival overgrowth tissues.[17] It is reasonable to speculate that LOX may be directly related to the pathogenesis of CsA-induced gingival overgrowth.

LOX, an extracellular enzyme, plays a key role in the post-translational modification of collagens and elastin, catalyzing inter- and intra-crosslinking reactions.[12] Because the crosslinked ECM is highly

resistant to degradative enzymes, it is thought that overexpression of LOX may cause severe fibrotic degeneration. Many reports have clearly demonstrated that LOX is consistently and dramatically upregulated in a variety of fibrotic diseases.[9,13–17] To the best of our knowledge, this is the first report of LOX expression being upregulated in CsA-induced gingival overgrowth specimens compared with normal gingival tissues. Strong immunostaining for LOX was detected in fibroblasts, epithelial cells, and inflammatory cells. LOX deposition is associated with CsA-induced gingival overgrowth, suggesting that it may play an important role in ECM turnover. From this phenomenon, we propose that CsA-induced gingival overgrowth may be due to the increased synthesis and deposition of ECM proteins, their altered degradation, or both.

Many studies have suggested that plaque-induced inflammation is associated with the onset or severity of drug-induced overgrowth,[18,19] and histologic findings have shown the presence of some level of inflammatory infiltrate in overgrown gingival tissues.[7,8] Here, the expression of LOX increased with the grade of inflammation in CsA-induced gingival overgrowth specimens. The LOX protein has consistently been found to be highly expressed in rat inflamed oral lesions *in vivo*, while normal non-inflamed periapical tissue contained no LOX-positive cells.[20] Our results suggest that CsA may predispose tissues to fibrosis via LOX overexpression in an inflammatory environment.

At present, no effective antifibrotic therapy is available that can be used for patients with CsA-induced gingival overgrowth. β-Aminopropionitrile is an irreversible inhibitor of LOX,[21] an extracellular enzyme that promotes crosslink formation in nascent fibrils of both collagen and elastin by conversion of lysine and hydroxylysine side chain residues into aldehydes. β-Aminopropionitrile was found to significantly decrease the collagen content in bleomycin-induced pulmonary fibrosis in rats.[22,23] Based on experimental evidence, anti-LOX activity may be suitable as an antifibrotic therapeutic target to prevent or delay CsA-induced gingival overgrowth.

As far as we know, this is the first attempt to evaluate the role of LOX expression in CsA-induced gingival overgrowth *in vivo*. We have demonstrated that LOX is elevated in CsA-induced gingival overgrowth compared with normal gingival tissues. LOX expression was significantly higher in CsA-induced gingival overgrowth specimens with higher levels of inflammatory infiltrates. CsA may predispose tissues to gingival overgrowth in inflammatory environments. More detailed *in vitro* and *in vivo* studies are needed to clarify the roles of LOX in CsA-induced gingival overgrowth in humans.

Acknowledgments

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ELSEVIER

ORIGINAL ARTICLE

Cytologic effects of primary tooth endodontic filling materials

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Background/purpose: Primary tooth endodontic filling materials should be biocompatible with periodontal tissue. The purpose of this study was to analyze the biologic effects of different endodontic filling materials for primary teeth on a human osteosarcoma cell line (U2OS).
Materials and methods: Experimental groups comprised different mixes of endodontic filling materials: zinc oxide-eugenol (ZnOE)+formocresol (FC); calcium hydroxide [$Ca(OH)_2$]+FC; $Ca(OH)_2$+iodoform+deionized water; $Ca(OH)_2$+iodoform+camphorated parachlorophenol (CPC); $Ca(OH)_2$+CPC; and Vitapex. These were prepared and used to fill special glass rings, which were subsequently eluted in 10 mL of cell culture medium at 37°C in a 5% carbon dioxide-in-air atmosphere for 24 hours. Cell culture medium alone was used as the control group. A DNA fragmentation assay was performed to determine the genotoxicity of each mix of materials. The level of cyclooxygenase (COX)-2 protein expression, the extent of dental material-elicited inflammation of U2OS cells, and the degree of mitogen-activated protein (MAP) kinase expression were determined using Western blot analysis.
Results: The results revealed that no DNA breakage was apparent after U2OS cells were treated with the various materials. COX-2 band expression dramatically declined in the ZnOE+FC group compared with the control group, although high levels of expression of the COX-2 band were noted for the $Ca(OH)_2$+FC and $Ca(OH)_2$+iodoform+CPC groups. Band levels of extracellular signal-regulated kinase (ERK-1 and ERK-2) expression declined in the ZnOE+FC and $Ca(OH)_2$+CPC groups compared with the control group. p53 and caspase-3 protein bands appeared in all experimental groups.
Conclusion: The cytotoxic mechanism of endodontic filling materials on U2OS cells was induced by means of activation of the p53 and caspase-3 apoptosis signaling pathways.

Introduction

Primary tooth endodontic filling materials are required to be resorbable and nontoxic to periapical tissues and the permanent tooth germ. According to reports by manufacturers of these materials, many different kinds of filling materials matching such requirements are currently available on the

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market. Such materials include calcium hydroxide [$Ca(OH)_2$], zinc oxide (ZnO), ZnO-eugenol (ZnOE) either with or without the incorporation of formocresol (FC), and iodoform pastes, such as Kri-1 paste (a mixture of iodoform and camphorated parachlorophenol [CPC]) and Vitapex paste [a mixture of 40.4% iodoform, 30.3% $Ca(OH)_2$, and 22.4% silicone]. The respective manufacturers all claim that these materials are biocompatible.

The use of ZnOE or ZnO to fill root canals of primary teeth was first described by Sweet in 1930. Such agents have been demonstrated to have antibacterial effects against pure cultures of certain bacteria, as reported by a number of studies.[1,2] However, Prashar et al.[3] reported that clove oil was highly cytotoxic to human skin cells at concentrations as low as 0.03% (v/v) with up to 73% of this effect being attributable to eugenol. Eugenol and FC components have been demonstrated to be toxic to cultured mammalian cells.[3,4] Similar cytotoxicity results were found in our previous work, and the addition of eugenol or FC to different endodontic filling materials revealed different degrees of toxicity toward a human osteosarcoma cell line (U2OS).[5]

The results of an animal study indicated that purified eugenol elicited less tissue necrosis and inflammation at all different exposure levels and for all different exposure times than was the case for commercially available eugenol. The degree of inflammation elicited by various ZnOE mixtures was strongly influenced by the quantity of free eugenol present in the mixture.[6] Eugenol compounds have various biologic effects including both antioxidant and anti-inflammatory activities.[7–10]

In 1997, Fuks et al.[11] compared the pulpal responses of baboon teeth to ferric sulfate and FC. Biologic outcomes of both tested chemical agents appeared to be equivalent 6 weeks after exposure to these materials, with 60% of the teeth from each test group showing mild inflammation. Cotes et al.[12] confirmed a similar inflammatory response of rat teeth after exposure to ferric sulfate and FC. Two studies of histologic impacts of FC upon dental pulp following pulpotomy showed chronic inflammation and necrosis of the radicular pulp,[13,14] while other animal models revealed the systemic distribution of FC from the pulpotomy site[15] and allergic and/or mutagenic properties of formaldehyde.[16]

The eukaryotic transcription factor nuclear factor (NF)-κB plays a primary role in general inflammation and the immune response.[17] NF-κB is a critical regulator of cyclooxygenase (COX)-2 expression in many different cell lines.[17,18] The intracellular signaling cascades controlling NF-κB activation are reported to be highly complex and involve a distinct set of kinases. Of the potential protein kinases involved in the activation of NF-κB, the activity of mitogen-activated protein (MAP) kinases, such as extracellular signal-regulated kinase (ERK), p38 and c-Jun N-terminal kinase/stress-activated protein kinase signaling pathways, have been well characterized.[19,20]

Our purpose was to analyze the biologic effects of different endodontic filling materials upon primary teeth. We attempted to evaluate the genotoxicity of these dental materials, their ability to stimulate an inflammatory reaction in test cells, and the possible mechanisms of their action in a U2OS.

Materials and methods

Materials and sample preparation

Following our previous investigation,[5] the experimental group comprised six different endodontic filling material formulations, as listed in Table 1. These different endodontic filling material formulations were: (1) 6 g ZnO + 1 mL eugenol + 1 mL FC; (2) 6 g $Ca(OH)_2$ + 1 mL FC; (3) 6 g $Ca(OH)_2$ + 0.6 g iodoform + 2 mL deionized water; (4) 6 g $Ca(OH)_2$ + 0.6 g iodoform + 2 mL CPC; (5) 6 g $Ca(OH)_2$ + 2 mL CPC; and (6) Vitapex (Neo Dental Chemical, Tokyo, Japan), a non-mixing type of material which was tested as

Table 1. Experimental groups and the composition of the experimental root filling materials

	Material	Composition	Dilute solution	Immersion time (hr)
Control group	Medium	McCoy's medium	NA	NA
Group 2	Vitapex [$Ca(OH)_2$ + iodoform + silicone oil]	0.5 g	1 mL McCoy's medium	24
Group 3	ZnOE + FC	6 g/1 mL/1 mL	1 mL McCoy's medium	24
Group 4	$Ca(OH)_2$ + FC	6 g/1 mL	1 mL McCoy's medium	24
Group 5	$Ca(OH)_2$ + iodoform	6 g/0.6 g	1 mL McCoy's medium	24
Group 6	$Ca(OH)_2$ + iodoform + CPC	6 g/0.6 g/2 mL	1 mL McCoy's medium	24
Group 7	$Ca(OH)_2$ + CPC	6 g/2 mL	1 mL McCoy's medium	24

$Ca(OH)_2$ = calcium hydroxide; ZnOE = zinc oxide-eugenol; FC = formocresol; CPC = camphorated parachlorophenol.

supplied by the manufacturer. Freshly mixed materials were placed in glass rings (2 mm in height, 6 mm in diameter) and allowed to set for 24 hours at 37°C in a humidified chamber. Five samples of each endodontic filling material were then eluted in 10 mL of cell culture medium for 24 hours at 37°C, in a 5% carbon dioxide-in-air atmosphere. Test materials were diluted by adding an appropriate volume of culture medium to achieve a final concentration of 5 μL/mL. Culture medium with no additional experimental material served as the control group.

DNA fragmentation assay

The DNA fragmentation assay was based on the method of Fady et al.[21] as described previously. Following treatment with selected endodontic filling materials, U2OS cells were lysed in 10mM Tris-HCl (pH 7.5), 100mM ethylenediaminetetraacetic acid (EDTA), 0.5% sodium dodecyl sulfate (SDS), and 100 μg/mL of proteinase K for a period of 18 hours at 37°C. DNA was then extracted twice with phenol/chloroform/isoamyl alcohol, precipitated in ethanol, centrifuged (30 minutes at 10,000*g*), and re-suspended in Tris-EDTA buffer containing ribonuclease at a concentration of 100 μg/mL for a period of 1 hour at 37°C. Following a second extraction in phenol/chloroform/isoamyl alcohol, and precipitation in 70% ethanol, the DNA was suspended in Tris-EDTA buffer, and 5–10 μg of DNA per lane was electrophoresed in a 1% agarose gel for 2 hours at a potential difference of 45 V. Gels were subsequently visualized following treatment with ethidium bromide.

Inflammatory protein and COX-2 protein evaluation

Using Western blot analysis, cell lysates derived from U2OS cell cultures were collected. In this assay, lipopolysaccharide was added to U2OS cells as a positive control. McCoy's medium was used as a negative control. The protein assay was performed as described in our previous study.[22] Briefly, U2OS cells were solubilized in SDS-solubilization buffer (1mM $MgCl_2$, 50mM Tris-HCl, 5mM EDTA, pH 7.5, 0.5% Triton X-100, 2mM phenylmethylsulfonyl fluoride [PMSF], and 1mM *N*-ethylmaleimide) for 30 minutes on ice. Following this, cell lysates were centrifuged at 12,000*g* and 4°C, and the protein concentrations were determined using Bradford reagent; bovine serum albumin (BSA) was used as the standard. Equivalent amounts of total protein per sample of cell extracts were run on a 10% SDS polyacrylamide gel electrophoresis (PAGE) assay and immediately transferred to nitrocellulose membranes. The membranes were then blocked with phosphate-buffered saline (PBS) containing 3% BSA for 2 hours, rinsed, and incubated with the primary anti-COX-2 antibody diluted 1:1000 in PBS containing 0.05% Tween 20 for a period of 2 hours. Following three washes with Tween 20 for 10 minutes each, the membranes were incubated for 1 hour with a biotinylated secondary antibody (polyclonal anti-rabbit immunoglobulin G [IgG] for COX-2) diluted 1:2000 in the same buffer used for washing. Then membranes were washed again as described above and treated with a 1:2000 streptavidin-peroxidase solution for 30 minutes. A β-actin antibody was used as the control for Western blotting. Following a series of three further washing steps, the extent of the immunologic reactions which had taken place was determined by diaminobenzidine. The relative intensities of the obtained bands were determined with a densitometer (AlphaImager 2000, Alpha Innotech, San Leandro, CA, USA).

Analysis of MAP kinases: ERK, p53, and caspase-3

After U2OS cells had been exposed to endodontic filling materials, culture plates were washed once with cold PBS. Five million U2OS cells were collected and lysed in 50 μL of lysis buffer (1% Triton X-100, 0.5% NP40, 10mM EGTA, 0.2mM Na_3VO_4, 0.2mM NaF, and 0.2mM PMSF) for a period of 30 minutes. Cell lysates were cleared at 15,000*g* for a period of 15 minutes at 4°C. Twenty-five micrograms of protein from each sample was collected and boiled for 5 minutes in 1×SDS gel-loading buffer (125mM Tris, pH 6.8, 5% glycerol, 28mM SDS, 1% β-mercaptoethanol, and 0.006% bromophenol blue). Proteins were separated by 12.5% SDS-PAGE and transferred onto polyvinylidene difluoride membranes. The membranes were blocked for 1 hour at room temperature in 3% BSA, 5% nonfat dried milk, 10mM Tris (pH 7.5), 100mM NaCl, and 0.1% Tween 20. Following four washes in TBS-T buffer (10mM Tris, pH 7.5, 70mM NaCl, and 0.1% Tween), the membranes were incubated with 0.5 μg/mL rabbit antibody overnight. Following four further washes with TBS-T buffer, the membrane was overlain with a second antibody for 1 hour, and then washed with TBS-T buffer for 20 minutes. The resultant films were scanned and quantified using a densitometer and the SCION image program (Scion, Frederick, MD, USA).

Results

Cell morphology

After treating U2OS cells with different endodontic filling materials for different time periods, there were changes in U2OS cell morphology, as presented in Fig. 1. U2OS cells in the ZnOE+FC group

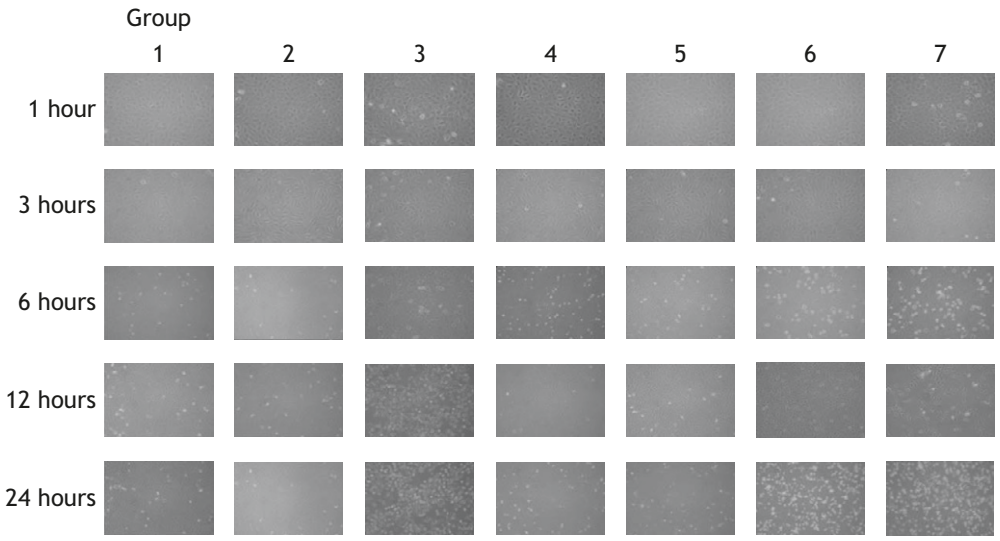

Fig. 1 U2OS cell morphologic changes following treatment with different formulations of endodontic filling materials at different time intervals (1, 3, 6, 12 and 24 hours of exposure to the test dental materials). Lane 1 represents the control group, lane 2 the Vitapex group, lane 3 the zinc oxide-eugenol (ZnOE)+formocresol (FC) group, lane 4 the $Ca(OH)_2$+FC group, lane 5 the $Ca(OH)_2$+iodoform group, lane 6 the $Ca(OH)_2$+iodoform+camphorated parachlorophenol (CPC) group, and lane 7 the $Ca(OH)_2$+CPC group.

appeared to have become condensed, revealing cell separation and a decline in cell number after treatment for 12 hours. Cell death followed treatment of cells with $Ca(OH)_2$ +iodoform+CPC for 24 hours. Virtually all cells in the $Ca(OH)_2$ +CPC group became somewhat star-shaped and quite condensed following 12 hours of treatment; and following 24 hours of treatment, significant cell separation was apparent. For the remaining experimental groups (groups in lanes 1, 2, 4 and 5 of Fig. 1), cell morphology post-treatment appeared to be normal with no significant changes in cell growth.

DNA fragmentation assay

No DNA-ladder bands appeared following the gel assay in any experimental group or the control group (Fig. 2). The DNA-breakage assay for U2OS cells revealed no ladders after treatment with the various dental materials, suggesting that after exposure to those dental materials, there was no evidence of any DNA fragmentation.

COX-2 protein expression

The strength of U2OS cell COX-2 protein expression (in order from high to low expression) for the six experimental groups in Fig. 3 was: lane 6>lane 4>lane 5>lane 7>lane 2>lane 3. The level of COX-2 band expression in U2OS cells from the ZnOE+FC group was virtually zero, but COX-2 expression in the $Ca(OH)_2$+FC and $Ca(OH)_2$+iodoform+CPC groups was quite high (Fig. 3).

MAP kinase expression

ERK (ERK-1, and ERK-2) kinase expression in U2OS cells from the ZnOE+FC and $Ca(OH)_2$+CPC groups was substantially lower than that in the control group. p53 and caspase-3 protein bands appeared in the Western blot assay of all experimental groups (Fig. 4).

Discussion

Endodontic filling materials and genotoxicity

The results of the DNA-fragmentation study showed that none of the tested formulations of endodontic filling materials were genotoxic to cultured U2OS cells (Fig. 2). Similar results were reported in a study by Ribeiro et al.[23] Their findings suggested that FC, paramonochlorophenol and $Ca(OH)_2$ do not promote DNA damage in L5178Y mouse lymphoma cells.

Although the eugenol and FC components of the tested endodontic filling materials were previously shown to be toxic agents in cultured mammalian cells,[3,4] these materials do not appear to be genotoxic to L5178Y mouse lymphoma cells or U2OS cells.[23] The lack of genotoxicity of FC on cell lines might be related to the material containing cresol, because cresol is a chemical inhibitor of reactive oxygen species synthesis.[24]

Eugenol was found to be genotoxic to a cultured human hepatoma cell line (Hep G2) in an *in vitro*

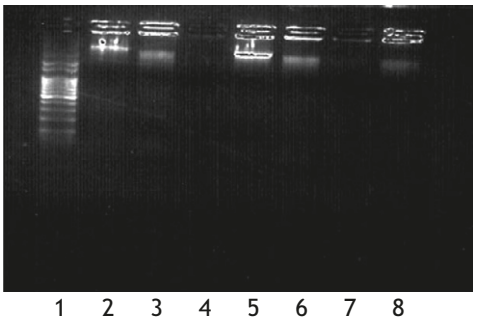

Fig. 2 DNA fragmentation assay results for U2OS cells treated with different formulations of pulpectomy materials. Lane 1 represents the marker, lane 2 the control group, lane 3 the Vitapex group, lane 4 the zinc oxide-eugenol (ZnOE)+formocresol (FC) group, lane 5 the $Ca(OH)_2$+FC group, lane 6 the $Ca(OH)_2$+iodoform group, lane 7 the $Ca(OH)_2$+iodoform+camphorated parachlorophenol (CPC) group, and lane 8 the $Ca(OH)_2$+CPC group.

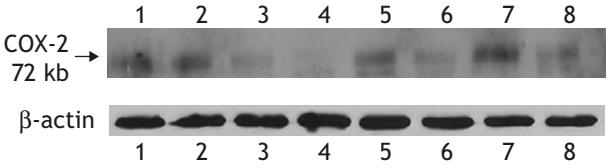

Fig. 3 Inflammation protein cyclooxygenase (COX)-2 expression subsequent to U2OS cells being treated with different formulations of pulpectomy materials, as revealed by Western blot analysis. Lane 1 represents the control group, lane 2 the lipopolysaccharide group, lane 3 the Vitapex group, lane 4 the zinc oxide-eugenol (ZnOE)+formocresol (FC) group, lane 5 the $Ca(OH)_2$+FC group, lane 6 the $Ca(OH)_2$+iodoform group, lane 7 the $Ca(OH)_2$+iodoform+camphorated parachlorophenol (CPC) group, and lane 8 the $Ca(OH)_2$+CPC group.

study.[25] A similar finding was reported by Maralhas et al.[26] who studied the effects of eugenol upon V79 cells using a chromosomal-aberration analysis. In contrast to those results, our results demonstrated no DNA fragmentation in U2OS cells.

$Ca(OH)_2$ did not exhibit genotoxicity toward astrocytes, as reported in our previous comet assay.[27] Similarly, $Ca(OH)_2$ did not induce DNA damage in mammalian cells.[23] Analogously, our results suggest that $Ca(OH)_2$-containing endodontic filling materials were not genotoxic in U2OS cells.

A study by Chang et al.[28] showed that CPC was toxic to cultured human pulp fibroblasts, but was incapable of inducing genotoxicity towards such cells. Here, test groups 6 and 7 contained CPC but did not appear to elicit any genotoxicity toward cultured U2OS cells.

Iodine, the main component of iodoform, induced morphologic transformation[29] and sister chromatid exchanges but not unscheduled DNA synthesis (UDS)

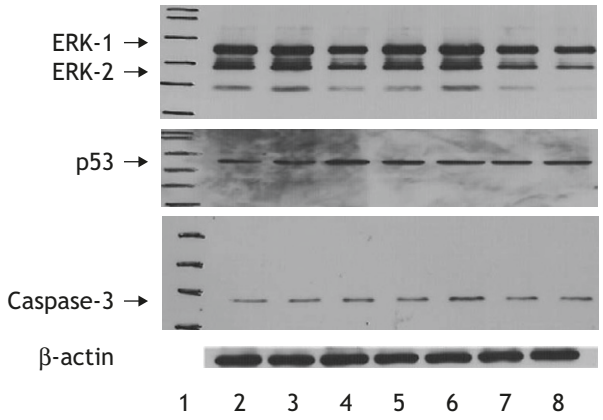

Fig. 4 MAP kinase expression of U2OS cells treated with different formulations of pulpectomy materials for 24 hours. Lane 1 represents the marker, lane 2 the control group, lane 3 the Vitapex group, lane 4 the zinc oxide-eugenol (ZnOE)+formocresol (FC) group, lane 5 the $Ca(OH)_2$+FC group, lane 6 the $Ca(OH)_2$+iodoform group, lane 7 the $Ca(OH)_2$+iodoform+camphorated parachlorophenol (CPC) group, and lane 8 the $Ca(OH)_2$+CPC group.

in Syrian hamster embryo cells.[30] Iodoform-containing dental materials (experimental groups 2, 5 and 6) did not appear to elicit any DNA fragmentation in cultured U2OS cells.

Endodontic filling materials induce U2OS-cell COX-2 protein expression

No COX-2 protein expression following exposure of U2OS cells to ZnO+eugenol+FC or to Vitapex was apparent (Fig. 3). This suggests that these types of dental material do not elicit U2OS cell inflammation. This is consistent with the results of a rat-tooth study by Cotes et al.[12] They suggested from the histology of the pulpal healing process after a pulpotomy in rat teeth that FC combined with ZnOE showed the smallest pulpal inflammatory response. Furthermore, purified eugenol elicited less necrosis and inflammation than commercial eugenol, as was reported in an animal study.[6] In this study, test group 3 produced little inflammation, which might be due to the purified eugenol content.

Apart from experimental group 3, all other test groups contained a $Ca(OH)_2$ component. Levels of COX-2 protein expressed by U2OS cells varied between the different study groups. Similar results were reported in a study by Nelson Filho et al.,[31] who demonstrated that when mast cells were exposed to all $Ca(OH)_2$-containing formulations, there was some degree of inflammatory response. Pitts et al.[32] found that after placing material in the apex of a tooth, giant cells but no inflammatory cells were found adjacent to any remaining $Ca(OH)_2$ particles. Segura et al.[33] found that $Ca(OH)_2$ inhibited macrophage function and reduced the inflammatory

reaction in periapical tissue and in dental pulp when it was used in root canal therapy or in direct pulp capping and pulpotomy, respectively.

Iodoform paste is bactericidal to microorganisms in the root canal and loses only 20% of its potency over a 10-year period. Kawakami et al.[34] found that Vitapex showed no necrotizing effect in rat subcutaneous tissue implantation. Similar results were noted in the present study; COX-2 protein expression did not occur in U2OS cells from the Vitapex group. Hence it would appear that Vitapex does not induce U2OS cell inflammation.

CPC is a tissue irritant.[35] Llamas et al.[36] reported that two different phenolic compounds inhibited adherence of rat peritoneal macrophages to the surface of proximate plastic tubes, suggesting that CPC may alter certain macrophage functions. We revealed that materials containing CPC elicited enhanced COX-2 protein expression by U2OS cells.

COX-2 expression related to MAP kinase expressions

No previous investigations relating to the effects on signaling pathways of cells stimulated with certain endodontic filling materials appear to have been published. Thus, it would be both appropriate and interesting to investigate the process of MAP kinase expression of cells exposed to various primary tooth endodontic filling materials.

When certain biomaterials come into contact with cells, the materials activate kinase signaling pathways of the cell. Here, levels of expression of ERK, p53 and caspase-3 protein kinase were evaluated following treatment with various endodontic filling materials. Significant ERK expression by U2OS cells was noted for all test groups. Thus, we can conclude that the materials used stimulated U2OS cell proliferation and differentiation.

Both p53 and caspase-3 proteins were expressed by U2OS cells in all test groups. Although we previously demonstrated that these test materials elicited various levels of cytotoxicity,[5] we again note that the specific cell death mechanism responsible for such cytotoxicity is still unknown. The specific cell death mechanism may be related to activation of p53 and/or caspase-3 protein kinase in U2OS cells. After exposure to certain endodontic filling materials, U2OS cells may undergo apoptosis and proceed through certain mechanisms which ultimately result in cell death. Similar results were reported by Kitamura et al.[37] who concluded that all $Ca(OH)_2$-containing dental capping agents were able to actively induce apoptosis during pulp wound healing.

Here, we observed no test material-elicited genotoxicity in any of the experimental groups. Furthermore, we noted that p53 induced COX-2 protein expression in all experimental groups except test group 2. It was previously proposed that COX-2 is the ultimate downstream target of p53, and that COX-2 activation is mediated by p53's induction of heparin-binding epidermal growth factor-like growth factor, which subsequently activates the Ras/Raf/MAPK pathway.[38] In 2005, Choi et al.[39] reported that p53 induces COX-2 expression, and COX-2 inhibits p53- and genotoxic stress-induced apoptosis. Furthermore, it was also reported that COX-2 inhibits DNA damage-induced apoptosis through direct regulation of p53 function.

Conclusion

Here, we demonstrate that the tested endodontic filling materials were not genotoxic toward U2OS cells, but they caused COX-2 inflammatory protein expression in all tested groups except for the ZnOE+FC group. Furthermore, the study also showed that the cytotoxic mechanism following U2OS cell exposure to the tested endodontic filling materials was induction of cell apoptosis.

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ORIGINAL ARTICLE

Fracture load of provisional fixed partial dentures with long-span fiber-reinforced acrylic resin and thermocycling

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Background/purpose: The purpose of this study was to evaluate the fracture load and fracture pattern of fiber-reinforced long-span acrylic resin provisional fixed partial dentures (FPDs) with different fiber types and reinforcement lengths after thermocycling.
Materials and methods: Eighty standardized four-unit FPDs of polymethyl methacrylate resin were fabricated on a metal jig and evenly divided into eight groups. The control and comparison groups were unreinforced acrylic specimens and specimens reinforced with 4-mm steel wire, respectively. The six experimental groups comprised acrylic FPDs reinforced with 4-, 18-, and 30-mm glass fiber (FibreKor) or polyethylene fiber (Construct).
Results: One-way ANOVA results revealed statistically significant differences between the fracture loads of the experimental and control/comparison groups ($P<0.05$). Two-way ANOVA results revealed that among the experimental groups, there were statistically insignificant differences between different reinforced fiber types and lengths ($P>0.05$). It is worth noting that increasing the reinforced fiber length was less important for enhancement of fracture load than the location of fiber reinforcement in the long-span acrylic resin FPDs. All samples displayed partial fracturing after thermocycling. The mean fracture loads of the experimental groups were ~50% higher than those of the control/comparison groups.
Conclusion: The results of this study indicate that fiber reinforcement with FibreKor/Construct enhanced the fracture load of long-span acrylic resin provisional FPDs after thermocycling.

Introduction

In recent years, self-curing acrylic resins have extensively been applied in clinical use for provisional fixed partial dentures (FPDs). The main reasons for the popular application of polymethyl methacrylate (PMMA) restorations are their economy, ease of operation, and the ability to reconstruct the shape of

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defects. PMMA also has the advantage of superior esthetic color and feasibility in relining and repair.[1,2]

The unsatisfactory strength of self-curing acrylic resins results in failure or partial breakage of provisional FPDs over time. Traditional PMMA resin provisional FPDs typically fracture owing to heavy occlusal stress. This results in clinical inconvenience due to the need to rehabilitate or replace the FPDs, which is much more difficult for the dentist. The frequent mechanical failures of provisional fixed prostheses usually cause inconvenience, loss of time, and embarrassment for both dental clinicians and patients.[3,4]

In order to improve the drawbacks of acrylic FPDs and prevent failure in clinical situations, enhancing FPD's volume of reconstruction and strengthening the acrylic resin with a metal plate have been investigated. However, the end results either violated the physiologic contours of provisional FPDs or involved overly complicated clinical procedures. Embedding a solid metal framework in a provisional FPD can reduce the limited space available and result in increased vertical thickness of the FPD, especially in the pontics.[5–7] Some studies attempted fiber application in order to strengthen provisional dental resins. Compared with metals, fiber reinforcement possesses superior mechanical, esthetic and cohesive characteristics, and has the advantage of being a lighter-weight composite. Previous studies on fiber reinforcement typically emphasized strengthening removable overdentures.[8,9]

Using fiber-strengthened provisional dental resins, Ramos et al.[10] employed polyethylene fiber (Ribbond; Ribbond Inc., Seattle, WA, USA) to strengthen PMMA resin. After fiber strengthening, which was accomplished by embedding fibers at a depth of one-third on the tension side of the test bar, samples had notably increased failure strength. The surfaces of early fibers were plasma-treated or sandblasted in an attempt to enhance their adhesive ability.[11] The use of glass fibers to strengthen provisional dental resins has also been investigated. Chung et al.[12] stated that two factors, fiber quantity and the reinforcement site, affect provisional FPD strength. Vallittu[13] used different woven forms of glass fibers to strengthen PMMA powders and n-butylmethacrylate liquids when constructing FPDs. The results suggested that glass fiber reinforcements considerably increased the fracture resistance of provisional FPDs, even though the fibers were embedded in unfavorable sites. Similarly, Fatma and Marwa[14] concluded that glass fibers can increase the fracture strength of overdentures. Despite this, even after surface treatment with plasma, radiation or chemicals, polyethylene fibers have yet to demonstrate efficient surface adhesion with resins.[10,15] Nohrstrom et al.[16] investigated the influence of the position of fiber reinforcement on the fracture resistance of interim FPDs. The results showed that the length of the span and quantity of fibers significantly affected the fracture load. *In vitro* studies in which material fatigue was induced by thermocycling suggested that the main cause of fractures of interim FPDs is probably fatigue of the material.

As conclusively demonstrated in all of the above-mentioned studies, the location of the fiber reinforcement is one of the main factors affecting the strengthening efficiency. Detailed information about recently developed commercial fibers, such as the effects of different surface treatments, the location of fiber reinforcement and strengthening properties of different fiber lengths, are not currently available.

The aim of this investigation was to determine the fracture load of long-span fiber-reinforced acrylic resin provisional FPDs using two types of fiber, and to investigate the influences of fiber length and reinforcement location on the fracture load after thermocycling.

Materials and methods

A stainless steel metal jig was made to simulate mandibular four-unit FPDs in which the first premolar and second molar functioned as abutments. The distance between the distal axes of the premolar to the mesial surface of the molar abutment was 20 mm. A four-unit FPD was made on the metal jig with an inlay pattern of wax. The cast mold of the FPD was duplicated with polysiloxane putty impression material (condensation type), Coltoflax (Coltene, Altstatten, Switzerland), using the metal jig with a four-unit FPD pattern of wax. An FPD of PMMA, Tempron (GC Corp., Tokyo, Japan), was then prepared using this putty mold as a standard model to ensure that the FPDs made for the fracture load test had the same dimensions and shape as the standard acrylic model. The detailed dimensions of the mandibular four-unit FPD made using the metal jig are shown in Fig. 1A.

The provisional FPDs of self-cured PMMA resin were reproduced using a condensation type of putty, polysiloxane (Coltoflax; Coltene, Altstatten, Switzerland), as the impression material. Before reproducing the resin specimens, impression materials embedded in a type IV stone mold (Fujirock EP; GC Dental Industrial, Tokyo, Japan) were used to create a negative reproduction. To avoid deformation of the impression/stone mold and ensure that the dimensions of the specimens were exactly duplicated, each impression/stone mold was discarded after producing five samples.

The resin powder-to-liquid ratio was 2.0 g to 1.0 mL, as suggested by the manufacturer. The PMMA

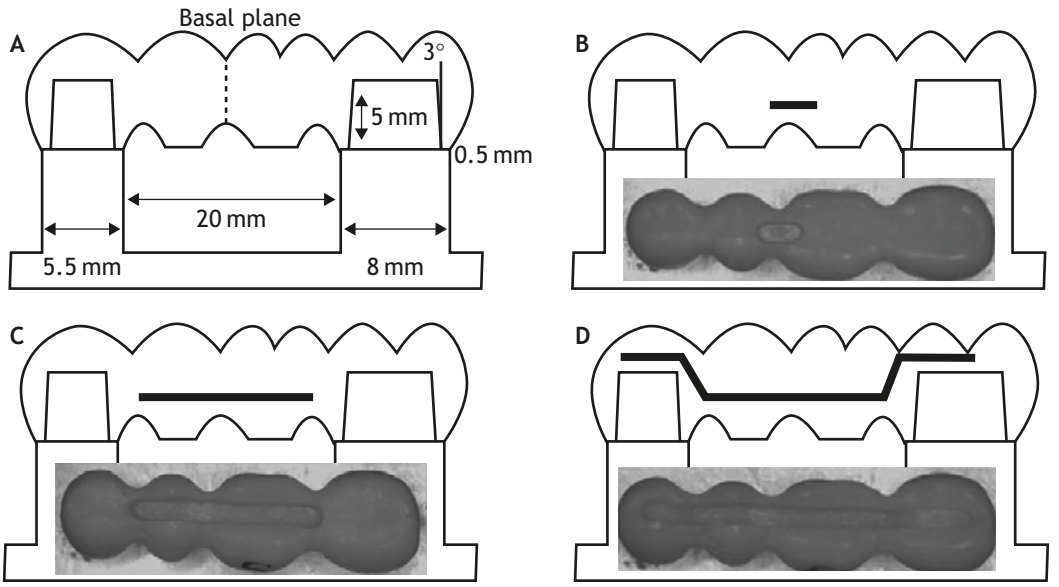

Fig. 1 Dimensions of the four-unit provisional fixed partial dentures (FPDs) on a metal jig. (A) The tension side between the two pontics represents the baseline location for fiber reinforcement. Lateral and occlusal views of FPDs with fiber-reinforced lengths of (B) 4 mm, (C) 18 mm, and (D) 30 mm.

resin mixture was hand-mixed for 5 seconds, and the cavity of the FPD impression/stone mold was immediately filled. The impression/stone mold was then fixed with a compressor and stored in water at 50°C for 10 minutes to allow complete polymerization of the FPDs. Once FPDs were removed from the mold, they were immersed in distilled water at 37°C for 24 hours and air-dried for an additional day at room temperature. The dimensions of all FPDs were measured using Digimatic Caliper (Mitutoyo, Tokyo, Japan), followed by proper finishing to obtain sample differences of <0.1 mm.

In our preliminary experiment, almost all fractures in samples ($n=10$) were concentrated on the tension side of the connector between the two pontics. The plane between the pontics and a line perpendicular to the long axis of the FPDs was set as the "basal plane" of fiber reinforcement. As shown in Fig. 1A, the basal plane was the central line of the fiber-reinforced FPD between the pontics. The 2.5×4.0 mm (width $\times$ depth) slots with lengths of 4, 18 or 30 mm were prepared in FPDs using a flat-end tungsten carbide bur mounted on a milling machine (Bachmann milling unit model 82; Cendres & Metaux, Biel-Bienne, Switzerland) (Fig. 1B–D). All provisional FPDs with slots of different lengths were reproduced using a self-cured PMMA resin, as per previous procedures.

The control group ($n=10$) consisted of intact self-cured PMMA resin FPDs without fiber reinforcement, and the comparison group ($n=10$) included FPDs impregnated with $4.00\times0.41\times0.56$ mm (length $\times$ width $\times$ depth) steel wires. The six experimental groups (each $n=10$) consisted of acrylic resin FPDs impregnated with 4, 18 or 30 mm lengths of polyethylene fiber (Construct; Kerr UK Ltd., Peterborough, UK) or glass fiber (FibreKor; Jeneric/Pentron Inc., Wallingford, CT, USA). The reinforced fibers of different lengths were immersed or wetted in the resin, as suggested by Vallittu,[17] before being embedded in pre-prepared FPDs slots. Additional acrylic resin was then added to the slots that were embedded with fibers to restore the original morphology of the FPDs. Demolded samples, with or without fiber impregnation, were thermocycled (600 cycles of 5°C/55°C for 2 minutes/cycle) (Long Wha Enterprise, Kaohsiung, Taiwan) and air-dried for 24 hours at room temperature prior to the loading test.

The FPDs were mounted on a metal jig and loaded with a steel ball 6 mm in diameter in the region of the central fossa of the first molar pontic. Prior to measuring the fracture resistance, all FPDs were loaded with a 30 N force to obtain an equal fit in the jig. Thin-film aluminum foil, 0.5 mm thick, was placed between the loading point and the FPDs to ensure even loading. The fracture loads were measured using a universal testing machine (LS 500; Lloyd Instruments, Hampshire, UK) with a crosshead speed of 5.0 mm/minute. The force causing the first fracture in the FPD was considered the fracture load. The point of fracture was determined from the force-deflection curve.

In order to evaluate the bonding condition between fibers and the resin matrix in the FPDs after thermocycling, the fracture surfaces obtained from selected test specimens were examined using a field emission scanning electron microscope (JSM 5300; JEOL, Tokyo, Japan).

One-way ANOVA was used to evaluate the statistical significance of the fracture load data. A two-way ANOVA comparison was used to determine the significance of variations in fiber type and reinforcement length span. Results were considered statistically significantly different at $P<0.05$. The JMP 5.0 software package (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses.

Results

The load required to fracture the FPDs varied from 676 to 382 N, as indicated in Table 1. According to the results of the mean fracture load, the 30-mm FibreKor fiber-reinforced sample and the steel wire-reinforced specimen, respectively, had the highest and lowest strength values. The control (non-fiber reinforcement) and comparison (steel wire reinforcement) groups showed no significant differences ($P>0.05$). The fracture loads of the 4-mm Construct and FibreKor fiber length reinforcements were larger than those of the control and comparison groups by about 47% and 59%, and 48% and 60%, respectively. The experimental groups of fibers reinforced with a short length of 4 mm, medium length of 18 mm and long-span length of 30 mm had significantly higher fracture strengths (in terms of the provisional FPDs) than the control and comparison groups ($P<0.05$ by one-way ANOVA; Table 1). There were no significant differences between the Construct and FibreKor fiber types with constant fiber length reinforcement (4, 18 and 30 mm) ($P>0.05$). Two-way ANOVA analysis of the experiment groups demonstrated no statistically significant differences in fracture load when fiber types or reinforced lengths were compared with one another ($P>0.05$) (Table 2).

Further investigation of fracture patterns was necessary for consideration of clinical reparability of provisional FPDs. Based on clinical observation, the fracture patterns of acrylic FPDs with or without fiber reinforcement were classified into three types (Fig. 2). The first type of fracture pattern was "catastrophic failure", in which the pontics were

Table 1. Mean fracture loads of long-span provisional FPDs with fiber and metal wire reinforcements, and the unreinforced control (each group $n=10$)

Group	Fracture load, mean (SD) (N)	One-way ANOVA and *post hoc* statistical analysis*
30-mm Fb	676.7 (66.4)	A
18-mm Fb	661.9 (68.2)	A
18-mm Cs	651.4 (46.5)	A
30-mm Cs	612.0 (81.7)	A
4-mm Fb	610.8 (63.2)	A
4-mm Cs	607.4 (75.8)	A
Control	413.2 (63.5)	B
Wire	382.6 (77.6)	B

*Groups with the same letter do not significantly differ at $P>0.05$. Cs = Construct; Fb = FibreKor.

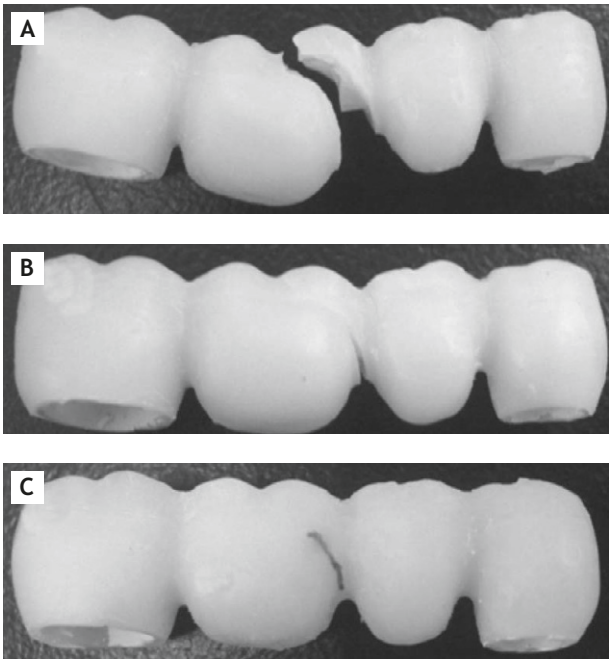

Fig. 2 Fracture patterns of provisional fixed partial dentures: (A) catastrophic, (B) bent and (C) partial fracture patterns.

Table 2. Two-way ANOVA statistical analysis of fiber-reinforced provisional fixed partial dentures containing different fiber lengths (4 mm, 18 mm, and 30 mm) and fiber types (FibreKor and Construct)

Variation	*df*	Sum of squares	*F*	P*
Main effects combined	5	45,905.2	1.99	0.095
Fiber type	1	10,296.6	2.23	0.141
Fiber length	2	24,365.7	2.64	0.081
Two-way interactions	2	11,242.9	1.22	0.304
Error	54	249,071.4		

*Not statistically significant at $P>0.05$.

sheared off by the compressive load. The second type of fracture pattern was "bent failure", in which an observable gap was detected between the pontics. The samples were, however, still held together by the fiber reinforcements. The third type of fracture pattern was "partial fracture", in which the specimens remained intact and only fracture lines could be detected. After thermocycling, all samples displayed partial fracture patterns, and the cracks were concentrated on the tension side of the pontics regardless of whether or not they were reinforced.

A clear interface between the wire and resin matrix on the fracture surface of the steel-wire-reinforced sample was observed by scanning electron microscope (Fig. 3A). This interface suggested poor linkage or incorporation of the wire-reinforced additive and resin matrix. As the cracks grew or extended across the resin matrix, they resulted in sample fracture. The fractured surfaces of the polyethylene fiber-reinforced samples are shown in Fig. 3B. The construct was easily delaminated from the woven form even though the fiber had many attached resin particles. The best combination of reinforced fiber and resin matrix was the glass fiber (FibreKor) specimens. The fracture surface (Fig. 3C) clearly revealed that the fiber fasciations had not separated and were still closely interlocked with the resin matrix. No spaces were found between the fiber and matrix, even under higher magnitude imaging.

Discussion

In a pilot study, the mean fracture load of long-span provisional FPDs without fiber reinforcement declined by about 34% (from about 622 to 413 N) after thermocycling, with the fracture type changing from catastrophic to partial failure patterns. The changing trend of the fracture pattern might have been due to the hydrophilic properties of the resin, which reduced the stiffness of the provisional FPDs after thermocycling.[18]

In early clinical applications, a scaffold of metals was used with resins as a restorative composite.[19,20] Vallittu and Lassila (1992) revealed that metals largely increased the fracture strength of dentures without thermocycling.[21] The main reason for the relatively low fracture strengths in our comparison group was that the metal wire was not chemically bonded to the PMMA resin matrix. The poor fit was due to incoherent interfaces, which were even more prevalent after thermocycling.

It is worth noting that the mean fracture load of the comparison group was even lower than the non-reinforced provisional FPD control group, although this difference was not significant. The expansion and shrinkage of different materials may have been caused by thermal shock due to stress build-up between the incoherent interfaces of the wires and acrylic resin. This effect allowed us to group the materials (Fig. 3A), and it provided an explanation for why the metal strengthening effect

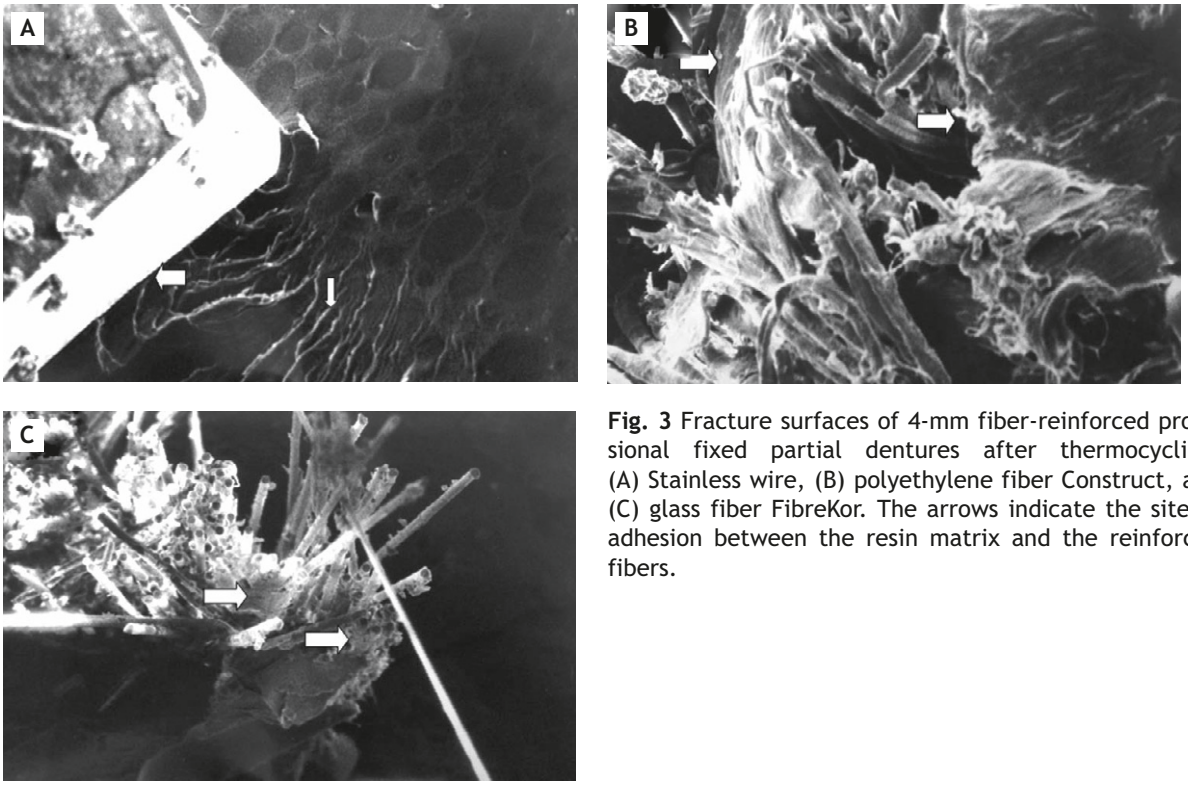

Fig. 3 Fracture surfaces of 4-mm fiber-reinforced provisional fixed partial dentures after thermocycling. (A) Stainless wire, (B) polyethylene fiber Construct, and (C) glass fiber FibreKor. The arrows indicate the site of adhesion between the resin matrix and the reinforced fibers.

was not obvious. The fractured surfaces of wire-impregnated provisional FPDs demonstrated ductile fracture striations, as indicated by the arrows in Fig. 3A. The fracture-resistant ability of the wires was not functional, and thermal fatigue, resulting in cracks, propagated through areas of the resin matrix.[22]

The mean fracture load values of the glass fiber-FibreKor specimens were larger than those of polyethylene fiber-Construct-reinforced 4-unit provisional FPDs of the same length. However, statistical analysis revealed only an insignificant difference between these two groups. The results suggested that both types of fibers, but not the steel wires, had the same capacity for reinforcement. The best reinforcement material in the above groups was the pre-silanated surface treatment which had excellent fracture-resistant abilities, even after thermocycling.

The fracture load did not significantly differ among the experimental groups (Table 2), although the microstructure studies showed that glass fiber (FibreKor) specimens possessed more compact structures than polyethylene fiber (Construct) specimens (Fig. 3B and C). The arrows in the images indicate the interfaces between fibers and resin matrices (Fig. 3B); the woven forms of the fiber constructs are derived from the matrix and broke up after bending failure. It is worth noting that although the microstructures varied with the fiber type (i.e., FibreKor and Construct fibers), the fracture loads were approximately equivalent. These phenomena could have been due to the fibers efficiently retarding crack propagation on the tension side of the provisional FPDs, and the location of the reinforced fiber being more important for improving fracture load resistance of acrylic FPDs than the reinforced fiber type that underwent pre-silanated treatment.

The drilled slots coinciding with the basal plane of the fiber-reinforced provisional FPDs were developed to enhance fracture strength, thus ensuring that the fiber-reinforced location was on the tension side of the provisional FPDs (Fig. 1A). The fracture loads for the 4-mm fiber-reinforced experimental groups were almost 50% higher than those of the control and comparison groups. The 18-mm and 30-mm length fiber-reinforced groups had larger mean fracture loads than the 4-mm groups. However, increasing the lengths of fiber reinforcements did not obviously enhance the strength, as shown by the statistical analysis. We propose that reinforcement of long-span provisional FPDs with different fiber lengths increases the strength of connectors between pontics when extended to both sides of the abutments and to the occlusal surface of both abutments (Fig. 1B–D). The significantly higher fracture loads as a consequence of fiber addition suggest that the strengthened location is much more important than the reinforcement length. Kanie et al.[23] also suggested that fiber-reinforced dentures should withstand greater tension on the concentration side in order to produce an efficient strengthening effect. In our results, regardless of length, the experimental samples demonstrated a fracture strength that was 50% higher than the control group after the weaker site was reinforced with fibers.

Here, polyethylene fiber (Construct) and glass fiber (FibreKor) specimens of different reinforced lengths embedded on the tension side of the long-span provisional FPDs were shown to raise the fracture load to >600 N after thermocycling. This value is important, because the mean masticatory force in the human molar area is 500–600 N.[24,25] From a clinical application viewpoint, selection should be based mainly on the type of fiber-reinforcement surface treatment method used; operational convenience and economic considerations should also be considered. Despite the fact that provisional FPDs were strengthened with different fiber lengths, differences in fracture resistance were not statistically significant. This emphasizes the importance of reinforcing the weakest location in acrylic resin long-span provisional FPDs.

Owing to the limitations of this study, the effects of periodontal ligaments and proprioceptors, and complicated occlusal contact patterns in the mouth were not investigated. Therefore, additional studies based on randomized well-controlled clinical trials are needed.

The commercial polyethylene-fiber Construct and glass-fiber FibreKor demonstrated excellent adhesive capabilities with acrylic dental resin. The fracture load for these fibers was 50% greater than non-fiber or steel wire additive-reinforced long-span provisional FPDs. With regard to the fracture load of long-span provisional FPDs, the weakest location was the tension side between the pontics. The location of fiber strengthening was much more important than the length of reinforcement or fiber type with regard to fracture loads in long-span acrylic resin provisional FPDs after thermocycling.

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ORIGINAL ARTICLE

Relationship between oral status and maximum bite force in preschool children

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Background/purpose: The purpose of this study was to determine correlations between maximum bite force and several variables, including age, sex, body height, body weight, caries index, occlusal pattern, vertical occlusal relationship, number of teeth in contact and maximum mouth opening, among healthy 4–6-year-old preschool children.
Materials and methods: A total of 201 preschool children aged 4–6 years were selected from two kindergartens. The collected data included the oral checking and bite force measuring records. Whole oral records and measurements of bite force were taken and analyzed.
Results: Growth variables, such as height and weight, correlated with sex and bite force. Although there was no significant difference in bite force among the three age levels (4, 5 and 6 years old), there were significant differences in growth variables. Oral status variables, such as the number of maxillary posterior teeth in contact and maximum mouth opening, showed significant positive correlations with bite force.
Conclusion: By combining the results of this study, it was concluded that associations of bite force with factors like age, maximum mouth opening and the number of teeth in contact were clearer than for other variables such as body height, body weight, occlusal pattern, and tooth decay or fillings.

Introduction

After widespread oral health education and increased use of fluoride mouth rinses among the younger population, the incidence of tooth decay in children in Taiwan decreased.[1] However, this drop still lags behind targets set by the World Health Organization. It is known that poor oral health can lead to severe tooth decay and early loss of teeth, which can then lead to crowded teeth and malocclusion. A previous study[2] showed that if children have good mastication ability, food is more easily digested. Nutrition is important to the growth and development of children, and digestion affects nutrition. People will choose soft food if they cannot chew effectively, eventually causing malnutrition and insufficient fiber, mineral and vitamin intake. One study[3] showed that 56% of such patients have digestive problems. Masticating malfunction can also lead to other diseases caused by malnutrition.[4]

Investigators[5,6] have suggested that maximum bite force is affected by the masticatory system, and it is generally accepted that a better masticatory system results in a stronger bite force. Oral status can affect mastication. Severely decayed and missing teeth are detrimental to mastication

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and weaken the function of masticatory muscles. Until now, there have been few studies investigating bite force in preschool children. The aim of this study was to investigate relationships between maximum bite force and variables including tooth decay, missing teeth, tooth fillings, occlusal pattern, number of maxillary posterior teeth in contact, and maximum mouth opening in preschool children.

Materials and methods

In total, 201 preschool children aged 4–6 years were selected. Oral examinations were performed according to the principles and methods indicated by the World Health Organization. Dentists and investigators from the Oral Health Science Research Institute, Kaohsiung Medical University, Kaohsiung, were trained to examine the children. An oral examination and measurement of bite force were carried out and recorded. Data collected included the oral check-up and bite force records, and are described as follows:

1. General information included sex, age, body height, and weight.
2. Oral status included tooth decay, tooth fillings, missing teeth, occlusal pattern, vertical occlusal relationships, maximum mouth opening, and the number of maxillary posterior teeth in contact.

   For the occlusal pattern, three classes were defined based on occlusal anterior–posterior relationships: (1) in class A, the terminal plane of the second primary molar is flush with the upper and lower distal margin surfaces; (2) in class B, the distal occlusion of the second primary molar with the lower second primary molar is situated in the posterior position, and there is a distal step for the distal part of the second primary molar with a large overjet; and (3) in class C, the mesial occlusion of the lower second primary molar and lower second primary molars is in the anterior position. There is also a mesial step of the lower second primary molar with an edge-to-edge or reversed bite incisor relationship.

   Three types of overbite were classified for the vertical occlusal relationship, according to the upper and lower incisors' occlusion: normal, deep, and open. A normal bite is defined as the vertical overlap not extending beyond half of the clinical crown length of the lower incisor during biting. A deep bite is defined as the vertical overlap of the anterior teeth extending beyond more than half of the clinical crown of the lower incisor during biting. An open bite is defined as there being no vertical overlap or there being a gap between the upper and lower incisors during biting.

   Maximum mouth opening is the vertical distance between the upper and lower central incisors when the mouth is open as wide as possible. If there was no central incisor, then the lateral incisor was used for the calculation.

   The primary and first permanent molars on both sides were used for measurement of the number of maxillary posterior teeth in contact. Articulating paper (0.0024 inches; Bausch, Pulpdent, Watertown, MA, USA) was used to measure the number of upper and lower molars in contact. An upper and lower molar in contact were defined as one pair, with a maximum of six pairs.
3. Bite force was assessed using the bite force MPM-3000 machine (SCAIME, Annemasse, France) to measure the maximum bite force of the primary molars on the left and right sides. There was a 10-second break between the two measurements. The maximum value measured was defined as the maximum bite force.

A database was designed using Microsoft Access, and data were analyzed with JMP 5.01 statistical software (SAS Institute, Cary, NC, USA). Numerical variables of body height, body weight, maximum mouth opening, tooth decay, tooth fillings, missing teeth, and maximum bite force measurements on the left and right sides were described, and the mean and standard deviation were recorded. Categorical variables were described and analyzed by proportion. Comparison between numerical variables was performed using the paired *t* test. Differences at the 5% level of probability were considered statistically significant. ANOVA was used to analyze the relationships between age and body height and weight, and bite force. Multiple regression was used to analyze relationships between the bite force and all variables including sex, height, weight, tooth decay, tooth fillings, missing teeth, number of maxillary posterior teeth in contact, occlusal pattern, and maximum mouth opening.

Results

In total, 201 preschool children with an average age of 5.2 years were selected from two kindergartens in Kaohsiung County. General information about the children is listed in Table 1. The average height of these children was 109.65±6.19 cm (Table 2). The average height of boys was 110.50±5.76 cm and that of girls was 109.02±6.44 cm. There was no statistically significant difference in height between sexes. The average weights of boys and girls were 19.73±3.31 kg and 18.77±3.41 kg, respectively, and this difference was statistically significant. There were also significant differences in height

and weight of children aged 4, 5 and 6 years. Further analysis showed that 6-year-olds had an obvious height difference from children aged 4 and 5 years. Weight followed the same trend.

In terms of the maximum mouth opening (Table 2), the average opening distance was 3.54±0.48 cm. The average mouth opening distance of boys (3.61±0.51 cm) was greater than that of girls (3.48±0.45 cm), but this difference was not statistically significant. There was also no significant difference between different age groups (children aged 4, 5 and 6 years had average opening distances of 3.51±0.44 cm, 3.58±0.51 cm and 3.41±0.51 cm, respectively; P=0.2812). The average bite force on the left side was 4.16±3.63 kg, and on the right side 4.47±4.11 kg (Tables 2 and 3). The average maximum bite force was 5.69±4.19 kg. Although the maximum bite force of boys was larger than that of girls, the difference was not statistically significant. There was also no significant difference between the bite force on the left and right sides of either boys or girls (Table 4).

Comparing the maximum bite force of preschool children by age (Table 3), no statistically significant differences among 4-, 5- and 6-year-olds were revealed. There was also no significant difference in the average left-side bite force. However, the average right-side bite force significantly differed with age (3.48±3.39 kg, 5.03±4.36 kg and 6.26±4.93 kg, respectively, for 4-, 5- and 6-year-olds; P=0.0056). Further analysis showed that 4-year-old children had an average bite force on the right side that significantly differed from those of 5- and 6-year-old children.

When comparing different occlusal patterns, vertical occlusal relationships and the number of maxillary posterior teeth in contact with the bite force (Table 5), no difference was found in the bite force attributable to any of these conditions. With respect to overall tooth condition (Table 6), the average number of decayed teeth in preschool children aged 4–6 years was 3.93±3.81 (4.06±3.75 for boys and 3.83±3.87 for girls). The average caries index was 4.79±4.09 (4.87±4.03 for boys and 4.72±4.15 for girls). There were no notable differences in sex or age with respect to tooth decay, missing teeth, tooth fillings, the caries index, caries filling rate or caries prevalence rate.

The regression analysis showed relationships of different variables with the maximum bite force and bite force on both sides (Table 7). Related analyses and a collinearity analysis demonstrated that the condition index was <30 (13.79), which implies that there was no collinearity problem with the data. The one variable that positively affected the maximum bite force was age, as 6-year-olds had a

Table 1. Sex, age, occlusal patterns, vertical occlusal relationships, and the number of maxillary posterior teeth in contact of study participants (*n*=201)

Variable	*n* (%)
Sex	
Male	86 (42.79)
Female	115 (57.21)
Age (yr)	
4	87 (43.28)
5	96 (47.76)
6	18 (8.96)
Occlusal pattern	
Class A	179 (89.05)
Class B	10 (4.98)
Class C	12 (5.97)
Vertical occlusal relationship	
Normal	176 (87.56)
Open bite	4 (1.99)
Deep bite	21 (10.45)
Number of maxillary posterior teeth in contact	
0–2	36 (17.91)
3	11 (5.47)
4	150 (74.63)
5 or 6	4 (1.99)

Table 2. Comparison of body height, body weight, maximum bite force (MBF) on the left side, MBF on the right side, MBF and maximum mouth opening between boys and girls*

Variable	All (*n*=201)	Boys (*n*=86)	Girls (*n*=115)	P
Body height (cm)	109.65±6.19	110.50±5.76	109.02±6.44	0.0929
Body weight (kg)	19.18±3.39	19.73±3.31	18.77±3.41	0.0462
MBF on the left side (kg)	4.16±3.63	4.69±3.92	3.77±3.35	0.0739
MBF on the right side (kg)	4.47±4.11	4.54±4.30	4.42±3.98	0.8341
MBF (kg)	5.69±4.19	6.12±4.38	5.37±4.02	0.2093
Maximum mouth opening (cm)	3.54±0.48	3.61±0.51	3.48±0.45	0.0568

*Data are presented as mean±standard deviation.

Table 3. Comparison of body height, body weight, maximum bite force (MBF) on the left side, MBF on the right side, MBF and maximum mouth opening among children at different ages*

Age (years)	Body height (cm)	Body weight (kg)	MBF on the left side (kg)	MBF on the right side (kg)	MBF (kg)	Maximum mouth opening (cm)
All (n=201)	109.65±6.19	19.18±3.39	4.16±3.63	4.47±4.11	5.69±4.19	3.54±0.48
4 (n=87)	105.48±4.53	17.53±2.69	3.96±3.51	3.48±3.39	5.00±3.89	3.51±0.44
5 (n=96)	112.25±5.10	20.18±3.18	4.29±3.63	5.03±4.36	6.07±4.24	3.58±0.51
6 (n=18)	115.97±5.63	21.83±3.93	4.48±4.29	6.26±4.93	7.06±5.30	3.41±0.51
P†	<0.0001	<0.0001	0.7728	0.0056	0.0796	0.2812
Significant pairs‡	6>5 years 6>4 years 5>4 years	6>4 years 5>4 years		6>4 years 5>4 years		

*Data are presented as mean±standard deviation; †ANOVA; ‡Tukey pairwise comparisons.

Table 4. Comparison of the maximum bite force (MBF) on the left side with that on the right side for different sexes and ages*

Variable	MBF on the right side (kg)	MBF on the left side (kg)	P
Sex			
All (n=201)	4.47±4.11	4.16±3.63	0.2757
Male (n=86)	4.54±4.30	4.69±3.92	0.7459
Female (n=115)	4.42±3.98	3.77±3.35	0.0697
P	0.8341	0.0739	
Age (yr)			
4 (n=87)	3.48±3.39	3.96±3.51	0.2420
5 (n=96)	5.03±4.36	4.29±3.63	0.0573
6 (n=18)	6.26±4.93	4.48±4.29	0.1923
P	0.0056	0.7728	

*Data are presented as mean±standard deviation.

Table 5. Comparison of the maximum bite force (MBF) on the left side, MBF on the right side and MBF, with occlusal patterns, vertical occlusal relationships and the number of maxillary posterior teeth in contact (MPTC)*

Variable	MBF (kg)	MBF on the right side (kg)	MBF on the left side (kg)
All (n=201)	5.69±4.19	4.47±4.11	4.16±3.63
Occlusal pattern			
Class A (n=179)	5.82±4.18	4.59±4.13	4.27±3.67
Class B (n=10)	3.21±2.59	2.28±2.24	2.27±2.18
Class C (n=12)	5.87±4.94	4.50±4.77	4.15±3.79
P	0.1580	0.2243	0.2376
Vertical occlusal relationship			
Normal (n=176)	5.82±4.29	4.59±4.20	4.21±3.70
Open bite (n=4)	7.22±1.63	4.70±3.90	6.79±1.44
Deep bite (n=21)	4.35±3.36	3.40±3.35	3.25±3.00
P	0.3915	0.4497	0.1784
MPTC			
0–2 (n=36)	4.24±3.83	3.14±3.27	3.02±3.56
3 (n=11)	5.90±3.47	4.37±2.59	4.73±3.90
4 (n=150)	6.05±4.28	4.78±4.35	4.46±3.61
5 or 6 (n=4)	4.98±3.56	4.91±3.65	1.65±0.38
P	0.1364	0.1976	0.0794

*Data are presented as mean±standard deviation.

Table 6. Comparison of the numbers of decayed, missing and filled teeth, caries index, caries filling rate, and caries prevalence rate of the different sexes and ages*

Variable	No. of decayed teeth (d)	No. of missing teeth (e)	No. of filled teeth (f)	Caries index (deft)	Caries filling rate (%)	Caries prevalence rate, *n* (%)
Sex						
All (*n*=201)	3.93±3.81	0.16±0.64	0.70±1.51	4.79±4.09	16.79±29.92	144 (71.64)
Male (*n*=86)	4.06±3.75	0.17±0.65	0.64±1.47	4.87±4.03	14.46±28.00	63 (73.26)
Female (*n*=115)	3.83±3.87	0.15±0.64	0.74±1.55	4.72±4.15	18.57±31.35	81 (70.43)
P	0.6821	0.7728	0.6449	0.7972	0.3990	0.6606
Age (yr)						
4 (*n*=87)	3.77±3.88	0.18±0.77	0.45±0.96	4.40±3.98	15.14±30.49	60 (68.97)
5 (*n*=96)	4.15±3.85	0.13±0.49	0.86±1.84	5.14±4.27	16.16±27.84	71 (73.96)
6 (*n*=18)	3.56±3.42	0.22±0.73	1.00±1.61	4.78±3.62	27.23±36.87	13 (72.22)
P	0.7300	0.7533	0.1185	0.4826	0.3592	0.7545

*Data are presented as mean±standard deviation.

Table 7. Regression coefficient (β), P value and confidence interval (CI) between the growth and oral status variables with the maximum bite force (MBF), MBF on the right side, and MBF on the left side*

Variable	MBF		MBF on the right side		MBF on the left side	
	β	CI	β	CI	β	CI
Sex	0.79	(–0.38, 2.00)	0.06	(–1.13, 1.24)	0.94	(–0.13, 2.01)
Age (6 *vs*. 4 years)	3.61‡	(1.02, 6.20)	3.87‡	(1.34, 6.39)	1.73	(–0.54, 4.01)
Age (5 *vs*. 4 years)	1.42	(–0.11, 2.93)	1.64†	(0.15, 3.12)	0.58	(–0.76, 1.92)
Body weight	0.16	(–0.11, 0.42)	0.17	(–0.09, 0.43)	0.14	(–0.09, 0.38)
Body height	–0.13	(–0.29, 0.04)	–0.11	(–0.26, 0.05)	–0.10	(–0.24, 0.04)
Tooth decay	0.03	(–0.13, 0.50)	0.07	(–0.09, 0.23)	0.03	(–0.12, 0.17)
Tooth missing	–0.76	(–1.68, 0.17)	–0.57	(–1.47, 0.33)	–0.69	(–1.51, 0.12)
Tooth filling	–0.19	(–0.58, 0.19)	–0.25	(–0.62, 0.13)	–0.06	(–0.40, 0.28)
Occlpatt (class A *vs*. class B)	0.67	(–1.84, 3.18)	0.27	(–2.18, 2.71)	0.08	(–2.12, 2.28)
Occlpatt (class C *vs*. class B)	0.13	(–3.32, 3.59)	–0.37	(–3.74, 3.00)	–0.37	(–3.41, 2.67)
VOR (normal *vs*. deep bite)	1.03	(–1.00, 3.05)	0.66	(–1.31, 2.63)	1.10	(–0.68, 2.87)
VOR (open bite *vs*. deep bite)	1.73	(–2.83, 6.28)	0.21	(–4.23, 4.64)	2.89	(–1.11, 6.88)
Maximum mouth opening	0.95	(–0.30, 2.20)	1.32†	(0.10, 2.53)	–0.11	(–1.20, 0.99)
MPTC (3 *vs*. 0, 1 or 2)	1.19	(–1.72, 4.09)	0.96	(–1.87, 2.71)	1.32	(–1.23, 3.87)
MPTC (4 *vs*. 0, 1 or 2)	1.63	(–0.07, 3.33)	1.71†	(0.06, 3.36)	1.32	(–0.17, 2.81)
MPTC (5 or 6 *vs*. 0, 1 or 2)	–1.22	(–5.81, 3.38)	–0.61	(–5.08, 3.86)	–2.37	(–6.41, 1.66)

*Condition index = 13.79 (<30). †P<0.05; ‡P<0.01. Occlpatt = occlusal pattern; VOR = vertical occlusal relationship; MPTC = number of maxillary posterior teeth in contact.

significantly stronger bite force than 4-year-olds. Variables that significantly affected the bite force on the right side included age (6 years *vs*. 4 and 5 years *vs*. 4 years), maximum mouth opening, and four versus zero, one or two maxillary posterior teeth in contact. However, no variable was found to have a substantial relationship with the bite force on the left side.

Discussion

Male and female preschool children aged 4–6 years showed significant differences in both height and weight (Table 3). This shows that growth development is notable even at this early age. This difference was even more obvious among the different age groups. The peak of growth development may lie somewhere between 4 and 5 years old.

Although class A and C children had higher average bite forces on both sides and a higher maximum bite force than class B children, the differences were not statistically significant. This indicates that there might not be a strong relationship between different occlusal patterns and bite force. These results are similar to the conclusions drawn by Ahlgren,[7] Ahlgren et al.,[8] and Kiliaridis et al.[9] With respect to the vertical occlusal relationship, the bite force

on both sides and the maximum bite force were highest in open-bite individuals and lowest in deep-bite individuals. These findings differ from those of a study by Sassouni[10] in adults and a study by van Spronsen et al.[11] in children. It was postulated that since the children selected in this study were younger than those from previous published studies, their ability to control their masticatory muscles might not be fully developed. It should be noted that there were only four children in the open-bite group, causing other factors to deeply affect the final result.

To study the number of maxillary posterior teeth in contact, the number of tooth contacts was divided into four groups for measurement. Although there were no significant differences in the bite force on the left and right sides or in the maximum bite force in these four groups, a higher number of maxillary posterior teeth in contact was associated with a stronger bite force. This finding is similar to the results of a study by Ingervall and Minder[12] of children aged 7–16 years.

There was no significant effect of age or sex on maximum mouth opening. This result differs from that of a study by Sun[13] using children aged 9–12 years. This can be explained by the fact that the jawbone and masticating muscles of preschool children are still in early development. However, a regression analysis and stepwise analysis both showed that maximum mouth opening had positive relationships with bite force on the right side and maximum bite force. This suggests that the larger the mouth opening, the stronger the maximum bite force is. Fields et al.[14] previously reported such a relationship in adults.

In comparing the left and right bite forces of both sexes and at different ages, we found the average bite force on the left and right sides to be 4.2–4.5 kg with no significant differences. The average maximum bite force was 5.7 kg. We measured the bite force of both the left and right sides and chose the higher value as the maximum bite force in order to increase the accuracy of the measurement. Results demonstrated that sex did not result in significant differences in bite force on the left and right sides or on maximum bite force. Shiau and Wang[15] previously found that the maximum bite force of male children aged 7–20 years was 31.6 kg, whereas that of females averaged 22.4 kg. Chen[16] found the maximum bite force of male children aged 6–13 years to be 20.51 kg and that of females to be 14.77 kg. Sun[13] studied children ranging 9–12 years old and found a significant difference between the maximum bite force of male and female children. However, Kiliaridis et al.[9] found no difference in the maximum bite force between male and female children aged 7–9 years. Ingervall and Minder[12] showed that there was a positive relationship between bite force and age in female children after eliminating factors of the collinearity problem. Our study found that an increase in bite force was related to an increase in age from 4–6 years in preschool children. Although no significant differences were observed in the left side and maximum bite forces, there were major differences in the bite force on the right side. Bite force on the right side of children aged 5 and 6 years was significantly larger than that of children aged 4 years. Most studies previously examined children aged ≥6 years and made only limited comparisons, while our results showed that the bite forces of children aged 4–6 years significantly differed.

The average number of decayed teeth in the children studied was 3.93, and the average caries prevalence rate was 71.64%. Both the average number of decayed teeth and the prevalence rate were higher in boys than girls, and the number of decayed teeth increased with age. In addition, the number of tooth fillings and the caries filling rate also increased with age. This suggests that the tooth decay rate is still high, and that caries filling therapy should increase with age.

A regression analysis was used to examine the relationship of left and right side bite forces and maximum bite forces with variables including age, sex, height, weight, tooth decay, missing teeth, tooth fillings, occlusal pattern, vertical occlusal relationship, maximum mouth opening, and number of maxillary posterior teeth in contact. Only the maximum bite force and bite force on the right side had a significant relationship with age (using the 4-year-old group as the control), maximum mouth opening, and four versus zero, one or two maxillary posterior teeth in contact. However no significant relationship was found between the left side bite force and any other variables. It was postulated that this might have been due to the habit of chewing on one side.

Several studies[12,13,15,16] have shown that there is a significant difference in bite force between boys and girls, in which the bite force of males is considerably stronger than that of females. However, some investigators did not find such a difference.[9,17–19] Helle et al.,[18] Linderholm et al.[20] and Ranta et al.[21] suggested that bite force is positively related to growth factors such as age, body height, and body weight. Shiau and Wang[15] found that growth development affects hand grasp force more than bite force. Here, apart from the difference in the bite force on the right side due to age, there were no significant differences in bite force with sex, body height or body weight. Kampe et al.[22] compared the bite force of filled and normal teeth in teenagers aged 16–18 years and found that there

was no difference between them. In a study of 390 children aged 6–13 years, Chen[16] found that the bite force was negatively related to the caries index. Sun[13] found that among male children aged 9–12 years, bite force was positively related to the total number of teeth, and that among both male and female children, maximum mouth opening was positively related to bite force. Ingervall and Minder[12] found that the number of maxillary posterior teeth in contact was positively related to bite force in children aged 7–16 years. In particular, the molars had a stronger relationship with bite force than contact of other teeth. We found positive relationships between maximum mouth opening and the number of molar teeth in contact (4 *vs*. 0, 1 or 2) with bite force.

There were significant differences in body height among the different age groups (age 6 years >5 and 4 years, age 5 years >4 years), and also found a significant difference in weight between the 4-year-olds and both older age groups. Given the rate of growth and development among preschool children, such differences are expected. However, sex, height or weight did not have a significant relationship with maximum bite force. Also, there was no significant difference in the bite force on the left or right side for any group. The bite forces on the right side of 5- and 6-year-old children were greater than that of 4-year-olds.

Interestingly, there were no significant associations of tooth decay, tooth fillings, occlusal patterns or vertical occlusal relationship with bite force. Missing teeth negatively affected bite force on the left side. It also showed that maximum mouth opening was positively related to both the bite force on the right side and the maximum bite force. In addition, four versus zero, one or two maxillary posterior teeth in contact was also positively related to the right side and maximum bite forces.

It is difficult to accurately measure the bite force of preschool children. Preschool children do not have fully developed motor control ability; therefore, measuring bite force can be affected by environmental and psychologic factors. This means that guidance is necessary to ensure the consistency of the tests.

With regards to oral status, bite force was positively related to the number of maxillary posterior teeth in contact and negatively related to missing teeth. This suggests that teeth are crucial for proper mastication. In addition, maximum mouth opening is related to the temporomandibular joint and masticating muscles. Overall tooth decay was not related to the strength of bite force. This suggests that the severity of tooth decay may be more important than the number of teeth exhibiting decay, and the number of decayed tooth surfaces should replace the number of decayed teeth in future studies.

Conclusion

1. There were significant differences among 4-, 5- and 6-year-old preschool children in body height (6>5 years, 6>4 years, and 5>4 years) and body weight (6>4 years and 5>4 years).
2. No difference in maximum bite forces was found between the right and left sides. When comparing the maximum bite forces among different ages, maximum bite force values of 6- and 5-year-old children were significantly greater than those of 4-year-old children.
3. Oral status variables such as the number of maxillary posterior teeth in contact and maximum mouth opening showed significant positive correlations with bite force.
4. Results showed that missing teeth were negatively related to the maximum bite force.

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ELSEVIER

ORIGINAL ARTICLE

Maximum mouth opening of ethnic Chinese in Taiwan

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Background/purpose: Maximum mouth opening (MMO) is a significant diagnostic reference for many clinical conditions. However, the number of relevant studies is limited, and the relationship between age and MMO has not yet been established. The purpose of this study was to measure and analyze the MMO of ethnic Chinese adults in Taiwan and to examine the possible relationship between age and MMO.
Materials and methods: A total of 1442 adult ethnic Chinese (Taiwanese) subjects aged 20–80 years (705 males, 737 females) were randomly selected. Subjects were divided into three age groups: 20–39 years (young), 40–59 years (middle), and ≥60 years (senior), and their maximum voluntary mouth opening was measured. Independent sample *t* test and one-way ANOVA followed by the *post hoc* Scheffé test were used to examine differences in MMO relative to sex and age groups. A simple linear regression model was used to estimate the relationship between MMO and age.
Results: The average MMO for sample subjects was 49.10±6.30 mm, and the MMO of males (49.92±6.55 mm) was significantly larger than that of females (48.32±5.95 mm; $P<0.001$). MMO significantly decreased with increasing age, regardless of sex. The average MMO values were 51.11±6.47 mm, 48.45±5.76 mm, and 46.62±5.71 mm for the young, middle and senior age groups, respectively ($P<0.001$). In the regression model, it was estimated that for every 10 years, MMO decreased by about 1.4 mm in males and 0.9 mm in females. For the age range of 20–80 years, the regression equation is: MMO (mm) = 56.60 − 0.14 × age, for males; and MMO (mm) = 52.33 − 0.09 × age, for females.
Conclusion: Within the limits of this study, we concluded that both sex and age have significant influences on the MMO value of ethnic Chinese in Taiwan, and age is a significant predictor of MMO measurements.

Introduction

Maximal mouth opening (MMO) is an important diagnostic factor for dental clinicians. Limitation of mouth opening can be related to many conditions such as temporomandibular disorders, odontogenic infection, oral malignancies, oral submucous fibrosis and trauma, and can cause varying degrees of difficulty in managing and treating patients. MMO is a practical diagnostic reference especially for those with temporomandibular joint problems.[1,2] In addition, measurements of MMO can also provide necessary information for oral instrument design. Despite the clinical significance of MMO, the number of relevant studies is limited, and some would need a substantially larger sample size for valid generalizability.

Research has shown that measurements of MMO can significantly vary with age,[3–7] sex,[3–5,8] and

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Table 1. Number of cases in each of the age and sex groups

	No. of cases		
	Young (20–39 years)	Middle (40–59 years)	Senior (≥60 years)
Male (n=705)	288	240	177
Female (n=737)	296	285	156
Total (n=1442)	584	525	333

stature.[9–12] Age may be an important predictor of MMO measurements,[3,4,6] but the relationship between age and MMO has not yet been established. The purpose of this study, therefore, was to measure and analyze the MMO of ethnic Chinese adults in Taiwan and to examine the possible relationship between age and MMO.

Materials and methods

In total, 1442 adult ethnic Chinese (Taiwanese) patients aged 20–80 years (705 males, 737 females) from the Dental Department of Shin Kong Wu Ho-Su Memorial Hospital were randomly selected. All of the study subjects were generally healthy and had bilateral natural tooth stops in the anterior and posterior dentition. Patients with a history of temporomandibular disorders, head trauma, head or neck tumors, oral submucous fibrosis or congenital abnormalities in the maxillofacial area, or class III malocclusion (anterior crossbite) were excluded from the study.

Before measuring MMO, subjects were asked to rest in the visiting area for at least 15 minutes. Then, they were put in the supine position in a dental chair. Maximum voluntary mouth opening was accomplished by instructing the subjects to open their mouth as wide as they could. The linear distance between the incisal edge of the same side of the upper and lower central incisors (inter-incisal distance) was measured using a Boley gauge. For each subject, three measurements were successively made within 1 minute, and the highest value of the three measurements was recorded. The overbite was recorded too, but its implications were not analyzed in this study.

The subjects were divided into three age groups of 20-year intervals for both males and females: 20–39 years (young), 40–59 years (middle), and ≥60 years (senior). Independent sample t test and one-way analysis of variance (ANOVA) followed by *post hoc* Scheffe's test were used to examine differences in MMO relative to sex and age groups. A simple linear regression model was used to estimate the relationship between MMO and age.

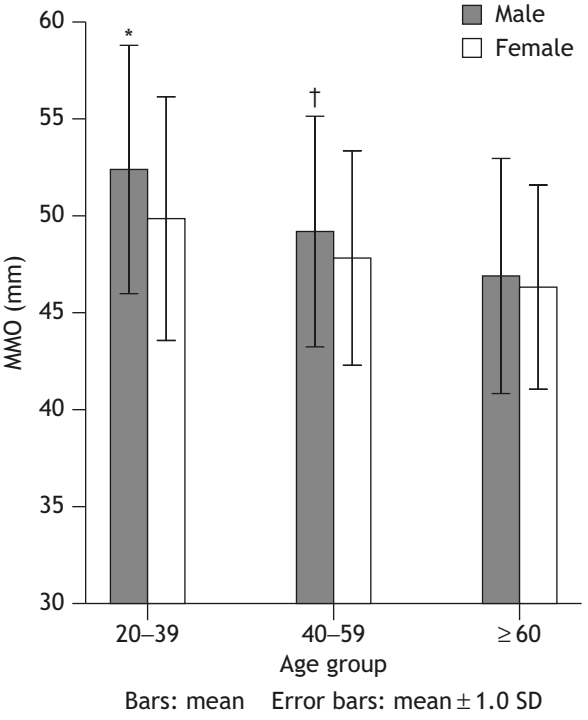

Fig. 1 Comparison between mean male and female maximum mouth opening (MMO) values by age group. *P<0.001 by independent sample t test; †P<0.01 by independent sample t test.

A two-sided P value of <0.05 was considered statistically significant.

Results

The mean age of the 1442 subjects was 46.2±15.6 years, and females (46.1±15.2 years) were slightly younger than males (46.4±16.0 years). The number of cases in each age group and the sex of those cases are presented in Table 1. The young and middle age groups were composed of 584 (40%) and 525 (36%) subjects, respectively, while the senior group was composed of fewer (333, 24%) subjects.

A comparison between the means of male and female MMO values by age group is illustrated in Fig. 1, and the mean values of MMO by age group are presented in Table 2. The average MMO for

Table 2. Mean value of maximum mouth opening by age group*

	Maximum mouth opening (mm)			P†
	Young (20–39 years)	Middle (40–59 years)	Senior (≥60 years)	
Male	52.39±6.40	49.19±5.95	46.90±6.07	<0.001
Female	49.86±6.29	47.82±5.54	46.32±5.27	<0.001
Total	51.11±6.47	48.45±5.76	46.62±5.71	<0.001

*Data are presented as mean±standard deviation; †analysis of variance.

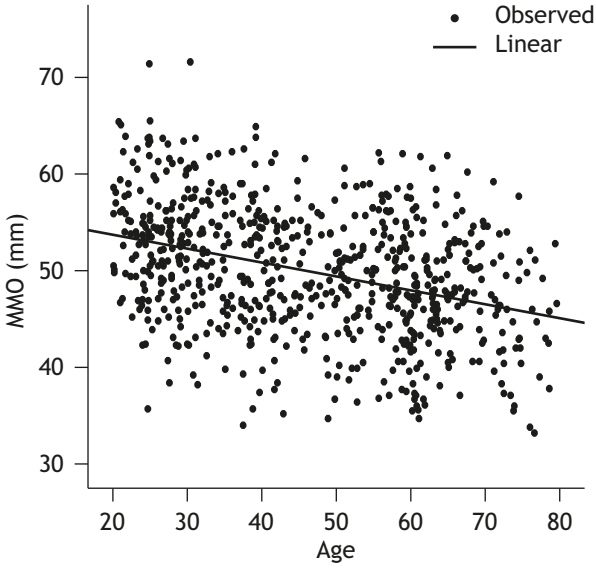

Fig. 2 Scatter and linear regression diagrams of maximum mouth opening (MMO) for males. MMO (mm) = 56.60 − 0.14 × age (age, 20–80 years). The slope, which represents the change in MMO with increasing age, was highly significant (P < 0.001).

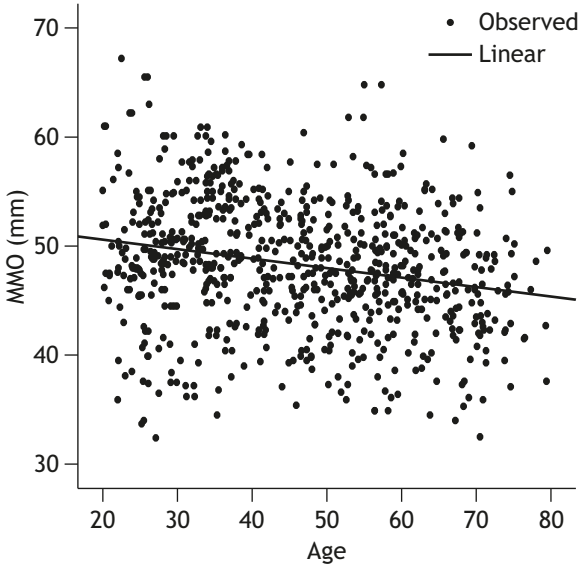

Fig. 3 Scatter and linear regression diagrams of maximum mouth opening (MMO) for females. MMO (mm) = 52.33 − 0.09 × age (age range, 20–80 years). The slope, which represents the change in MMO with increasing age, was highly significant (P < 0.001).

all 1442 subjects was 49.10±6.30 mm. There was a significant difference in MMO between males and females for both the young (P < 0.001) and middle age groups (P = 0.007), but not for the senior group (P = 0.352) (Fig. 1). However, the average MMO of males (49.92±6.55 mm) was significantly larger than that of females (48.32±5.95 mm; P < 0.001).

There were significant decreases in MMO with increasing age, regardless of sex (Table 2). Average MMO values were 51.11±6.47 mm, 48.45±5.76 mm and 46.62±5.71 mm for the young, middle and senior age groups, respectively (P < 0.001).

The tendency of MMO to decrease with age was obvious, as shown in Figs. 2 and 3 which depict scatter and linear regression diagrams in which the slopes on both graphs were negative (P < 0.001). In the regression model, it was estimated that for every 10 years, the MMO decreased by about 1.4 mm for males and 0.9 mm for females. The degree of the reduction was slightly less for females than males. Regression equations were deduced by calculating the regression coefficient and intercept. For the age range of 20–80 years, the regression equation was: MMO (mm) = 56.60–0.14 × age, for males; and MMO (mm) = 52.33–0.09 × age, for females.

Discussion

MMO varies among individuals. Research has found that MMO is influenced by a number of factors including age, sex, and anthropometric measures such as stature,[9–12] the size of the mandible[13–15] and the cranial base.[13] We found that both age and sex had significant influences on the MMO of ethnic Chinese in Taiwan.

When measuring MMO, head position is an important factor.[16–18] Higbie et al.[16] described how MMO decreases in the order of forward, natural and retracted head positions. Here, all subjects were

Table 3. Comparison of maximum mouth opening (MMO) values from different studies

Author	Country	No. of cases	Age (years)	Mean MMO (mm)
Sheppard and Sheppard[6]	USA	200	16–70	49.8
Agerberg[4]	Sweden	200	18–25	55.9 (male), 53.2 (female)*
Mezitis et al.[3]	Greece	1160	18–70	52.85 (male), 48.34 (female)
Cox and Walker[19]	Nepal	700	18–68	47.1
Placko et al.[10]	France	228	18–84	50.77

*Includes overbite correction.

placed in a supine position for measuring in order to eliminate the possible influence of different head positions.

MMO has been described either as the inter-incisal distance[3,6,10,19] or as the inter-incisal distance plus the overbite.[4] Measurement of the inter-incisal distance plus overbite means measurement of the vertical distance traveled by the mandible. However, as pointed out by Mezitis et al.,[3] the functional opening of the mouth is more important, because this is the value that actually affects chewing and dental treatment. Hence, the inter-incisal distance was used as the MMO measurement in this study.

The inter-incisal distance during active opening was used as the MMO measurement in most studies.[6,11,19] An advantage of the incisal edge distance measurement is that the measuring point is relatively more permanent and more easily determined. An extraoral measurement was also used in some studies. Wood and Branco[20] compared direct and extraoral measurements, and concluded that direct measurements using a ruler were more precise and accurate.

Among the three measurements taken for each subject, the first measurement of MMO was generally greatest in this study. This might have been caused by decreasing muscle power with succeeding measurements. However, some authors do not agree with that.[3,9,19,21] Passive mouth opening by an investigator was also employed in some studies, and the value of MMO was found to be greater than that of active MMO.[14,15,22] This may have been because of the application of external force during measurement.

The correlation between MMO and stature is controversial. Some studies described a positive relationship,[9–12,14] while others did not.[4,13] Westling and Helkimo[14] mentioned that MMO is relatively dependent on the size of the mandible, which is obviously greater in males. Since human males are generally taller and larger than females, it is conceivable that MMO would be larger in males. In addition, Dijkstra et al.[15] pointed out that differences in MMO may be attributed to the mandibular length. The majority of MMO studies, including the present study, demonstrated a sex difference between males and females.[3–5,8] Whether or not the difference is attributable to a variation in stature remains unclear. Further investigation is required to clarify possible correlations among these factors.

Studies have shown that MMO steadily increases after birth until adulthood,[6,9,12] and then gradually decreases as aging progresses.[3–7] We also found a trend for reduction of MMO with age for adult ethnic Chinese people. This decrease was about 1.4 mm in males and 0.9 mm in females for every 10 years after entering adulthood. The cross-sectional study design of our investigation, however, might not be able to demonstrate the actual MMO decrease in an individual. Longitudinal studies are required to confirm this tendency.

In addition to the factors mentioned above, racial differences are another issue of concern. Studies of MMO from different countries are described in Table 3. There is a wide range of average MMO values in different studies. Although MMO values of Asians seem to be smaller than those of Caucasians, we could find no obvious racial differences when comparing the present study results with others.

There are two limitations of this study. First, study subjects were selected from one medical center in Taipei City. Almost one-third of our dental patients come from satellite cities and nearby towns, and many of the inhabitants there are immigrants from central and southern Taiwan. To a certain extent, therefore, the sampled subjects may be representative of the island's population. Second, we deliberately classified subjects' ages into three categories to simplify the analyses, which might have influenced the results. However, this should not be a major caveat to our results considering the relatively large sample size.

In summary, within the limits of this study, we concluded that both sex and age have significant influences on the MMO value of ethnic Chinese in Taiwan, and age is a significant predictor of MMO

measurements. Further research should be carried out to determine the underlying factors that contribute to such variations.

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