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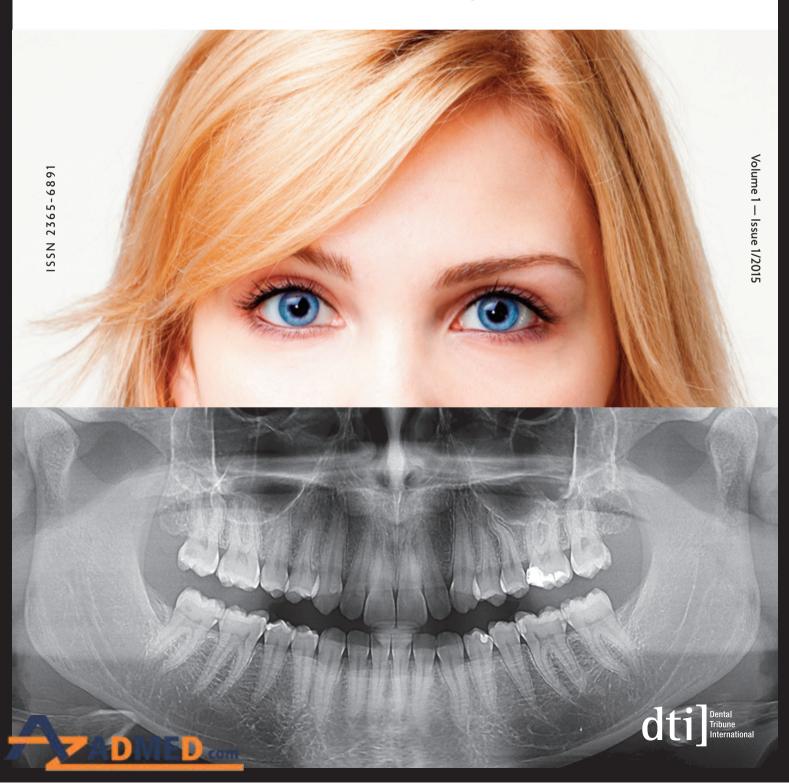
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Journal of

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Journal for periodontology, implant dentistry, dental prosthodontics and maxillofacial surgery





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Journal of

Oral Science & Rehabilitation

Dear reader,

Launching a new journal is never easy, especially in times in which a multitude of them are being published. The *Journal of Oral Science* & *Rehabilitation* originated from the efforts of a large group of researchers involved in the development of implant dentistry. Since the mid-1980s, the concept of osseointegration has had a profound influence on treatment planning in dentistry, markedly changing it. It is my view that implant dentistry has been developed to the point that it should be considered an independent dental specialty.

Even though implant dentistry is characterized by surgical aspects that fundamentally involve basic oral science, it should be considered the cornerstone of oral rehabilitation. In fact, while in the past oral rehabilitation aimed to replace missing crowns, implant dentistry has evolved to the restoration of the entire crown-root complex. This, in turn, means that this discipline not only addresses prosthetic issues, but also takes into consideration the biology of the soft and hard tissue.

The title of the journal, which refers to basic scientific knowledge and oral rehabilitation, conveys our attempts to illustrate the complexity of implant dentistry and our wish to develop a platform for researchers and clinicians so that implant dentistry may be considered an all-inclusive discipline that addresses all biological, clinical and aesthetic issues related to patients. The journal will encourage clinicians to play an active role as coordinators of oral rehabilitation, replacing their traditional view of themselves as primarily surgeons. Consequently, this will require a deeper understanding of oral surgery, oral biology, oral rehabilitation and stomatology, and we hope with this journal to contribute to the improvement of knowledge in these fields.

Prof. Ugo Covani Editor-in-Chief

Prof. Ugo Covani Editor-in-Chief

Professor of Implant Dentistry, University of Pisa, Italy Chairman of the Istituto Stomatologico Toscano, Versilia general hospital, Camaiore, Italy



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The Journal of Oral Science & Rehabilitation publishes original and highquality research and clinical papers in the fields of periodontology, implant dentistry, prosthodontics and maxillofacial surgery. Priority is given to papers focusing on clinical techniques and with a direct impact on clinical decision-making and outcomes in the above-mentioned fields. Furthermore, book reviews, summaries and abstracts of scientific meetings are published in the journal.

Papers submitted to the *Journal of Oral Science & Rehabilitation* are subject to rigorous double-blind peer review. Papers are initially screened for relevance to the scope of the journal, as well as for scientific content and quality. Once accepted, the manuscript is sent to the relevant associate editors and reviewers of the journal for peer review. It is then returned to the author for revision and thereafter submitted for copy editing. The decision of the editor-in-chief is made after the review process and is considered final.

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Immediate replacement of failed dental implants owing to periimplantitis

Abstract

Objective

This work aimed at determining whether immediate implant placement to replace infected implants can be a treatment method for periimplantitis.

Materials and methods

Immediate replacement of failed dental implants requires a conservative implant extraction technique capable of preserving as much viable soft and hard tissue as possible. An implant extraction kit was employed to extract safely dental implants failed owing to periimplantitis. The explantation socket was curetted and decontaminated before the immediate placement of new implants. The implants were then followed clinically and radiographically to assess their survival rate.

Results

Seven patients were treated to remove nine implants. The failed dental implants were extracted at a torque of 162 ± 41 N cm. The presence of dental plaque and metallic contamination due to surface cleaning was detected under a scanning electron microscope. The implants were followed for 50 ± 2 months after placement and 43 ± 3 months after loading. No implant failure was registered during this period. The mesial bone loss was 1.0 ± 0.8 mm and the distal bone loss was 1.0 ± 0.8 mm.

Conclusion

The survival of all implants and the minimal marginal bone loss would support this procedure for the immediate replacement of dental implants in sockets affected by periimplantitis.

Keywords

Periimplantitis, implant removal, immediate implant placement, implant survival.

Introduction

The high predictability of dental implants makes them the first choice for replacing missing teeth.¹⁻³ This, in addition to the long-term success of implant-supported fixed prostheses,⁴ results in the wide acceptance of implant therapy among the general population.

New improvements in clinical protocols can increase the predictability of implant therapy further and reduce rehabilitation time and cost. One such improvement is the graftless rehabilitation of missing teeth. Lazzara et al. have introduced the concept of immediate implant placement after tooth extraction.⁵ This procedure results in a reduction in the number of surgical procedures and in the time required to complete oral rehabilitation.^{5, 6} Also, immediate implant placement is one of the surgical procedures by which to achieve alveolar ridge preservation.⁴

Published data document the high success rate of immediate implant placement and support the predictability of the technique in the absence of periapical lesions.^{4,7-10} Even in the presence of periapical infection, recent research has shown that immediate placement of dental implants is possible provided there is adequate socket cleaning and decontamination.¹⁰⁻¹² In a recent randomized clinical trial, Montoya-Salazar et al. studied the influence of periapical infection on the success rate of immediately placed dental implants after tooth extraction.¹⁰ The infected sockets were curetted and decontaminated before implant placement.¹⁰ In the group of infected sockets, all implants placed were successfully osseointegrated and loaded. The three-year survival rate was 94.44% with no significant differences when compared with the noninfected socket group.¹⁰

Periimplant mucositis and periimplantitis are inflammatory diseases of bacterial origin, but bone loss only occurs in the case of peri-

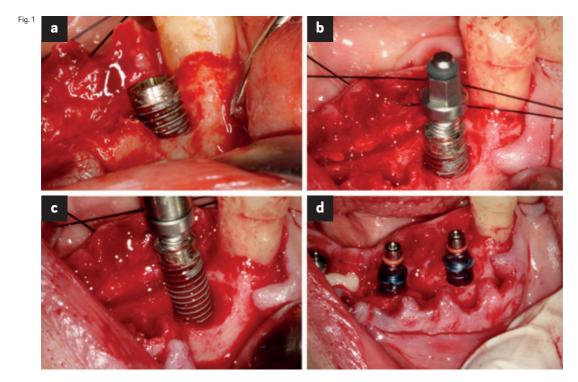


Fig. 1

(a) A nonmobile implant with advanced bone loss due to periimplantitis.
(b) An extraction ratchet placed into the implant connection.
(c) Implant removal by application of counterclockwise torque.
(d) The dental implant placed after careful curettage of the socket.

implantitis.¹³ The prevalence of periimplantitis varies between different studies and a prevalence (implant based) of 6.6-36.6% has been reported.¹⁴⁻¹⁸ Dental implant extraction may be indicated in cases of advanced bone loss around the implant. In these cases, could immediate implant replacement be considered?

No study has reported on immediate implant placement after the extraction of infected dental implants. This dearth could be related to the need for a predictable technique that permits conservative implant extraction that preserves most of the viable soft and hard tissue. At the same time, the technique should not damage the bony walls of the socket and thereby compromise the osseointegration of the new dental implant. A kit for implant extraction has been developed to fulfill the above-mentioned requirements and to enhance the possibility of achieving adequate implant stability.^{1,19}

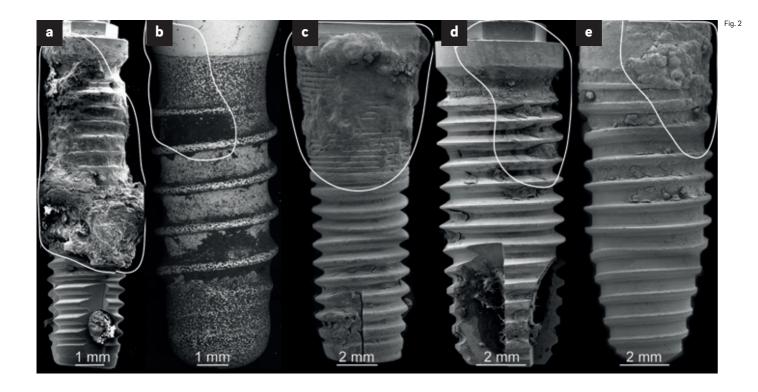
A clinical protocol that aims to decrease the bacterial load by curetting and decontamination of the socket, maintain the regenerative capacity of the surrounding alveolar walls, and achieve primary stability would result in favorable outcomes for immediate replacement of failed dental implants. In this article, we analyze the outcomes of this clinical protocol. To that end, failed, nonmobile, infected dental implants were extracted using an implant extraction kit and new

implants were immediately placed in replacement of these. Plasma rich in growth factors was placed in the explantation socket before implant placement. The extracted dental implants were analyzed under a scanning electron microscope and the patients were followed for four years.

Materials & methods

Outcome criteria

In order to achieve the objectives of the study, demographic and anamnesis data were obtained from the patients' records. Implant failure was defined as any implant lost owing to failure to achieve osseointegration or to loss of acquired osseointegration. The patient was the statistical unit for the description of demographic data. The implant was the statistical unit for the statistical description of implant location and removal torque. For the new implants, data on insertion torque, failure and marginal bone loss were collected. Implant length was used as a reference to calibrate the linear measurements on the digital panoramic radiograph. Implant survival rate was analyzed using the Kaplan-Meier method. All the statistical analyses were performed using the SPSS for Windows statistical software package (Version 15.0; SPSS, Chicago, Ill., U.S.).



Surgical protocol

In all of the patients, the same surgical protocol was followed. All of the patients received prophylactic antibiotic medication before and after surgery. Infiltrative anesthesia was administered and incisions were made to elevate a full-thickness flap (**Fig. 1**). Implant explantation was carried out using an implant extraction kit (BTI Biotechnology Institute, Vitoria, Spain). A ratchet was first engaged into the implant connection and the removal torque was exerted by a wrench in a counterclockwise direction, maintaining a perpendicular position of the assembly in relation to the implant platform (**Fig. 1**).^{1, 19}

After implant removal, the explantation socket was carefully curetted to remove any granulation tissue and immediate placement of a new implant was performed only in those sockets in which the four bony walls were preserved **(Fig. 1)**. Bone drilling for placement of the new implant was performed to remove only 0.2–0.5 mm of bone. An implant with a wider diameter than that of the failed implant was then placed using a surgical motor set at an insertion torque of 25 N cm and the implant placement was then continued manually to finish **(Fig. 1)**. Activated fraction 2 of plasma rich in growth fac-

tors (PRGF-Endoret; BTI Biotechnology Institute, Vitoria, Spain) was placed in the socket before implant placement. The surgical site was then covered with a fibrin membrane before flap closure.

In order to obtain plasma rich in growth factors,²⁰ peripheral blood was extracted by venipuncture into two 9 ml extraction tubes containing 3.8% sodium citrate (BTI Biotechnology Institute, Vitoria, Spain) and processed according to the manufacturer's instructions. To activate platelets and fibrin formation, 50 µl of calcium chloride solution (PRGF Activator, BTI Biotechnology Institute, Vitoria, Spain) per milliliter of plasma was employed. Activated fraction 1 (platelet count comparable to the peripheral blood) was employed to prepare a fibrin membrane that was compressed (Fibrin compactor, BTI Biotechnology Institute, Vitoria, Spain) to provide a thin and consistent membrane to cover the surgical site before flap repositioning and suturing with a 5-0 monofilament nylon suture. Activated fraction 2 (platelet count two to three times higher than peripheral blood) was injected into the implant bed and was used to humidify the dental implants before placement. Followup visits were scheduled to remove sutures, detect any surgical complications and fabricate the implant-supported prosthesis.

Fig. 2

Scanning electron micrographs of five implants removed owing to periimplantitis. All of the implants showed clear signs of bacterial contamination and plaque formation, accompanied by vertical bone loss. Full implant images have been reconstructed using three to five scanning electron micrographs. The areas where bone resorption around the implants occurred are highlighted (white line). A scale bar is indicated for every implant image.



Scanning electron microscopy

The extracted implants were studied under a scanning electron microscope (SEM, Quanta 200FEG, FEI, Eindhoven, Netherlands). Owing to the presence of organic components on the surface after explantation, the samples were fixed with a 2.5% glutaraldehyde solution (Sigma-Aldrich, St. Louis, Mo., U.S.) in phosphatebuffered saline (PBS, Sigma-Aldrich, St. Louis, Mo., U.S.) for 8h. The samples were then dehydrated by sequential immersion in serial diluted solutions of 0, 10, 30, 50, 70, 90 and 100 % v/v of ethanol in water. Dehydrated samples were then air-dried, carbon-coated in a sample preparation chamber with a sputtering system (Gatan Alto 1000E, Gatan, Abingdon, UK) and examined by SEM. Images were taken at 20 kV acceleration voltage. The SEM-attached energy-dispersive X-ray unit served to analyze the elemental composition of the surface remnants.

Results

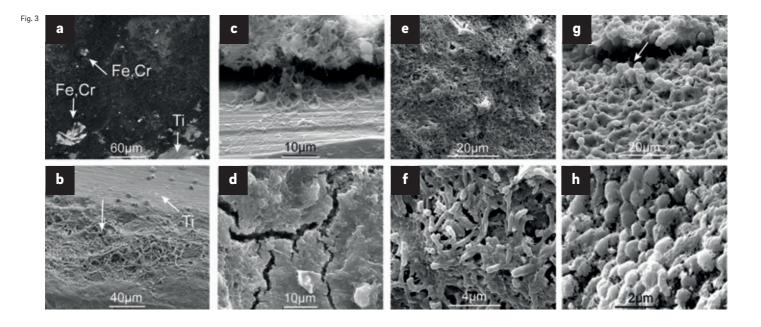
Seven patients with nine dental implants failed owing to periimplantitis were treated according to the previously described protocol. Six patients were females and the mean age was 61 ± 4 years. All patients were nonsmokers.

Six of the failed dental implants were in the maxillae. Four of the maxillary implants were in the anterior region and all of the mandibular implants were in the posterior region. The average extraction torque of the failed dental implants was 162 ± 41 N cm.

All of the explanted implants were analyzed by scanning electron microscopy. Figures 2 and 3 show representative sets of SEM images of the explanted implants. All of the implants were scanned completely at several magnifications. The lower magnification was used to obtain a general image of each of the implants extracted (Fig. 2). In these general images, traces of dental plaque can clearly be observed at the coronal parts of the implants. The area depicted over the implants (white line) corresponds to vertical bone defects detected before implant extraction. By increasing the magnification, details such as bacterial arrangements could be detected (Fig. 3). These were mainly cocci (Fig. 3: g & h) and bacilli (Fig. 3: **b-f)**, although more sensitive techniques are needed to correctly identify the particular bacterial taxonomy. Biofilms disrupted by dehydration during the preparation of the samples could also be clearly identified (Fig. 3: d). In a in Figure 3, energy-dispersive X-ray spectroscopy (EDX) showed the presence of residue of inorganic materials on the implant surface, mainly iron and chrome. These particles could come from stainless-steel surgical tools used to attempt to eliminate the plaque adhering to the surface. From the image, we can clearly see that not only did the biofilm remain on the surface, but these procedures also left contaminants on the implant surface. In **b** in Figure 3, it can be seen how the plaque preferentially

Fig. 3

Scanning electron micrographs showing details of the surfaces of the removed implants shown in Figure 2. In some cases, EDX was performed to determine the composition of particles found on the titanium (Ti) surfaces. Several explants showed abundant microbial colonization and biofilm formation (b-h). In some cases, attempts at decontamination could be traced back to the surface of the implants: In a. we performed EDX spot analysis of the particles found on the surface and found that they corresponded to surgical tools (stainless steel: Fe, Cr). Bacterial accumulation was preferential in the rough parts of the implant surfaces (arrows in b and g).



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Periimplantitis: Immediate implant replacement

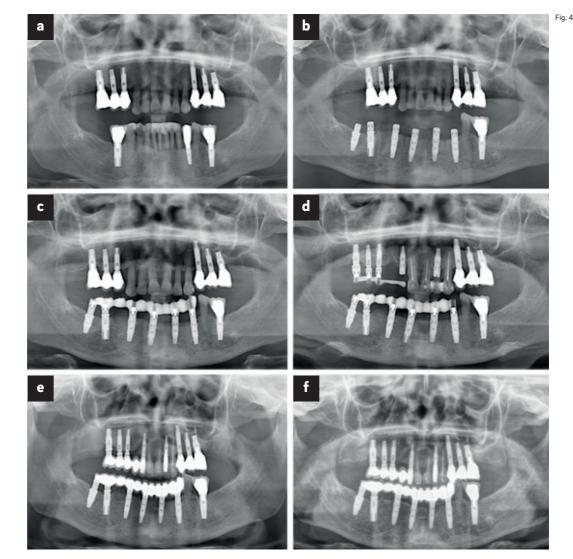
Fig. 4

(a) Preoperative panoramic radiograph showing the presence of bone resorption around the dental implants in position #34.

(b) The immediate placement of a new dental implant.
(c) The provisional prosthesis.
(d) The provisional prosthesis one year after seating.

(e) The seating of the definitive prosthesis.

(f) The clinical situation four years after implant placement.



formed on the rougher parts of the surface. Overall, the evaluation of both the post-extraction sockets and the SEM images of the implant surfaces found that most of the dental plaque remained adhered to the removed implant surfaces.

New dental implants were immediately placed at a torque of 36 ± 16 N cm. Only two implants were placed at a torque of < 25 N cm. Two short implants of 5.5 mm × 5.5 mm and 5.5 mm × 7.5 mm were placed. Three implants were 8.5 mm in length and had a diameter of 4.0, 4.5 and 5.5 mm, respectively. The rest of the implants were 10.0–13.0 mm in length and 3.75–5.0 mm in diameter, respectively.

The nine implants gave support to a single crown, four partial fixed prostheses and four

complete fixed prostheses. All of the partial and complete fixed prostheses were screw retained. The implant loading was immediate for the single crown and delayed for the partial and complete fixed prostheses.

The implants were followed for 50 ± 2 months after placement (range: 48–52 months) and 43 ± 3 months after loading (range: 40–48 months). No implant failure was registered during this period. The mesial bone loss was 1.0 ± 0.8 mm and the distal bone loss was 1.0 ± 0.8 mm. The marginal bone loss was measured on radiographs taken after 40 ± 6 months of implant loading. **Figure 4** shows a case that was treated according to the described protocol and followed for four years after implant placement.

Discussion

The results of this study support the immediate replacement of failed dental implants after extraction. The clinical protocol followed for the management of failed dental implants would enhance the possibility of osseointegration of dental implants placed in infected sites.

The positive outcomes of this protocol could be related to the decrease in the bacterial load through the removal of the infected implant. The SEM analyses showed that bacterial plaque still adhered to the implant surface upon removal, and this represents a first step in the cleaning of the extraction socket. Adequate socket curettage to remove any granulation tissue and the drilling of the socket would additionally contribute to the mechanical decontamination of the socket. Furthermore, placement of PRGF-Endoret in the socket could have had an antimicrobial effect. It has been reported that PRGF-Endoret has antimicrobial effects against Candida albicans, Enterococcus faecalis, Streptococcus agalactiae, Streptococcus oralis, Staphylococcus aureus and Staphylococcus epidermidis.^{21,22} All of these measures would reduce the risk of infection and early implant failure.

Implant primary stability is crucial for implant osseointegration and is the result of mechanical anchoring (direct contact) of the implant to the host bone.²³ Implant primary stability serves to prevent excessive implant micromovements and thus permit implant osseointegration.²⁴ The insertion torque of the dental implants placed in this study was 36 ± 16 N cm. Engelke et al. have concluded that an insertion torque of > 30 N cm is advisable to obtain adequate primary stability and a torque of ≤ 11 N cm is considered a risk factor that increases the likelihood of implant failure.²⁵

Different methods to remove osseointegrated dental implants have been described. Some of them include trephining a bone block in which the dental implant is present and the use of a thin bur at low speed with irrigation to separate the implant from the surrounding bone.^{1, 19} These methods have the limitation of being traumatic and of jeopardizing the explantation socket for future implant placement.

In this study, the use of an implant extraction kit was efficient and minimally invasive in removing dental implants while preserving the alveolar bone. This made it feasible to replace the failed implant immediately. This immediate replacement of failed implants reduced the number of surgical procedures required to treat the patient.

In a recent study,⁸¹ patients were treated with the same implant extraction kit to remove 158 nonmobile implants from the maxillae and the mandible.¹ With the kit, the conservation of hard and soft tissue is possible and implant failure can be resolved within a shorter period and at reduced cost by avoiding advanced tissue regeneration techniques.

Conclusion

Atraumatic implant explantation permitted the preservation of viable tissue and the immediate placement of a new implant. The implant survival and marginal bone loss outcomes would support the immediate placement of dental implants in a socket affected by periimplantitis.

Competing interests

EA is the Scientific Director of BTI Biotechnology Institute (Vitoria, Spain) and head of the Eduardo Anitua Foundation (Vitoria, Spain). MHA and RT are scientists at BTI Biotechnology Institute (Vitoria, Spain).

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Comparison of new bone formation

between biphasic β -TCP bovine vs. β -TCP bovine doped with silicon biomaterials in small and large defects: Experimental study in dogs

Abstract

Objective

The aim of this study was to assess the bone regeneration of critical-size mandibular defects filled with beta-tricalcium phosphate (β -TCP) bovine biomaterial in dogs compared with β -TCP bovine biomaterial doped with silicon at 12 weeks.

Materials and methods

The mandibular second, third and fourth premolars of six Beagle dogs extracted bilaterally were used in this study. Three experimental groups were evaluated: Test A (hydroxyapatite [HA]/ β -TCP granules alone), Test B (HA/ β -TCP granules plus 3% silicon) and controls (empty defect). The animals were sacrificed at eight and 12 weeks. Evaluation was performed by scanning electron microscopy, X-ray microtomography (μ CT) and histological and histomorphometric analysis.

Results

Histological evaluation showed a higher volume reduction in Test A compared with Test B (p < 0.05). Test B showed the highest values for cortical defect closure and bone formation around the granules, followed by Test A and the control group (p < 0.05).

Conclusion

Within the limitations of this animal study, it can be concluded that HA/ β -TCP plus 3% silicon increases bone formation in critical-size defects and the incorporation of 3% silicon reduces the resorption rate of the HA/ β -TCP granules.

Keywords

Bone graft, bone substitute, $\beta\text{-TCP},$ bone defect, dog, silicon.

Introduction

The reconstruction of osseous defects remains an important and unresolved issue in oral surgery. During the first year after tooth extraction, about 50% of the buccolingual ridge dimension will be lost.¹ Healing processes after dental extractions include the formation and maturation of blood clots, the infiltration of immature mesenchymal cells and the formation of a provisional bone matrix.² Immature bone becomes established quite early on in this process to be replaced later by mature trabecular bone.³ Processes of hard-tissue modeling and remodeling after tooth extraction have been studied in the dog model.^{4, 5} It was demonstrated that the socket was first occupied by a coagulum that was replaced by granulation tissue, provisional connective tissue and woven bone. More often during this healing period, bone loss occurs in the walls surrounding an extraction site with a reduction in the buccal alveolar crest.⁶⁻⁸

There are various alternatives available for the treatment of these osseous defects, the most traditional and long established of these being autogenous bone grafts, used to replace the lost bone. However, this technique has certain disadvantages given that the quantity of available bone is always limited and that it involves a second surgical site and thus increased cost and treatment time and may lead to further problems at the bone graft donor site, such as bleeding, infection and pain. For this reason, different graft materials have been developed that are intended to bring about new bone formation.⁹⁻¹² Among the alternatives available (allografts, xenografts or synthetic bone substitutes), synthetic materials can be ideal for bone regeneration given that many characteristics of such materials—mechanical properties, porosity, degradation rate and composition—can be modified according to the specific clinical requirements.^{13,14}

Biomaterial of porcine origin, which has high biocompatibility, stimulates the formation of new bone in contact with biomaterial particles. This material is used for maxillary sinus elevation prior to implant placement.¹⁵⁻¹⁷

According to Tadic and Epple, synthetic calcium phosphate bone substitutes, such as hydroxyapatite (HA), tricalcium phosphate (TCP) and biphasic calcium phosphate (BCP), offer excellent biocompatibility and are in common use as alternatives to autologous bone.¹⁸ In particular, BCP ceramics, consisting of mixtures of HA and beta-tricalcium phosphate (β -TCP), are widely used as bone substitutes. Although BCP and β -TCP are more resorbable than HA bioceramics, an even higher resorption rate is desirable for bone repair applications whenever complete implant osseointegration and bone replacement are required in the midterm.

Recently, ceramics doped with silicon at different rates have become a subject for research because of the biological benefits of silicon in their chemical composition.¹⁹ Zou et al. have reported that silicon-doped BCP enhances osteoconductivity and has been found to be nontoxic *in vivo* at concentrations as high as 50,000 ppm, producing no adverse effects in rats.²⁰

Furthermore, it has recently been postulated that silicon in the form of nanoparticles could even be bioactive and beneficial to the skeleton, although the mechanisms by which silicates regulate skeletal development and function remain unknown.²¹ The addition of silicon to TCP can improve stability, provide better structural properties and stimulate new bone formation in small animal models.^{22, 19} The literature, however, includes few examples of *in vivo* research into the benefits of incorporating 3% silicon nanoparticles into HA/β-TCP porous granular structures.

The purpose of this *in vivo* study was to evaluate the biological effects of the incorporation of 3% silicon nanoparticles into HA/ β -TCP by histological and histomorphometric analysis, scanning electron microscopy and X-ray microtomography (μ CT) evaluation in canine bone defects.

Materials & methods

Animals

Six male beagle dogs of 1.5 years of age and weighing 12–13 kg each were used in the study. The experiment protocol was designed in accordance with the Spanish and European guidelines for animal experiments. The experiment was approved by the Ethics Committee for Animal Research of the University of Murcia (Spain), in accordance with the European Union Council Directive of Feb. 1, 2013 (R.D.53/2013).

Surgical procedure

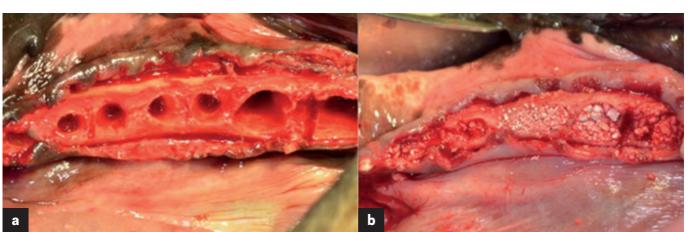
The animals were pre-anesthetized with acepromazine (0.12%–0.25 mg/kg), buprenorphine (0.01 mg/kg) and medetomidine (35 mg/kg). The mixture was injected intramuscularly into the femoral quadriceps. Then an intravenous catheter was inserted (22- or 20-gauge diameter) into the cephalic vein, and propofol was infused at a slow constant infusion rate of 0.4 mg/kg/min. Conventional dental infiltration anesthetic (articaine 40 mg, 1% epinephrine) was administered at the surgical sites. These procedures were carried out under the supervision of a veterinary surgeon.

Teeth extraction and grafting procedures

In both quadrants of the lower jaws, the second, third and fourth premolars (PM) and first molars (M1) were used as experimental sites. The alveoli corresponding to PM2, PM3 and PM4 were classified as small defects and M1 as large defects, respectively.

Teeth were sectioned with a carbide tungsten drill; the roots were removed with forceps, without damaging the remaining bony walls. Sulcular marginal incisions were made along the vestibular and lingual areas adjoining the alveoli, separating tissues to make the crestal hard-tissue walls visible **(Figs. 1a & b)**.

Prior to graft placement, the external dimensions of the post-extraction sockets (diameter) were measured using a caliper and recorded. The mean alveolar ridge measurements of the extraction sockets were as follows: 3.8 ± 0.21 mm (PM2), 4.0 ± 0.5 mm (PM3), 4.1 ± 1 mm (PM4) and 5.6 ± 0.07 mm (M1).



Figs. 1a & b

Post-extraction sockets of premolars PM2, PM3, PM4 and molar M1 **(a)**; 4BONE XBM granules, 4BONE XBM plus 3% silicon and control sites **(b)**.

Biomaterials

The study used 4BONE XBM granules (MIS Implants Technologies, Bar-Lev, Israel), a widely available bone substitute. This material consists of a completely synthetic bone graft material composed of 60% HA and 40% β -TCP, and features 70% interconnected macroporosity and microporosity. It is available as granules of 0.5-1 mm in size and is packaged in syringes that must be hydrated with physiological saline prior to use (following the manufacturer's instructions; Fig. 1b). Two different forms of this material were used: 4BONE XBM bovine granules in their manufactured form (without modification) and 4BONE XBM bovine granules plus 3% silicon, which was prepared by immersing 50 g of 4BONE XBM sequentially in a liquid solution containing 3% silicon nanoparticles for 2 h. Afterwards, the hydrated granules obtained were heated to 134 °C for 1 h to dry the liquid content and to sterilize the material prior to use.

Synthesis of hydroxyapatite

Hydroxyapatite was synthesized by solid-state reaction from a stoichiometric mixture of anhydrous calcium hydrogen phosphate (CaHPO₄, Sigma-Aldrich, St. Louis, Mo., U.S.) and calcium carbonate (CaCO₃, Sigma-Aldrich, St. Louis, Mo., U.S.) with an average particle size of < 15 μ m and a Ca/P ratio of 1.72. The mixture of CaHPO₄ and CaCO₃ was heated in a platinum crucible at 1,200 °C for 6 h at a heating rate of 10 °C/min, followed by cooling at a rate of 6.5 °C/min until it had reached room temperature. The obtained material was ground and characterized by X-ray diffraction.

Synthesis of tricalcium phosphate

Tricalcium phosphate was synthesized by solidstate reaction from a stoichiometric mixture of anhydrous CaHPO₄ (Panreac, Barcelona, Spain) and CaCO₃ (Fluka) with an average particle size of < 15 μ m and a Ca/P ratio of 1.60. The mixture of CaHPO₄ and CaCO₃ was heated in a platinum crucible at 1,000 °C for 12 h, followed by slow cooling. The obtained material was ground and characterized by X-ray diffraction.

Study design

The alveoli (small defects and large defects) corresponding to the right hemi-mandible were used as controls and were filled with 4BONE XBM granules, after rehydration with sterile saline, and the left hemi-mandible defects (small defects and large defects) were filled with 4BONE XBM granules doped with 3% silicon as a second test material. In summary, three treatment groups were created:

(i) bone defects filled with 4BONE XBM granules alone (Test A)
(ii) small defects filled with 4BONE XBM granules doped with 3% silicon (Test B)
(iii) control bone defects.

Samples were allocated to test groups using randomization software (Research Randomizer).²³

Tissue flaps were repositioned without tension-free adaptation using interrupted and horizontal mattress sutures for wound closure (Monofil 4-0, Ancladén, Barcelona, Spain). During the first week after surgery, the animals were medicated with amoxicillin (500 mg b.i.d.) and

Figs. 1a & b

ibuprofen 600 mg t.i.d.) administered systemically. The sutures were removed after two weeks. The dogs received a soft diet and a plaque control regimen that included tooth cleaning with the use of toothbrush and dentifrice, and administration of a 0.2% chlorhexidine solution three times a week until the end of the experiment (eight and 12 weeks).

Animal euthanasia

The animals were euthanized at eight (three animals) and 12 weeks (three animals) by means of an overdose of Sodium Pentothal (Abbott Laboratories, Chicago, Ill., U.S.).

Micro-CT evaluation

Immediately after sacrifice at eight or 12 weeks, µCT evaluation was performed to evaluate the residual volume of graft material. Each specimen was placed on the scanning platform of a GE eXplore Locus µCT scanner (GE Healthcare, Piscataway, N.J., U.S.) and 360 X-ray projections were collected (80 kVp, 500 mA, 26 min total scan time). The projection images were preprocessed and reconstructed into 3-D volumes (20 µm resolution). Each volume was scaled to Hounsfield units using a calibration phantom containing air and water (phantom plastic); a plug within the phantom containing HA was used as a bone mimic for bone mineral/density calculations. The 3-D data was processed and rendered (isosurface/maximum intensity projections) using MicroView (GE Healthcare). Volumes were imported into MATLAB (R2009b, MathWorks, Natick, Mass., U.S.) for automated batch analysis. Briefly, a fixed cylindrical volume of interest (14 mm diameter, 5 mm height) was applied to each volume. As each volume was calibrated using a fixed standard, calcium phosphate, cortical bone, trabecular/woven bone and scaffold content were determined using predefined Hounsfield unit thresholds (> 3,000, 2,000-3,000, 750-2,000, and 300-750, respectively).

Residual graft material was calculated as the graft/total bone volume × 100, expressed as a percentage at eight or 12 weeks for both test groups **(Table 1)**.

Sample processing

The soft tissue of each mandible was dissected to leave exposed the bone surfaces. Each

 Residual volume
 8 weeks (Mean ± S.D.)
 12 weeks (Mean ± S.D.)

 Test A
 63.72 ± 5.1%
 43.91 ± 1.2%

 Test B
 76.22 ± 1.6%*
 58.53 ± 1.1%*

Statistical significance was set at p < 0.05.

mandible was block-sectioned and the tissue fixed with 4% formalin. The samples were dehydrated in a graded ethanol series. The blocks were infiltrated with Technovit 7200 resin (Heraeus Kulzer, Hanau, Germany) and polymerized with ultraviolet light.

The polymerized blocks were then sectioned in a buccolingual direction. Three slices were obtained per site and reduced by micro-grinding and polishing using an EXAKT grinding unit (EXAKT, Norderstedt, Germany) to an even thickness of approximately 15–30 lm. The slides were stained with the Lévai–Laczkó technique; the entire circumference of each section (containing bone, grafted granules and connective tissue) was traced manually to create individual regions of interest.

Histomorphometric analysis

The percentages of residual graft material, connective tissue and new bone were calculated in relation to the total measurement area (socket walls). The central portion of each core was selected to avoid any potential bias. In this way, both the coronal (remaining native host bone) and the apical portions were excluded from analysis (using a safe margin of 1.5–2 mm). Histomorphometric measurement of the samples was conducted using ImageJ software (developed by the U.S. National Institutes of Health, Bethesda, Md.). Descriptive evaluation and morphometric measurements were performed under a Nikon Eclipse 80i microscope (Tekno Optik, Huddinge, Sweden) equipped with the Easy Image 2000 system (Tekno Optik) using 91-94 lenses (Fig. 2).

Histomorphometric evaluation

Prepared samples were photographed with a digital camera using a 20× motorized optical microscope (BX51, Olympus, Japan). These photographs were combined using computer software (cellSens Dimension, Olympus, Japan) to

Table 1 Residual volume of graft material at eight and 12

weeks.

At 12 weeks, both test groups showed a reduction in the volume of material in comparison with eight weeks.

Test B showed slower resorption expressed as higher residual volume in comparison with **Test A** at eight and 12 weeks.

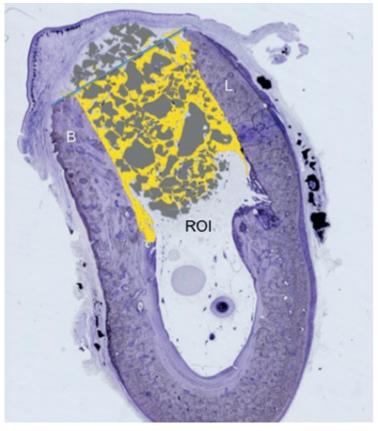


Fig. 2 Region of interest (ROI)

Each defect was identified by one orifice marked with amalgam. The ROI was a polygon delimited by the lateral walls of the defect on both sides, coronally by the upper cortical and inferiorly by the basal cortical (5× magnification, Lévai– Laczkó staining). CDC = cortical defect closure; CT = connective tissue; NB = new bone; MB = mature bone. obtain high-resolution images of the entire sample. Regions of interest (ROI) were manually delimited to facilitate identification of the different tissues present in each sample. The following variables were recorded at the two study times (eight and 12 weeks):

Cortical defect closure: Percentage of new bone present within the original defect walls in the ROI. **Residual material:** Percentage of granules present inside the ROI in relation to the total area **(Fig. 2)**.

Connective tissue: Connective tissue or the connective tissue space present inside the ROI expressed as percentage.

New bone: Percentage of new bone present inside the marrow space and between the granules in the ROI.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics software (Version 20.0; IBM Corp., Armonk, N.Y., U.S.). After descriptive analysis, the Mann–Whitney *U* test was used to evaluate the significance of differences between Test A and Test B.

The Friedman test is the nonparametric equivalent of a one-sample repeated measures design or a two-way analysis of variance with one observation per cell. Values were recorded as mean \pm standard deviation. The Student's *t*-test was applied to compare mean averages and to quantify relationships between differences.

Friedman tests the null hypothesis that k related variables come from the same population. For each case, k variables are assigned the rank 1 to k. The statistic is based on these ranks. Equal means were regarded as the null hypothesis, while the existence of significant differences between means acted as an alternative hypothesis. As significant differences between the means did exist, the null hypothesis was rejected. Significance was set at p < 0.05.

Results

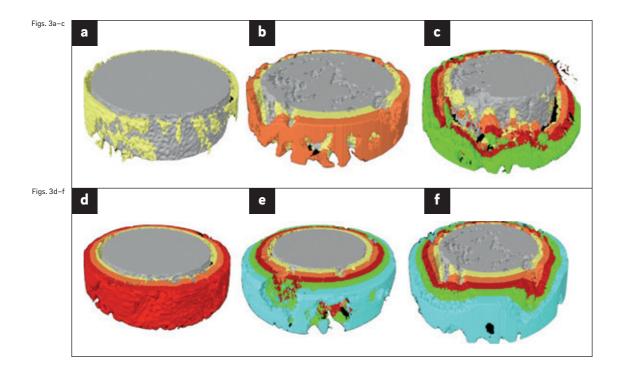
At eight and 12 weeks, no animals had been lost and the surgical zones showed no signs of inflammation. In all experimental sites, healing was uneventful. After eight and 12 weeks of healing, keratinized mucosa was observed covering the edentulous zones without dehiscences or exposure of bone or graft granules. The histomorphometric and histological results of new bone formation, residual graft and connective tissue after eight and 12 weeks of healing are described below.

Micro-CT evaluation

At eight weeks, Test B showed a higher residual volume of graft material (76.22 \pm 1.6%) in comparison with Test A (63.72 \pm 5.1%; **Figs. 3a–c**). A higher reduction in volume was observed at 12 weeks in Test B, which maintained almost 58.53 \pm 1.1% of the original volume while supporting bone formation compared with Test A (43.91 \pm 1.2%; **Figs. 3d–f & Table 1**).

Histological description at eight weeks

Cortical defect closure: All groups showed an approximation of the borders and a subsequent reduction of the original critical-size defect. Test B showed the highest defect closure ($68.71 \pm 1.2\%$); a mixture of new bone and granules formed the new cortical bone. Test A showed partial closure ($58.95 \pm 3.4\%$) and the control group showed the lowest defect closure ($11.23 \pm 1.8\%$).



Both test groups showed significant defect closure in comparison with the control group (p < 0.05; **Table 2**).

Residual material: Test B showed a higher percentage of residual material than did Test A (p < 0.05; **Table 2**).

Connective tissue: Connective tissue was higher in the control group (87.32 \pm 1.4%) compared with Tests A and B (p < 0.05; **Figs. 4a–c & Table 2**). **New bone:** New bone grew at the defect borders and between the particles in both Tests A and B. In the control group, new bone was only present at the defect borders. Bone formation commonly started in and around the 4BONE XBM graft particles. The highest amount of new bone was found in Test B, followed by Test A and the control group (p < 0.05; **Table 2**). Histological description at 12 weeks

Under fluorescence microscopy, in both groups at 12 weeks of healing, the presence of a hardtissue bridge that sealed the coronal part of the extraction socket was observed. The bridge was due to a continued small amount of new bone formation with some areas of mature bone. The material favored the growth of new bone in two different ways: first, by creating a new bridge between the defect walls and, second, through the actions of its components. At 12 weeks, the defect had completely closed in the group treated with 4BONE XBM plus 3% silicon compared with the group treated with 4BONE XBM alone. This marginal bridge was mainly of woven bone with areas of lamellar bone and some 4BONE XBM granules included inside the new bone (Figs. 4d-f).

Figs. 3a-c Micro-CT evaluation of the test groups at eight weeks.

The images represent a comparison between the two study times and the different materials tested. Dotted circles show the initial defect size, illustrating the reduction in graft volume in all groups. The control group is not shown, as control defects did not receive any graft material (a). Test A showed an increased reduction in the graft volume (b). Test B showed a medium-volume reduction (c).

Figs. 3d-f Micro-CT evaluation of the test groups at 12 weeks.

The images represent a comparison between the two study times and the different materials tested. Dotted circles show the initial defect size, illustrating the reduction in graft volume in all groups. The control group is not shown, as control defects did not receive any graft material (d). Test A showed an increased reduction in the graft volume (e). Test B showed a medium-volume reduction (f).

Table 2

Histomorphometry at eight weeks.

Post hoc multiple comparisons showed that cortical defect closure and residual material were higher for **Test B**, connective tissue was higher for the control group, and new bone formation was higher for **Tests A** and **B** in comparison with the control group.

Table 2

Histomorphometry 8 weeks	Cortical defect closure (Mean ± S.D.)	Residual material (Mean ± S.D.)	Connective tissue (Mean ± S.D.)	New bone (Mean ± S.D.)
Controlª	11.23 ± 1.8%	_	87.32 ± 1.4% ^{*b, c}	14.87 ± 1.5%
Test A ^b	58.95 ± 3.4%	44.33 ± 2.1%	16.67 ± 1.7%	41.33 ± 1.2% ^{*a}
Test B°	68.71±1.2% ^{*b, a}	$49.86 \pm 3.2\%^{*b, a}$	12.87 ± 1.1%	45.78 ± 1.9%*ª

Statistical significance was set at p < 0.05.

Table 3 Histomorphometry in 12 weeks.

Post hoc multiple comparisons showed that defect closure and residual material were higher for **Test B**, connective tissue was higher for the control group, and new bone formation was higher for **Tests A** and **B** in comparison with the control group.

Histomorphometry 12 weeks	Cortical defect closure (Mean ± S.D.)	Residual material (Mean ± S.D.)	Connective tissue (Mean ± S.D.)	New bone (Mean ± S.D.)
Controlª	26.45 ± 1.5%	_	71.65 ± 1.6% ^{*b,c}	27.54 ± 2.1%
Test A ^b	78.23 ± 1.2%	34.36 ± 1.1%	14.28 ± 1.9%	53.29 ± 1.7%*ª
Test B°	86.11 ± 1.9% ^{*b,a}	36.24 ± 1.8% ^{*b,a}	10.37 ± 1.6%	$58.92 \pm 0.8\%^{*a}$

*Statistical significance was set at p < 0.05.

Cortical defect closure: All groups showed a reduction in the defect size in comparison with the eight-week study time. Test B showed the highest defect closure (86.11 \pm 1.9%). Test A showed increased closure (78.23 \pm 1.2%) in comparison with the eight-week study time, and the control group showed the lowest amount of defect closure (26.45 \pm 1.5%). Both test groups showed significant defect closure in comparison with the control group (p < 0.05; **Table 3**).

Residual material: Test B showed a higher percentage of residual material ($39.41 \pm 1.3\%$) in comparison with Test A ($35.78 \pm 2.9\%$) and the control group (p < 0.05; **Table 3**).

Connective tissue: Connective tissue was the highest in the control group (71.65 \pm 1.6%), and was lower in Tests A and B (p < 0.05; **Table 3 & Figs. 4d–f**).

New bone: New bone was observed at the centre of the defect and at the borders in Tests A and B; in the control group, no new bone formation was found. The highest amount of new bone was found in Test B, followed by Test A (p < 0.05; **Table 3**).

Discussion

The purpose of the present work was to evaluate the benefits of incorporating 3% silicon into the composition of a biphasic synthetic graft material of HA/ β -TCP, used in critical-size defects in dogs' post-extraction defects. The current dog model has previously been used in several experiments in our laboratory to study various aspects of socket healing.^{4–6} In the studies referred to, woven bone started to form in the fresh extraction socket after one week of healing and after four weeks the socket was largely filled with woven bone (about 90% of the socket).

In our present study, cortical defect closure was evaluated by histological and histomorphometric tests at eight and 12 weeks. Excellent defect closure in both test groups with graft material granules surrounded by new bone was found.

The present experiment demonstrated that the early healing of an extraction socket grafted with HA/ β -TCP plus 3% silicon involved new bone formation and a coagulum was replaced by a provisional granulation tissue matrix in which new woven bone could be formed. The biphasic biomaterial was apparently involved in this process. HA granules were occupied by large active multinucleated cells that most likely removed calcium and phosphate ions from the small granules of the biomaterial. Thus, in the grafted sites, substantial amounts of newly formed bone could only be detected in the apical portion of the socket where the graft material was absent. In the remaining portions of the grafted sockets, a mildly inflamed provisional matrix surrounded the majority of the 4BONE XBM granules.

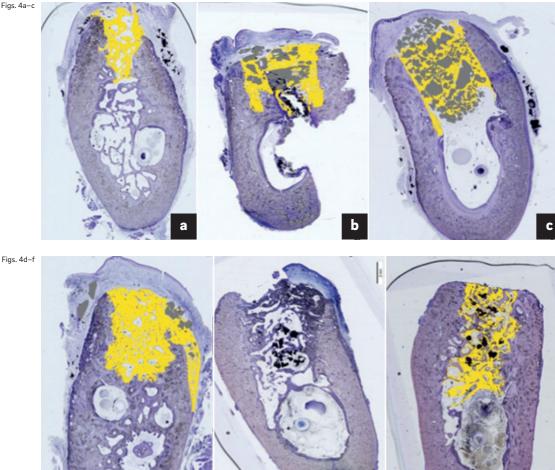
The present results agree with those obtained by El Backly et al., who compared the effects of platelet-rich plasma and a silicon-stabilized HA/ β -TCP scaffold on healing critical-size defects in rabbit calvaria, evaluating healing at four, eight and 16 weeks.²⁴

In the nongrafted control sites, large amounts of woven bone had formed in most compartments of the socket. This finding is in agreement with observations from similar experiments that investigated tissue modeling and remodeling in extraction sockets, as well as in mechanically produced defects in the alveolar ridge in dogs.^{4,5}

Most of the graft particles present in the test sites were surrounded by either a dense provisional matrix or newly formed woven bone, especially in the test group treated with 4BONE XBM plus 3% silicon. Most of the 4BONE XBM granules were in direct contact with immature woven bone.

The present study used CT and resin-embedded histology to evaluate healing evolution. The results showed that the silicon-stabilized

Table 3



closure and improved new bone formation. The material was also very stable. These results agree with research carried out by Kruse et al., who created noncritical-size defects in rabbit calvarias, filling them with three different materials: synthetic HA/silica oxide-based test granules, xenogenic HA-based granules, and synthetic HA/silica oxide-based granules.²⁵ It was found that the incorporation of silica into the HA provided comparable results to a standard xenogenic bovine mineral in terms of bone formation and defect bridging in noncriticalsize defects.

The residual material in the present study was higher in Test B, a finding that agrees with incorporating calcium silicate into β-TCP cemechanical properties. As demonstrated by 3 wt% dicalcium silicate ceramic after four

HA/β-TCP scaffold produced effective defect Velasquez et al., the addition of silicon to the β-TCP ceramic structure enhanced its properties by reducing its resorption rate and thus increasing the material's stability during the bone formation processes.¹² Similar results were obtained by Wang et al.,² who suggest that 50 or 80% silicon could promote bone regeneration by stimulating osteogenesis, angiogenesis, and the proliferation and differentiation of osteoblast-like cells.²⁶

The present study found connective tissue present in higher percentages in the control group in comparison with Tests A and B, which agrees with research carried out by De Aza et al., who implanted β -TCP and β -TCP doped with 3 wt% dicalcium silicate ceramic (B-TCPss) in several other studies that have affirmed that critical-size defects in rabbit tibiae.¹⁴ They observed organized collagen fibrils at the ment increases the material's stability and β-TCPss-bone interface for TCP doped with

Figs. 4a-c Histomorphometric comparison at eight weeks between all groups.

Control defect: Bone formation was observed only at the defect walls, and incomplete closure of the defect was observed (a).

Test A: Bone formation was observed around the peripherv of the granules; at the basal zone, new cortical formation supported by the granules was observed (b).

Test B: A reduction in the graft volume was observed, with increased bone formation around and inside the granules (c).

Figs. 4d-f Histomorphometric comparison at eight and 12 weeks between all groups.

Control defect: Bone formation was observed only at the defect walls, and complete closure of the defect was not observed (d).

Test A: Bone formation was observed around the periphery of and inside the granules (e).

f

Test B: A reduction in the graft volume was observed. with increased bone formation around and inside the granules (f).

weeks, whereas a collagen-free layer was present around the silicon-free β -TCP implants. These findings suggest that the incorporation of silicate ions into β -TCP ceramics promoted bone remodeling processes at the β -TCPssbone interface, so that the stability rate of the β -TCPss material decreased.¹⁴ Apparently, the organized collagen network facilitated the later mineralization of the collagen matrix, aided by the silica content. Moreover, the introduction of calcium silicate into porous TCP bioceramics is an effective way to prepare bioactive bone grafting scaffolds for clinical use and to control properties such as *in vivo* degradability and osteoinduction of TCP.²⁷

In the present study, new bone formation was higher in Tests B and A in comparison with the control group, which showed maximum new bone formation after 12 weeks. These results agree with earlier studies incorporating different kinds of calcium silicate into synthetic ceramic cements. The incorporation of dicalcium silicate (C_2S) into the structure of β -TCP improved the materials' integration and compatibility, facilitating its capacity to bond with natural bone and improving the rate of new bone formation in comparison with a C_2S -free β -TCP composition.¹² According to Velasquez et al., the *in vivo* behavior of β -TCP ceramic and C₂S-doped β-TCP compositions matched their in vitro behavior.¹² The bioactivity and biocompatibility of these ceramics de-

pended on their initial C₂S content. The results of the study suggest that doping of the β -TCP ceramic with 3% C₂S promotes bone mineralization during implantation into natural bone. Of all the compositions tested, the biphasic material doped with 3 wt% C₂S showed the greatest bioactivity both *in vitro* and *in vivo* and thus could be of interest for bone restorative purposes in specific applications.¹² It offers an ideal matrix in regenerative procedures and might be a promising candidate as an implant material in orthopedic, oral and maxillofacial applications owing to its mechanical and biological properties.¹²

Conclusion

Despite the limitations of this dog study, it may be concluded that the use of this biphasic material favors new bone formation and allows critical-size defects to heal without interfering in the regeneration process. The biphasic material with 3% silicon increased the dimensional stability of the graft, a feature that offers potential in areas that require dimensional stability and replacement by bone tissue.

Competing interests

The authors declare that they have no competing interests.

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Complications of postoperative swelling of the maxillary sinus membrane after sinus floor augmentation

Abstract

Objective

The aim of this article was to investigate postoperative swelling of the maxillary sinus membrane that occurred one week after sinus floor augmentation.

Materials and methods

Maxillary sinus floor augmentations were performed by the lateral window technique in 132 sites using betatricalcium phosphate (β -TCP) granules. Cone beam computed tomography (CBCT) scans were taken before surgery, the day of surgery, and one week, three months and one year after surgery. The proportion of the area of the postoperative swelling of the sinus membrane in relation to the remaining sinus cavity was determined and classified into three types: Type 1, less than one-third; Type 2, one-third to two-thirds; and Type 3, more than two-thirds of the remaining sinus cavity. The sites were divided into two groups based on the extent of lateral window coverage: Group 1, not completely covered; and Group 2, completely covered. The degree of migration of the β -TCP granules was evaluated and classified into three types: Type A, limited to the lateral window; Type B, limited to the adjacent tooth; and Type C, extending beyond the adjacent tooth.

Results

One week after surgery, swelling of the maxillary sinus membrane occurred in all 132 sites (100%). The proportion of postoperative swelling was Type 1 at 24 sites (18.2%), Type 2 at 65 sites (49.2%) and Type 3 at 43 sites (32.6%). In Group 1, the extent of migration was Type A at seven sites (38.9%), Type B at eight sites (44.4%) and Type C at three sites (16.7%). In Group 2, the extent of migration was Type A at 110 sites (96.5%), Type B at one site (0.9%) and Type C at three sites (2.6%).

Conclusion

A complication of this temporary swelling of the sinus membrane was the migration of β -TCP granules toward the buccal side through the lateral window. It is recommended that the lateral window be covered tightly to avoid the migration of bone substitute materials in the lateral window technique.

Keywords

Sinus floor augmentation, cone beam computed tomography, swelling of the sinus membrane, biological reaction, complication.

Introduction

Conventional postoperative evaluation using panoramic radiographs provides only 2-D information and may not be good enough to evaluate the outcomes of sinus floor augmentation precisely. Recently, cone beam computed tomography (CBCT) was developed, and it offers the advantage of clear image quality at very low patient radiation doses. CBCT has made it possible to evaluate biological reactions of the augmented area longitudinally using images taken in the same direction. However, there remains considerable disagreement about how to reduce the patient radiation dose from CBCT. The radiation dose depends on the CBCT unit, exposure voltage, exposure current and imaging volume. Okano et al. reported that the effective dose of the 3D Accuitomo (J. Morita, Kyoto, Japan) ranged from 18 to 66 µSv.¹ According to data

from Li, the effective dose from 3D Accuitomo was 54 µSv, while that of CB MercuRay (Hitachi Medical Systems America, Twinsburg, Ohio, U.S.) using a panoramic field of view was 560 µSv.² Lofthag-Hansen et al. reported that the calculated effective dose of 3D Accuitomo was 52-63 µSv with a volume size of 60 mm in diameter × 60 mm in length, tube voltage of 75 kV and tube current of 4.5–5.5 mA.³ Thereaugmentation using CBCT appears to be safe when the appropriate CBCT device and parameters are selected.

We found that the maxillary sinus membrane swelled one week after sinus floor augmentation. This previously unknown biological reaction could not be identified on the 2-D radiographs and has not been reported before. The aims of this clinical study were to investigate this postoperative swelling of the maxillary sinus membrane using CBCT and to evaluate its complications. Furthermore, methods to prevent these complications were considered.

Materials & methods

Selection and regulation of CBCT device

In order to limit the radiation dose, 3D Accuitomo was selected and an imaging volume size of 60 mm in diameter × 60 mm in length for the examination of maxillae was chosen. Furthermore. the tube voltage was set at 80 kV and the tube current at 2 mA for 17.5 s of exposure time. In this situation, the calculated effective dose was approximately 40 µSv.

Informed consent for CBCT scans

It was explained to all of the patients that the total radiation dose of five CBCT examinations was approximately 200 µSv and less than half of that of old-type CBCT scans. All of the patients understood the importance of CT evaluations of the sinus floor augmentation and consented to five CBCT scans over the first year after surgery.

Patients and surgery

The patient population was 112 and consisted of 35 males and 77 females who ranged in age from

25 to 77 years (mean age of 53.5 years). All patients were in good health, and 24 patients (21.4%) were smokers. The standard examination found no local or systemic contraindications to the maxillary sinus floor augmentation. In 20 patients (15.2%), the maxillary sinus floor augmentation was performed bilaterally, and the surgery was carried out in 132 sites in total.

All of the patients had been referred to our fore, postoperative examination of sinus floor clinic by their original dentists for sinus floor augmentation owing to insufficient bone volume of the posterior maxillae. Maxillary sinus floor augmentations were performed by the lateral window technique using only beta-tricalcium phosphate (β-TCP) granules (OSferion, Olympus Terumo Biomaterials, Tokyo, Japan) over the period of March 2006 to June 2012. The surgeries were performed by the same oral surgeon under local anesthesia with intravenous sedation. After the creation of the lateral window, the maxillary sinus membrane was detached from the surface of the maxillary sinus and elevated. The empty compartment created by elevating the sinus membrane was filled with β -TCP granules as the bone substitute material.

> The sites were divided into two groups based on the extent of lateral window coverage. The lateral window was not completely covered in Group 1 and was completely covered with a titanium mesh plate and microscrews or only a resorbable barrier membrane in Group 2.

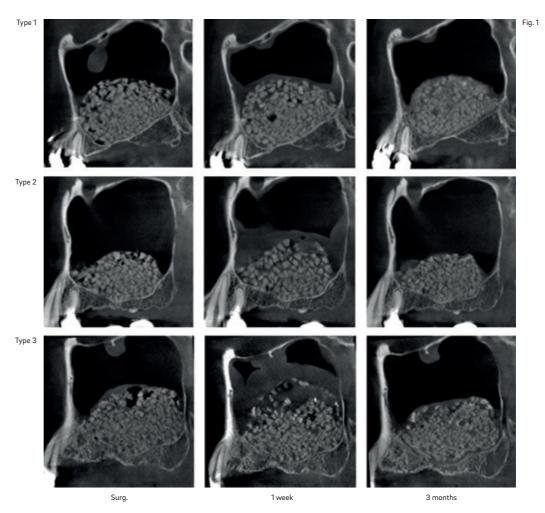
CBCT evaluation

The proportion of the area of the postoperative swelling of the maxillary sinus membrane that occurred one week after surgery in relation to the remaining sinus cavity was determined and classified into three types (Fig. 1):

- Type 1: Swelling of less than one-third of the remaining sinus cavity
- Type 2: Swelling of one-third to two-thirds of the remaining sinus cavity
- Type 3: Swelling of more than two-thirds of the remaining sinus cavity.

The degree of buccal migration of the β -TCP granules through the lateral window was classified into three types (Fig. 2):

Type A: Limited to the lateral window Type B: Limited to the adjacent tooth **Type C:** Extending beyond the adjacent tooth.



Results

CBCT evaluation Extent of postoperative swelling

Slight swelling of the maxillary sinus membrane, of up to 4 mm, was observed in 21 sites (15.9%) before the surgery. One week after surgery, postoperative swelling of the sinus membrane was observed in 132 sites (100%). Three months after surgery, the swelling of the sinus membrane had disappeared spontaneously **(Fig. 1)** in 127 sites (96.2%).

The number of sites according to the three types of postoperative swelling is shown in **Figure 3**. In approximately 80% of the sites, the extent of the postoperative swelling constituted more than one-third of the remaining sinus cavity:

Type 1: 24 sites (18.2%) **Type 2:** 65 sites (49.2%) **Type 3:** 43 sites (32.6%). Migration of β-TCP granules

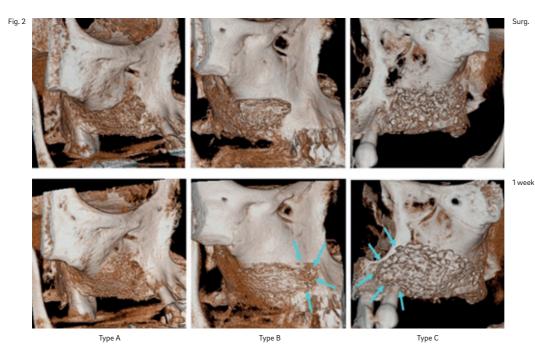
Migration of the β -TCP granules toward the buccal side through the lateral window was observed as a complication of the postoperative swelling of the maxillary sinus membrane.

Case 1 (52-year-old female, Group 1, Type 3, Type C, nonsmoker)

The sinus floor augmentation and the guided bone regeneration technique were performed simultaneously, and a barrier membrane was placed without covering the lateral window completely (**Figs. 4a–c**). One week after surgery, Type 3 swelling of the sinus membrane was observed and some β -TCP granules at the augmented area had disappeared (**Fig. 4d**, **red arrows**). According to the horizontal CBCT slice images, the β -TCP granules had migrated toward the buccal side through the lateral window and moved beyond the canine (**Fig. 4e**, **yellow arrows**). Ten days after surgery, intra-

Fig. 1

Classification of the postoperative swelling of the maxillary sinus membrane that occurred one week after the sinus floor augmentation. Three months after surgery, the postoperative swelling had disappeared spontaneously in 127 sites (96.2%).



Type A

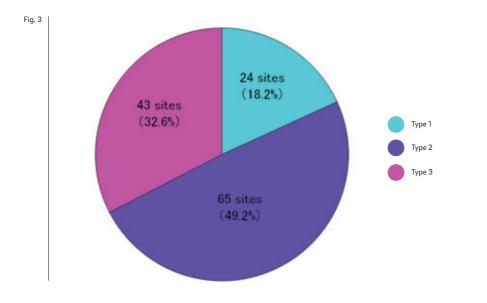


Fig. 2

Classification of the buccal migration of β -TCP granules through the lateral window.

Fig. 3

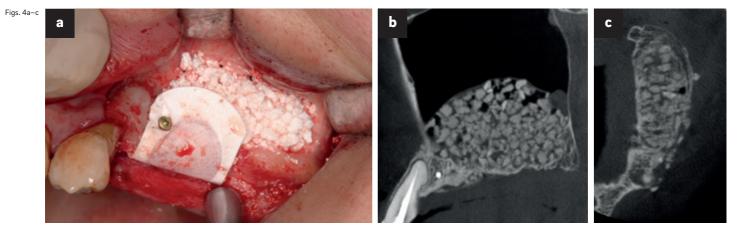
Incidence of the types of postoperative swelling of the maxillary sinus membrane (n = 132).

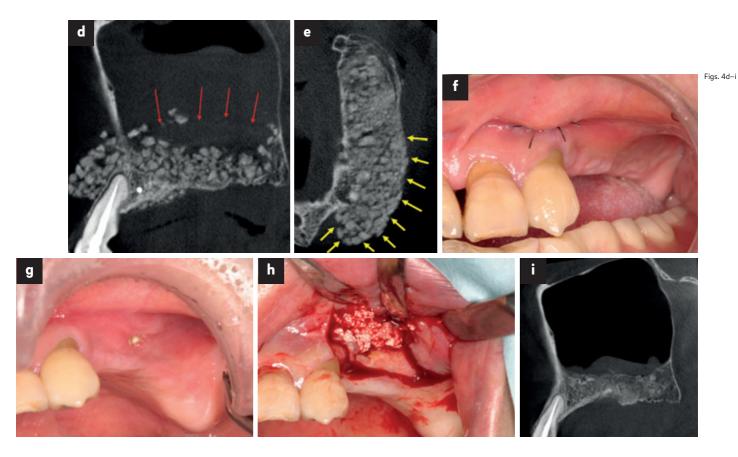
Figs. 4a-c

(a) The lateral window was not completely covered by the barrier membrane.

(b) A sagittal CBCT slice image taken on the day of surgery. The area to be augmented was filled with β -TCP granules.

(c) A horizontal CBCT slice image taken on the day of surgery.





Figs. 4d-i

(d) A sagittal CBCT slice image taken one week after surgery. Type 3 swelling of the maxillary sinus membrane had occurred. Some β -TCP granules had disappeared from the augmented area (red arrows).

(e) A horizontal CBCT slice image taken one week after surgery. The β -TCP granules had migrated toward the buccal side through the lateral window and moved beyond the canine (yellow arrows).

(f) An intra-oral photograph taken ten days after surgery. The swelling at the sulcus remained and a hard object was palpable underneath the gingiva.

(g) Six months after surgery, a β -TCP granule became exposed through the gingiva.

(h) The migrated β-TCP granules, the barrier membrane and the microscrews were removed.

(i) A sagittal CBCT slice image taken one year after sinus floor augmentation.

oral swelling remained (**Fig. 4f**) and something hard could be felt underneath the mucosa. As one β -TCP granule had become exposed, the migrated β -TCP granules and barrier membrane were removed six months after surgery (**Figs. 4g & h**). One year after surgery, the swelling of the sinus membrane remained and the volume of the augmented area had decreased considerably compared with that on the day of surgery (**Fig. 4i**).

Lateral window coverage and migration

The relationship between the degree of migration and lateral window coverage after one week of healing is shown in **Table 1**. In Group 1, β -TCP granules had migrated through the lateral window toward the buccal side in 11 sites (61.1%), and Type C had occurred in three sites (16.7%). In Group 2, Type A was seen in 110 sites (96.5%), and Type C was observed in three sites (2.6%) in spite of the full coverage of the lateral window.

Case 2 (70-year-old female, Group 2, Type 2, Type C, nonsmoker)

In this case, since the anterior wall of the maxillary sinus was very thin, the lateral window was covered with two collagen membranes and the wound was closed without a releasing incision (**Figs. 5a–d**). One week after surgery, Type 2 swelling of the sinus membrane was observed (**Fig. 5e**) and the β -TCP granules had migrated toward the buccal side from all directions (**Fig. 5f, blue arrows**).

Case 3 (43-year-old female, Group 2, Type 3, Type A, nonsmoker)

The sinus floor augmentation was performed and the lateral window was completely covered with a titanium mesh plate and fixed with three titanium microscrews (**Figs. 6a–d**). One week after surgery, Type 3 swelling of the sinus membrane was observed and the trap door had lifted up due to the pressure of the swelling (**Fig. 6e, yellow arrows**). However, no β -TCP granules had migrated through the lateral window (**Fig. 6e**), and the swelling disappeared spontaneously three months after surgery (**Fig. 6f**). One year after surgery, the titanium mesh plate and screws were removed and the implants were placed successfully (**Fig. 6g**).

Table 1	-

Figs. 5a-f

	Group 1 Not completely covered	Group 2 Completely covered
Sites	18 sites	114 sites
Туре А	7 sites (38.9%)	110 sites (96.5%)
Туре В	8 sites (44.4%)	1 site (0.9%)
Туре С	3 sites (16.7%)	3 sites (2.6%)

Table 1

Lateral window coverage and migration of β -TCP granules (n = 132).

Figs. 5a-f

(a) The area to be augmented was filled with β -TCP aranules.

(b) The lateral window was completely covered with two pieces of resorbable membrane.

(c) A sagittal CBCT slice image taken on the day of surgery.

(d) A volume-rendered image taken on the day of surgery.

(e) A sagittal CBCT slice image taken one week after surgery. Type 2 swelling of the maxillary sinus membrane had occurred.

(f) A volume-rendered image taken one week after surgery. The $\beta\text{-}\text{TCP}$ granules had migrated toward the buccal side from all directions (blue arrows).

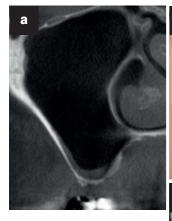
The volume of the augmented area had been retained, and a radiopaque line similar to that of cortical bone was observed at the newly formed floor of the maxillary sinus (Fig. 6h, blue arrows).

Discussion

CBCT has changed the possibilities of implant dentistry, especially in bone augmentation techniques. Its low radiation dose makes it possible to evaluate the augmented area longitudinally with images taken in the same direction. In this study, postoperative swelling of the maxillary sinus membrane was evaluated using CBCT at five stages. However, the radiation dose should be restricted even though that sinus membrane during sinus floor augmentaof CBCT is very low. Therefore, selection of the tion. Almost all of the patients reported no

CBCT device and the parameters to be used was very important to avoid the harmful influence of radiation on the patients' health.

The postoperative swelling of the maxillary sinus membrane, which occurred one week after the sinus floor augmentation, was an unknown biological reaction. It occurred in all 132 sites and disappeared spontaneously in 96.2% three months after surgery. In a monkey model,^{4, 5} inflammatory cellinfiltration was identified underneath the epithelial layer of the sinus membrane four days after sinus floor augmentation. At 20 days after surgery, the sinus mucosa presented a normal aspect with inflammatory infiltration of limited size. Thus, this temporary postoperative swelling of the sinus membrane was due to mechanical stimulation from elevation of the



Figs. 6a-h

(a) A coronal CBCT slice image taken before surgery.

(b) The area to be augmented was filled with β -TCP granules.

(c) A titanium mesh plate was placed over the lateral window and fixed with three microscrews.

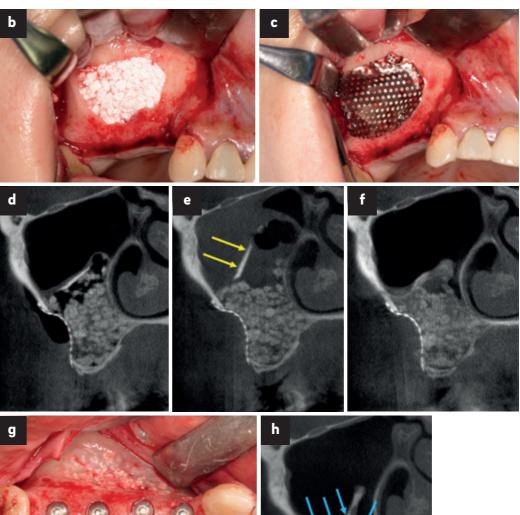
(d) A coronal CBCT slice image taken on the day of surgery.

(e) A coronal CBCT slice image taken one week after surgery. Type 3 swelling of the maxillary sinus membrane had occurred and the trap door had become dislocated (yellow arrows). However, the buccal migration of the β -TCP granules did not occur owing to the rigid coverage of the lateral window with the titanium mesh plate and screws.

(f) A coronal CBCT slice image taken three months after surgery. The postoperative swelling of the maxillary sinus membrane had disappeared spontaneously and the trap door returned to its original position.

(g) One year after surgery, the titanium mesh plate and screws were removed. The remaining β -TCP granules were observed at the lateral window and embedded in the newly formed bone. Four implants were placed successfully.

(h) A coronal CBCT slice image taken after placement of the implants. A radiopaque line similar to that of cortical bone was observed at the newly formed floor of the maxillary sinus (blue arrows).



symptoms concerning the postoperative swelling of the sinus membrane, and we had not previously observed this phenomenon.

In approximately 80% of the 132 sites, the extent of the postoperative swelling constituted more than one-third of the remaining maxillary sinus cavity. As shown in **Figure 1**, even if the size of the augmented area was almost the same, the extent of the swelling was different. These results suggest that the extent of the postoperative swelling did not depend on the area of the detachment, and it was difficult to predict the extent of swelling before surgery.

A complication of this postoperative swelling of the sinus membrane was migration of the β -TCP granules. This migration was brought about by the pressure of swelling and the direction of pressure was difficult to determine. When the pressure was toward the lateral window, the β -TCP granules migrated toward the buccal side of the alveolar bone through the lateral window. This migration of the granules led to the loss of β -TCP granules at the augmented area and resulted in unexpected poor bone formation as in Case 1. Therefore, bone substitute materials such as β -TCP granules act as a space maker for

Figs. 6a-h

bone augmentation in sinus floor elevation. Ahn Case 3. In the lateral window technique, it is et al. reported that little to no new bone formation was observed at the augmented area six months after sinus floor augmentation using blood-soaked collagen sponges as a space maker.⁶ Scala et al. concluded that the void initially occupied by the coagulum after sinus membrane elevation shrank substantially during the observation period.^{4, 5} Furthermore, Schweikert et al. reported the function of a titanium device as a space maintainer in sinus floor augmentation in monkeys.⁷ They concluded that shrinkage of the newly formed tissue was observed and the space-maintaining function of the device was in doubt. The current study found that the postoperative swelling of the sinus membrane occurred in 100% sites and the pressure of swelling was strong enough to migrate the β -TCP granules toward the buccal side. Therefore, blood-soaked collagen sponges or clots would have collapsed under the pressure of postoperative swelling of the sinus membrane.

The migration of bone substitute materials posed the risk of wound dehiscence and infection. In Case 2, the buccal migration of the β -TCP granules occurred even though the lateral window had been covered with two collagen membranes. When the postoperative swelling was Type 2 or 3, the pressure of the swelling was sufficiently strong to push the membranes out of the lateral window. Therefore, we now cover the lateral window with a titanium mesh plate and screws, as was done in sence Publishing; 2010.

recommended that the lateral window be covered tightly to avoid the migration of bone substitute materials through the lateral window.

Conclusion

One week after sinus floor augmentation, postoperative swelling of the maxillary sinus membrane occurred in all 132 sites. The swelling brought about the migration of the bone substitute materials. Furthermore, the migration of the β -TCP granules caused loss of volume at the augmented area and wound dehiscence. In order to avoid the migration of bone substitute materials, the lateral window should be covered tightly with a titanium mesh plate and screws for safety in the lateral window technique for sinus floor augmentation.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

Some photographs of Cases 1-3 were excerpted from Nosaka Y. Sinus floor elevation: avoiding pitfalls using cone-beam CT. Quintes-

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The prevalence and quantitative analysis of the Epstein-Barr virus in healthy implants and implants affected by periimplantitis: A preliminary report

Abstract

Objective

Viruses, in particular the Epstein-Barr virus (EBV), appear to play a role in the genesis and progression of human periodontitis and periimplantitis. The aim of the present study was to compare the presence of EBV in healthy periimplant sites or those affected by periimplantitis.

Materials and methods

From January 2013 to December 2014, 50 consecutive subjects with implants affected by periimplantitis and 50 subjects with healthy implants attending for a routine check-up or spontaneous visits during the study period in three private clinics (Rome and Genoa, Italy, and Belgrade, Serbia) were enrolled in this clinical study.

Quantitative real-time polymerase chain reaction assays for EBV were performed on every patient. The internal connections and external surfaces of the implants were evaluated. Independent sample *t*-tests or nonparametric Mann–Whitney *U* tests were performed to check for any statistically significant difference in each continuous variable between the two groups of patients (healthy vs. periimplantitis).

Results

Eighty-three patients (40 with healthy implants and 43 with periimplantitis-affected implants) concluded the study. The study evaluated 103 dental implants affected by periimplantitis and 197 healthy implants (mean time of loading: 5.31 ± 2.6 years). Although 28.6% of the healthy patients and 37.2% of the patients affected by periimplantitis presented at least one site with EBV, the differences were not statistically significant (p > 0.05).

Conclusion

This study did not find a clear link between periimplantitis etiopathogenesis and viral infection.

Introduction

Periimplantitis can be defined as an inflammatory process of the periimplant soft and hard tissue with or without primary infection, associated with clinically significant, progressing crestal bone loss after the adaptive phase after prosthetic loading.¹ Numerous studies have analyzed the bacterial flora associated with diseased implants to possibly understand the role played by the bacterial infection in the genesis of periimplantitis.^{2, 3} This research underlines a similar microbial profile between periimplantitis and periodontitis, with high numbers of periodontal pathogens in periimplant sites (Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens and Aggregatibacter actinomycetemcomitans) confirming data previously reported by Leonhardt et al.⁴

Viruses, in particular the Epstein–Barr virus (EBV) and other herpesviruses, appear to play a role in the genesis and progression of human periodontitis.⁵ Viruses infect periodontal B lymphocytes⁶ exerting diminished ability to defend against bacterial challenge and permitting the overgrowth of periodontopathic microorganisms.

Besides in periodontitis, recent studies have shown a correlation between periimplant infection and the presence of EBV. Jankovic et al. found a high prevalence of *Human cytomegalovirus* (HCMV) and EBV DNA in the subgingival plaque of periimplantitis sites, suggesting a possible active pathogenic role in periimplantitis.⁷ They showed a higher prevalence of EBV and HCMV in periimplantitis sites compared with healthy periimplant sites. In a split-mouth study, Verdugo et al. suggested that EBV may be a likely candidate in the etiopathogenesis of periimplant disease and that periimplantitis etiopathogenesis could be orchestrated and fueled anaerobic rods.⁸ The aim of the present study is to compare the presence of EBV in periimplantitis-affected and healthy periimplant sites.

Materials & methods

From January 2013 to December 2014, 50 consecutive subjects with implants affected by periimplantitis and 50 subjects with healthy implants attending for a routine check-up or spontaneous visits during the study period in three private clinics (Rome and Genoa, Italy, and Belgrade, Serbia) were enrolled in this clinical study.

The inclusion criteria were systemically healthy nonsmoker subjects treated with at least one implant that had been functioning for at least one year. Patients were periodontally healthy and had not taken any systemic antibiotics, antiinflammatory drugs or oral antimicrobial agents within the preceding six months.

Periimplantitis is commonly defined as reported by the Estepona consensus meeting: an infection with suppuration associated with clinically significant progressing crestal bone loss after the adaptive phase.¹ However, in the present study, to find a clinically feasible threshold, according to Renvert et al., periimplantitis was defined when an implant presented radiographic presence of bone loss of > 3 mm after implant integration, with a pocket probing depth of ≥ 4 mm, bleeding on probing and/or suppuration.9

This human case-control study was conducted in accordance with the Declaration of Helsinki and all subjects provided written informed consent prior to their entry into the study. It conformed with the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.10

All clinical examinations were performed by the same operators (LC, PP and MR) and subgingival plaque samples were collected with the **GUIDOR** Perio-Implant Diagnostic Kit (Sunstar Iberia, Barcelona, Spain). The sampling kit is intended for the collection and transport of samples containing periodontal and periimplant pathogens. Briefly, prior to subgingival plaque sampling, each tooth was isolated with cotton rolls. Absorbent paper points were inserted into the periodontal pockets. After 15s, these paper points were removed and placed into a 2 ml tube. The tubes containing the sample were sent to the Institut Clinident laboratory (France) in the pro-

by a combination of EBV and Gram-negative vided mailing envelopes. The internal connections and external surfaces of the implants were evaluated.

> Quantitative real-time polymerase chain reaction assays for the Epstein-Barr virus

Quantitative real-time polymerase chain reaction (PCR) assays were performed to detect the presence or absence of and quantify EBV DNA in the paper points. First, total DNA was isolated using the QIAxtractor DNA Plasticware and QI-Axtractor DX Reagents (Qiagen, Hilden, Germany) according to the manufacturer's guidelines. Then, real-time PCR was carried out for EBV using the Epstein-Barr virus quantitative Real Time PCR kit (Diagenode, Liège, Belgium) and the Rotor-Gene Q thermal cycling system (Qiagen, Hilden, Germany).

Briefly, quantitative real-time PCR assays were performed in a volume of 25 µl, composed of 12.5 µl of MasterMix Optima Multiplex 2X DNA, 2.5 µl of EBV primers and double-dye probe (FAM, emission 520 nm), 2.5 µl of internal control DNA, 2.5 µl of internal control primers and double-dye probe (Yellow Dye, emission 548 nm), and 5 µl of DNA extract or EBV-positive control or EBV-negative control or DNA Standard (for quantitative standard curve; all products by Diagenode, Liège, Belgium). Five EBV DNA dilutions were used for the standard curve (from 200 copies to 2,000,000 copies of EBV amplicon/PCR reaction).

Assays were carried out on the Rotor-Gene Q thermal cycling system with the following program: 50 °C for 2 min, 95 °C for 10 min, followed by 45 cycles of 15 s at 95 °C, and 60 s at 60 °C. Fluorescence signals (FAM, emission 520 nm; Yellow Dye, emission 548 nm) were measured every cycle at the end of the extension step. The resulting data were analyzed using Rotor-Gene Q Series Software (Qiagen, Hilden, Germany).

Statistical methods

Normality of variables was assessed by graphical methods (mean of histograms) and confirmed by the Shapiro-Wilk normality test and the Levene test of homogeneity of variance. All characteristics were summarized using mean (standard deviation) median (range) or frequencies (percentages).

		N (%)	Mean ± S.D.	Median [minmax.]
Group	Healthy	42 (49.4)		
	Periimplantitis	43 (50.6)		
Number of implants assessed per subject			3.5 ± 2.2	3.0 [1.0–13.0]
Number of EBV-positive implants			0.8 ± 1.4	0.0 [0.0-6.0]
Positivity %			25.1 ± 39.5	0.0 [0.0–100.0]
EBV-positive i	nternal surfaces	6 (7.1)		
Internal surfaces, EBV copies (highest value recorded)			2941.7 ± 4433.1	100.0 [100.0–9450.0]
EBV-positive external surfaces		25 (29.4)		
External surfaces, EBV copies (highest value recorded)			396643.8 ± 144106 8.4	100.0 [100.0–6840000.0]

Table 1

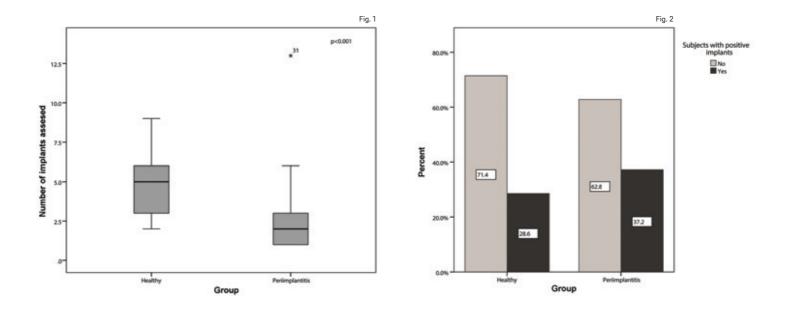
Clinical characteristics of the sample.

Table 2

Differences between the two groups of patients.

		Healthy			F	Periimplantiti	s	P-value
		Mean ± S.D	Median [minmax.]	N (%)	Mean ± S.D.	Median [minmax.]	N (%)	
Number of implants assessed per	subject	4.7 ± 1.8	5.0 [2.0-9.0]		2.4 ± 2.0	2.0 [1.0–13.0]		0.001*
Subjects with EBV-positive implants	Yes No			30 (71.4) 12 (28.6)			27 (62.8) 16 (37.2)	0.54
Number of EBV-positive implants		1.0 ± 1.7	0.0 [0.0-6.0]		0.6 ± 0.9	0.0 [0.0-4.0]		0.21
Positivity %		21.9 ± 37.7	0.0 [0.0–100.0]		28.3 ± 41.4	0.0 [0.0–100.0]		0.46
EBV-negative internal surfaces				39 (92.9)			40 (93.0)	1.00
EBV-positive internal surfaces				3 (7.1)			3 (7.0)	1.00
Internal surfaces, EBV copies (highest value recorded)		3216.7 ± 53 98.2	100.0 [100.0–9450.0]		2666.7 ± 4445.6	100.0 [100.0–7800.0]		1.00
EBV-negative external surfaces				30 (71.4)			30 (69.8)	1.00
EBV-positive external surfaces				12 (28.6)			13 (30.2)	1.00
External surfaces, EBV copies (highest value recorded)		805152.8 ± 2044335.3	2600.0 [100.0-6840000.0]		19558.5 ± 37449.4	100.0 [100.0–109200.0]		0.69

*A *p*-value of ≤ 0.05 was considered statistically significant.



Independent sample *t*-tests or nonparametric Mann-Whitney U tests were performed to check for any statistically significant difference in each continuous variable between the two groups of patients (healthy vs. periimplantitis).

Chi-squared tests or Fisher exact tests were used to assess whether a statistically significant change in proportions occurred in categorical variables between the two groups of patients in the same population.

All the analyses were computed using IBM SPSS Statistics software (Version 20.0; IBM Corp., Armonk, N.Y., U.S.). A *p*-value of ≤ 0.05 was considered statistically significant.

Results

At the end of the study, the total population consisted of 83 subjects (44 females, 39 males; mean age: 59.5 ± 11.3 years). Twentyfour females and 19 males were reported in the group with periimplantitis, and 20 females and 20 males were reported in the healthy patients. Seven patients belonging to the periimplantitis group and ten to the healthy group refused to undergo the microbiological analysis and for this reason were excluded from the studied sample.

The study evaluated 103 dental implants affected by periimplantitis and 197 healthy implants (mean time of loading: 5.31 ± 2.6 years). Clinical characteristics are shown in Table 1. In Table 2, all of the differences between the two groups of patients are expressed.

A statistically significant difference (p < 0.001) between the two groups (Fig. 1) was observed in the total number of implants assessed per patient, with a higher number in the healthy subjects (4.7 ± 1.8) compared with patients with periimplantitis (2.4 ± 2.0) . For the other evaluated variables, no statistically significant difference was detected (p > 0.05; **Table 2**).

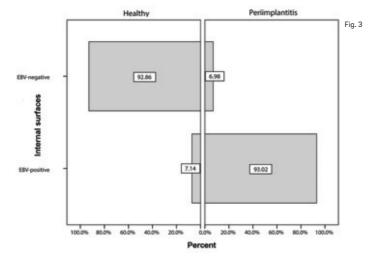
EBV was present in 12 patients (Fig. 2) in the healthy group (28.6%) and in 16 patients in the periimplantitis group (37.2%). Of the implants affected by periimplantitis, 28.3% were positive for EBV, as were 21.9% of the healthy implants (Table 2). However, the differences between the two groups were not statistically significant. The highest and the median values recorded for EBV were higher among the healthy subjects in both the internal and the external implant sites. Figures 3 and 4 show the distribution of EBV in the internal and external implant sites with lower contamination by EBV in the internal implant connections with respect to the gingival sulci.

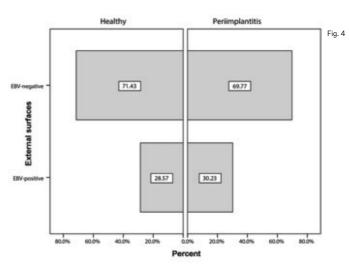
Fig. 1

Difference in the number of implants assessed between healthy patients and patients with periimplantitis.

Fig. 2

Subjects with EBV-positive implants in the two groups.





Discussion

Periimplantitis is one of the most controversial problems affecting the outcome of implant treatment. Different hypotheses have been proposed about its etiology and definition even if the term "periimplantitis" appears to have been improperly used to describe any periimplant bone loss, irrespective of the complexity of the numerous factors that may contribute to loss of marginal bone around implants.¹¹

Various authors have put forward the hypothesis that periimplantitis could be associated with EBV or HCMV. These viruses have been suggested to alter the local host immune response in combination with periodontopathic bacteria, with the potential to lead to periodontal and periimplant tissue destruction.⁷

In fact, it is well known that the great majority of the human population (more than 90%) are infected with EBV. This particular virus establishes specific communication with the host, changing the expression of its own genes in different cell types, depending on its differential status. During primary infection, EBV initially infects or al epithelial cells in the lytic form and subsequently infects B cells, where the virus assumes one of three different types of latency lifelong. Occasionally, the latent infection reactivates and changes into lytic infection. The differentiation of memory B cells with the EBV genome into plasma cells after antigen stimulation activates the lytic EBV infection. The reactivation most commonly occurs in tonsillar plasma cells, as well as in tonsillar B cells. This is one of the reasons that the saliva from immunocompromised but also immunocompetent persons often contains infectious EBV, with or without any signs of infection. EBV can change the immune response of the host with a specific influence on the immunopathogeneses of infection. The cytokines secreted during EBV infection can influence local immunopathology.

The results of the present study rejected the hypothesis that periimplantitis could be associated with EBV; in fact, no statistically significant differences were found regarding the presence of EBV in healthy or periimplantitis-affected sites (28.6% of the healthy patients and 37.2% of the periimplantitis-affected patients presented at least one site with EBV).

This is in contrast to previous studies, in which a significantly higher presence of EBV was found in subgingival samples from periimplantitis lesions than from healthy periimplant sites. In fact, both Jankovic et al. and Verdugo et al. found a significantly higher presence of EBV in the periimplantitis-affected sites.^{7,8}

The absence of significant differences between the groups could lead to the rejection of the hypothesis of the pathogenic key mechanism of viruses in the incidence of periimplantitis. However, this could be because the present study had a retrospective design. This limitation could jeopardize the detection of EBV, which is thought to activate immunological response only in the early stage of disease.¹² This could be indirectly confirmed by the fact that most parts of the sites in the periimplantitis group were under the limit of quantification. At the same time, interactions between EBV and herpesviruses could be supposed to have an effect on the host response.⁷

Fig. 3

Distribution of EBV in internal sites in both groups.

Fig. 4

Distribution of EBV in external sites in both groups.

Various authors have proposed that microbiological contamination of the internal implant connection indicates bacterial leakage along the implant-abutment interface, abutment-prosthesis interface, and restorative margins.^{13, 14} The results of the present study confirm viral leakage with the presence of EBV in the implant connection, even if with a lower detection frequency with respect to the periimplant sulci. Three periimplantitis-affected implants and three healthy implants presented with EBV with no statistically significant differences between the two groups. That slightly less of the virus was found in patients with periimplantitis-affected sites compared with healthy sites may indicate that the intensive local immune response in the case of periimplantitis reduces the amount of virus. However, in four sites, the virus was only found in the periimplant sulci. This could confirm the impressive turnover and interaction between bacteria and viruses, which could only be supposed in a retrospective study with one time point such as the present one. This particular interaction is likely mediated by cytokines produced during viral and bacterial infections. Tumor necrosis factor α, interleukin 1 and interleukin 6 have a great impact on the pathogenesis of periimplantitis. In this paper, we focused our attention on virus detection only and maybe this is the most important limitation of the study. For this reason, an observational prospective study focused also on bacteria could clarify the interactions between these microbiota and maybe the microbiological scenario of periimplantitis.

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Conclusion

Within the limits of the present study, no statistically significant differences were found regarding the presence of EBV in healthy or periimplantitis-affected sites. This study failed to find a link between periimplantitis etiopathogenesis and viral infection.

Competing interests

The authors declare that they have no conflict of interests related to this study. The study was partially supported by Institute Clinident (Aix-en-Provence, France), which provided technical support, and Sweden & Martina (Padua, Italy), which provided diagnostic test kits.

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of Epstein-Barr virus DNA and

Prevalence and quantitative analysis

Porphyromonas gingivalis associated

with Japanese chronic periodontitis

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Immunohistochemical osteopontin expression in bone xenograft in clinical series of maxillary sinus lift

Abstract

Objective

The objectives of this study were to examine osteopontin (OPN) expression in bone and anorganic bovine bone (ABB) in maxillary sinus grafts after six months of healing and to study its relationship to morphological and immunohistochemical results and to patient variables and habits.

Materials and methods

Forty maxillary sinus lift procedures were performed in 40 consecutive patients. Bone cores were obtained from implant receptor sites at implant placement for histological, morphometric and immunohistochemical studies.

Results

Histomorphometric analysis found 32.75 \pm 14.0% vital bone, 39.49 \pm 17.4% connective tissue, and 27.75 \pm 21.8% remnant ABB particles. OPN expression was diffuse in 77.5% (31/40) of ABB samples and focal in 22.5% (9/40); it was diffuse in 80% (8/10) of pristine bone samples and focal in 20% (2/10). OPN immunostaining of ABB particles was intense in 45% of maxillary sinus lift biopsies, moderate in 27.5%, mild in 10%, and absent in 17.5%. OPN expression was mainly detected at the interstitial boundary of bone with ABB particles and within osteocyte lacunae and bone canaliculi.

Conclusion

Immunohistochemical expression of OPN is related to bone remodeling and maturation changes in maxillary sinus lift procedures with ABB xenograft.

Keywords

Anorganic bovine bone, bone remodeling, intrasinus graft, immunohistochemistry, osteopontin.

Introduction

Osteopontin (OPN) human gene contains seven exons, spans ~11.1 kb, and maps to the long arm of chromosome 4 (4q13).^{1, 2} OPN is expressed by a single-copy gene as a ~34 kDa³ nascent protein that is extensively modified by posttranslational events; it is secreted as a noncollagenous acidic bone matrix of single-chain phosphoglycoproteins with diverse functions, including cell-binding activity⁴ and angiogenesis.⁵ OPN has calcium-binding properties and is expressed by cells in a wide variety of tissues, including bone, tooth and cartilage, and in activated macrophages and lymphocytes.⁶

Data are available on the structure, location and properties of OPN, but the biological function of this protein in bone remains uncertain. OPN influences bone homeostasis by different mechanisms. This polypeptide chain undergoes extensive post-translational modifications, including glycosylation, phosphorylation and sulfation, and the precise modification pattern depends on the species and tissues in which the protein is synthesized.⁷ The functional significance of post-translational modifications in OPN is poorly understood.

Bone remodeling is a regulated process in which removal via osteoclasts is followed by bone formation via osteoblasts.⁸ The presence of OPN has traditionally been interpreted as an indicator of bone formation. In bone, OPN is produced by osteoblastic cells at various stages of differentiation,⁹ including differentiated osteoblasts, and by osteocytes.^{10,11} The protein is primarily made by cells of osteoblastic lineage, and it is also expressed by fibroblastic cells in embryonic stroma¹² and at wound-healing sites.¹³ OPN is found *in situ* in osteoblasts and accumulates in mineralized bone matrix during endochondral and intramembranous ossification.¹⁴ It has also been reported to enhance osteoblastic differentiation and proliferation and increase alkaline phosphatase activity.¹⁵ Increased OPN expression at injury or infection sites likely results from the release of growth factors (e.g., platelet-derived growth factor) or cytokines (e.g., interleukin1) that activate different transcription factors, such as Fos and Jun, which are capable of upregulating OPN transcription.¹⁶

Hence, besides promoting bone formation, OPN has been implicated in bone resorption.¹⁷ Various mechanisms have been proposed to underlie this biological function. Phosphorylation of OPN appears necessary for the inhibition of biological crystal formation and for the formation of calcium carbonate crystals.⁷ OPN is a potent inhibitor of the mineralization process, because it is binding to hydroxyapatite (HA), inhibits the formation of HA crystals¹⁸ and the growth of HA crystals, ¹⁹ and promotes the inhibition of bone mineralization.²⁰ OPN plays an important role in osteoclastogenesis and osteoclast activity. Its expression is upregulated during the maturation of monocytes into macrophages,²¹ a process that presumably occurs as circulating monocytes extravasate and migrate through the tissue.¹⁶ Parathyroid hormone-induced RANKL signaling normally augments the number and activation of osteoclasts, but this increase is disrupted in the absence of OPN.²² The neutralization of OPN suppresses osteoclastogenesis in vitro, whereas its addition enhances osteoclastogenesis in OPN^{-/-} cells.²³ However, Chellaiah et al. reported an increase in the number of osteoclasts in OPN-/- mice as a compensatory mechanism for the decreased activity of OPN-/- osteoclasts, because OPN-deficient osteoclasts do not migrate and are unable to resorb bone.^{24,25} Hence, bone-resorbing activity could only partially be restored by exogenous OPN,²⁴ indicating that autocrine OPN is important for osteoclast activity.²⁶

Anorganic bovine bone (ABB) is a deproteinized, sterilized bovine cancellous bone comprising calcium-deficient carbonate apatite.²⁷ ABB is frequently utilized as a bone substitute in maxillary sinus lift procedures when insufficient autogenous cortical bone (ACB) is available for the graft.²⁸ ABB particles are similar to human cancellous bone in crystalline and morphological structure.²⁹ They are natural, osteoconductive bone substitutes that promote bone growth in periodontal and maxillofacial osseous defects. The particles provide a scaffold and a matrix for bone cell migration and are integrated into the natural physiological remodeling process. It has been suggested that deproteinized cancellous bovine bone can induce new bone formation through osteoinductive mechanisms.³⁰ It has also been reported that the application of ABB in a collagenous matrix induces the formation of membranous and endochondral bone in vivo and that ABB exerts high angiogenic activity.³¹ In previous studies, however, no OPN was detected in bovine bone slices, and no staining was observed in osteocytes, blood vessels, cement lines or typical sites of OPN expression.³² In an animal study, Araújo et al. described OPN expression in ABB particles during early healing of the post-extraction socket.¹³ Our group described a similar phenomenon in humans during late healing after sinus grafting, observing OPN expression not only in the ABB particles, but also within their canalicular system. These observations differ from previous findings in ultrastructural studies in a rat model that suggested that OPN accumulated at the mineral front and was progressively incorporated deeper into the bone, but by the further deposition of new bone matrix.33

The objectives of this study were to examine OPN expression in bone and ABB in maxillary sinus grafts after six months of healing and to study its relationship to morphological and immunohistochemical results and to patient variables and habits.

Materials & methods

Study design and subject recruitment

This clinical case series was reviewed and approved by the institutional review board of the University of Granada Faculty of Dentistry (Spain) prior to subject recruitment. The study was conducted according to the principles of the Declaration of Helsinki for experimentation with human subjects.³⁴

Totally or partially edentulous patients needing a sinus lift were screened and included in the study if they met the following inclusion criteria: age between 18 and 85 years, Physical Status I or II according to the American Society of Anesthesiologists,³⁵ absence of uncontrolled systemic disease or a condition known to alter bone metabolism (e.g., osteoporosis or diabetes mellitus), O'Leary plaque score of $\leq 20\%$,³⁶ and ≤ 5 mm of remaining bone height from measurement on a panoramic radiograph.^{37, 38} Exclusion criteria were the following: antibiotic intake in the previous three months, prescription for more than six months of medications known to modify bone metabolism (e.g., bisphosphonates or corticosteroids), pregnancy or intention to become pregnant at the time of the screening, the presence of an untreated chronic sinus condition (e.g., cyst or tumor) or sepsis, a history of cancer or radiation to the oral cavity, complications of these conditions affecting the sinus area, and consumption of > 10 cigarettes/day. Patients smoking up to 10 cigarettes/day³⁹ and alcohol consumers were included in the study. For the statistical analysis, patients who smoked \geq 1 cigarettes/day were considered smokers and those having \geq 1 alcohol-containing drinks/day (> 10 g of alcohol/day)⁴⁰ were considered alcohol consumers. Patients who met the inclusion and exclusion criteria were required to read, understand and sign the informed consent form before being enrolled in the study.

Surgical procedures

Patients were asked to take 875/125 mg amoxicillin/clavulanate (or, if allergic to penicillin, 300 mg clindamycin) t.i.d. for ten days, starting two days before the surgery to minimize infection risk. All surgical procedures were performed under local anesthesia (articaine with epinephrine 40/0.01 mg/ml, Sanofi-Aventis Deutschland, Frankfurt/Main, Germany). The procedure proposed by Galindo-Moreno et al.41 was followed, using a bone scraper (Safescraper, Meta, Reggio Emilia, Italy) to harvest ACB from the lateral wall and expose the Schneiderian membrane. After the membrane lift, sinus cavities were grafted with scraped ACB in combination with ABB particles sized between 250 and 1,000 µm (Geistlich Bio-Oss, Geistlich Pharma, Wolhusen, Switzerland); the ratio of ACB to ABB in the composite graft was 1:1 v/v.⁴² A maximum of 5 cc of graft material was used per sinus cavity. After bone grafting, an absorbable collagen membrane (Geistlich Bio-Gide, Geistlich Pharma, Wolhusen, Switzerland) was placed over the lateral aspect of the bony window. Flaps were then carefully approximated and sutured with 3-0 surgical silk (Laboratorio Aragó, Barcelona, Spain) by primary intention.

After a six-month healing period, a trephine (internal and external diameters of 3 mm and

4 mm, respectively) was used to harvest bone core biopsies from the alveolar crest in which implants were prosthetically planned. Implants (OsseoSpeed, Astra Tech, Mölndal, Sweden; Microdent, Microdent Implant System, Barcelona, Spain) were placed in a two-stage approach.

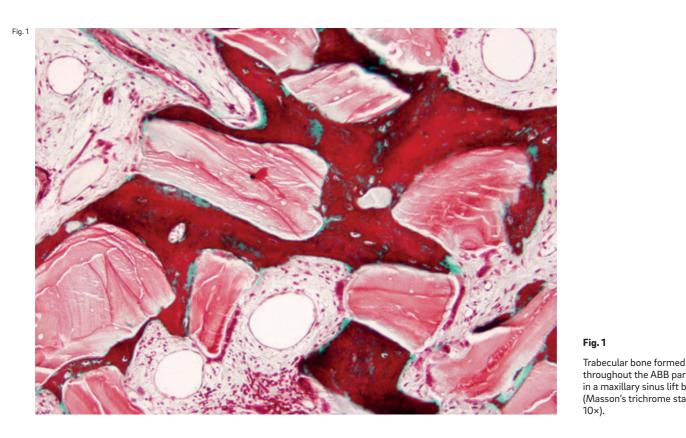
Histological study

The trephine biopsies were fixed in 10% buffered formalin for 24 h, decalcified in Decalcifier I (Surgipath Europe, Peterborough, UK), containing formaldehyde (10% w/v), formic acid (8% w/v) and methanol (1% w/v), for 24 h at 37 °C in an oven and embedded in paraffin. Then, 4 µm sections were cut along the central axis of the biopsies and dewaxed and hydrated for staining with hematoxylin-eosin, periodic acid-Schiff, Masson's trichrome and Goldner's trichrome. A millimeter scale in the eyepiece of a BH2 microscope (Olympus Optical, Tokyo, Japan) with a 40× objective was used to count osteoblasts, osteoclasts and osteocytes per mm². Results were expressed in terms of the number of positive cells per mm².

Bone histomorphometry was performed semi-automatically on Masson's trichromestained sections, assessing ten randomized images with a 10× objective, using a microscope equipped with a digital camera (DP70, Olympus Optical, Tokyo, Japan) connected to a computer and applying ImageJ software (Version 1.48; developed by the U.S. National Institutes of Health, Bethesda, Md.). Separate quantifications of vital bone, ABB particles and connective tissue were performed, expressing the results as percentages of each compartment.

Immunohistochemical analysis

Decalcified and paraffin-embedded sections were dewaxed, hydrated and heat-treated in 1mM EDTA buffer for antigenic unmasking. Sections were incubated for 60 min at room temperature with pre-diluted OPN polyclonal antibody to identify cellular and interstitial expression and with the following pre-diluted monoclonal antibodies (all from Master Diagnóstica, Granada, Spain): CD34 (clone QBEnd/10) to identify endothelial cells; CD56 (clone 56C04/123A8) to identify osteoblasts; tartrate-resistant acid phosphatase (TRAP; clone 26E5) to identify osteoclasts; CD68 (clone KP1) to identify monocytes and macrophages; and vimentin (clone V9)



Results

Histological and histomorphometric results

After six months, a normal woven and lamellar pattern of trabecular bone had formed throughout the graft in all patients who had received ABB plus ACB (1:1) grafts, and biopsies from the augmentation area contained this trabecular bone in different proportions. Image analysis revealed 32.75 ± 14.0% vital bone, 39.49 ± 17.4% connective tissue, and 27.75 ± 21.8% remnant ABB particles (Fig. 1). ABB particles were detectable in the trabecular bone in a slightly smaller proportion than in the original graft. In the pristine bone, $48.93 \pm 15.4\%$ was vital bone and 51.07 ± 20.8% connective tissue.

Immunohistochemical results

OPN expression was diffuse in 77.5% (31/40) of ABB samples and focal in 22.5% (9/40); it was diffuse in 80% (8/10) of pristine bone samples and focal in 20% (2/10). The presence of OPN immunostaining on ABB particles was intense in 45% of maxillary sinus lift biopsies, moderate in 27.5%, mild in 10%, and absent in 17.5%; it was distributed within the lacuno-canalicu-

to identify mesenchymal cells (as positive control). The immunohistochemical study was done on an automatic immunostainer (Autostainer 480S, LabVision, Fremont, Calif., U.S.), using the micropolymer-peroxidase-based method followed by development with diaminobenzidine (Ultravision Quanto, Master Diagnóstica, Granada, Spain). A millimeter scale in the eyepiece of a BH2 microscope (Olympus Optical, Tokyo, Japan) with a 40× objective was used to count the number of positive cells and vessels per mm².

Statistical analysis

The Shapiro-Wilk test was used to assess the normality of variables. After descriptive analysis, the Student's t-test or Welch test for unequal variances and the Spearman correlation coefficient (rho) were used to evaluate the significance of differences. Clinical, morphological and morphometric variables were compared between the presence and absence of OPN in cells and ABB particles using the Student's t-test. A p < 0.05 was considered significant. IBM SPSS Statistics for Windows (Version 20.0; IBM, Armonk, N.Y., U.S.) was used for the data analyses.

Fig.1

throughout the ABB particles in a maxillary sinus lift biopsy (Masson's trichrome stain, 10×).

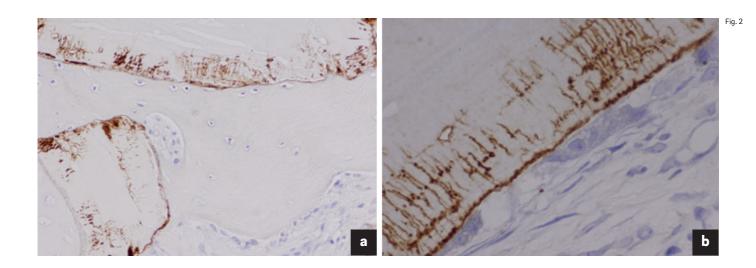


Fig. 2

Immunohistochemical expression of OPN.

(a) OPN location on boundary of ABB particles with trabecular bone (micropolymer-peroxidasebased method, 20×).

(b) Detail of OPN deposits in lacuno-canalicular network and location on boundary of ABB particles near to osteoclast (micropolymerperoxidase-based method, 60×). lar system of ABB particles and on their surface cient = 0.622, p = 0.008, Spearman test), and close to osteoclast-like cells (Fig. 2). osteoblasts (rho coefficient = 0.391, p = 0.048,

At six months, OPN expression was principally observed at the interstitial boundary of bone with ABB particles and within lacunae and bone canaliculi, forming a star shape **(Fig. 2)**, with no expression in the trabecular bone or interstitium. Cortical OPN expression was directly correlated with the number of osteocytes per mm² (rho coefficient = 0.405, p = 0.045, Spearman test), with OPN expression in cement lines (rho coefficient = 0.757, p < 0.001, Spearman test) and with OPN expression in osteocytes (rho coefficient = 0.432, p = 0.012, Spearman test).

A direct correlation was found between OPN expression in ABB particles and in monocytes and macrophages (CD68-positive cells; rho coefficient = 0.583, p = 0.009, Spearman test). OPN expression in osteocytes was inversely correlated with the number of osteoblasts (CD56-positive cells) per mm² (rho coefficient = -0.828, p = 0.042, Spearman test).

Avascular bed was formed in the nonmineralized tissue by vessels of different calibers in the graft area and by capillary vessels among the adipocytes in the bone marrow area, with a mean in the biopsies of 86.28 \pm 56.6 CD34positive vessels per mm². OPN expression in osteocytes was directly correlated with the number of vessels per mm² (rho coefficient = 0.828, *p* = 0.042, Spearman test).

TRAP expression was directly correlated with the count per mm² of osteoclasts (rho coefficient = 0.532, p = 0.015, Spearman test), monocytes and macrophages (rho coeffi-

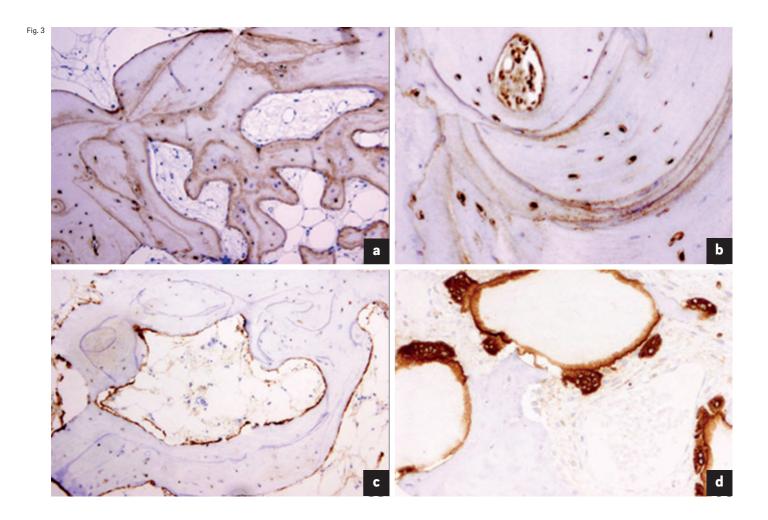
cient = 0.622, p = 0.008, Spearman test), and osteoblasts (rho coefficient = 0.391, p = 0.048, Spearman test). TRAP expression was correlated with the local and diffuse expression of OPN (rho coefficient = 0.439, p = 0.022, Spearman test; **Fig. 3**).

Discussion

In this study of bone xenografts in a clinical series of maxillary sinus lift, immunohistochemical OPN expression was detected not only in osteocytes and on ABB particles, but also within the lacuno-canalicular system of ABB particles and close to osteoclast-like cells on their surface.

Bone formation or resorption requires adhesion molecules (arginine–glycine–asparagine sequences), such as fibronectin, fibrinogen, vitronectin, Type I collagen, OPN or bone sialoprotein, to attach osteoblasts or osteoclasts to surfaces for remodeling.⁴³ Because ABB particles are free of proteins,⁴⁴ protein expression on the particles must derive from proteins absorbed from the environment. The above chemotactic factors may have stimulated and directed the migration of cells to the foreign material.¹³

Microchannel pores (< 10 µm) of ABB may be relevant for osteogenic cell attachment, migration, proliferation and differentiation.⁴⁵ At the same time, the inner surface of ABB particles becomes considerably enlarged, favoring the formation of new vessels and therefore the inward growth of new bone within the particles.³¹ A greater microporosity also expands



the scaffold surface and may enhance cytokine adsorption.46 The interconnectivity of the pores in ABB particles and their hydrophilic properties explain the presence of OPN within the lacuno-canalicular system. This explanation is supported by the distribution of OPN found in our samples from the surface to the core of the particles, with the lacuno-canalicular system of the remnant ABB particles clearly depicted by the staining. Although the biological relevance of this finding remains unclear, it may be related to the cellular recolonization and revascularization of ABB that has been observed after six months of healing.³¹ In this previous study, a direct correlation was found between OPN expression in osteocytes and CD34-positive endothelial cells (rho coefficient = 0.828, p = 0.042, Spearman test). As already noted, one of the functions of OPN is related to angiogenesis,⁵ which is impaired in OPN-deficient mice.⁴⁷ Images of ABB particles after six months of graft maturation were compatible with neovascularization and central re-

sorption, implying the integration of this biomaterial within the functional and biomechanical system of the neoformed bone.

Whereas most noncollagenous proteins are more or less homogeneously dispersed throughout bone, ultrastructural immunocytochemical studies have consistently found OPN to be predominantly distributed at cement lines in remodeling bone and at laminae limitans.48,49 These sites represent matrix-matrix and cell-matrix boundaries, respectively, and are therefore important in the bone formation process.⁶ Cement lines demarcate the boundary between older and newer bone and characteristically have a high OPN content. The origin of OPN in the cement line has been controversial, $^{\rm 32,\,50}$ and osteoclasts and osteoblasts may both be involved. OPN may initially play a role in osteoblast adhesion or early calcification events in the cement layer.⁵¹ Subsequently, the more diffuse distribution of OPN throughout the bone matrix may influence osteoclast activity during resorption and the transformation of woven and lamellar bone.⁶ Our findings support this proposi-

Fig. 3

(a) Immunohistochemical expression of OPN in osteocytes and cement lines in pristine bone (micropolymerperoxidase-based method, 10×).

(b) Detail of OPN expression in osteocytes (micropolymerperoxidase-based method, 20×).

(c) Immunohistochemical expression of CD56 in osteoblasts around trabecular bone (micropolymerperoxidase-based method, 10×).

(d) Immunohistochemical expression of TRAP in osteoclasts around ABB particles (micropolymerperoxidase-based method, 40×). tion, given that OPN expression in the cement lines may indicate bone formation or destruction as a function of the cells attached to the particle surface. However, this dual behavior of OPN remains unclear.

OPN can be expressed in numerous tissues⁵² and by multiple cell types,14 including osteoblasts and osteoclasts.53 Kunii et al. confirmed the expression of OPN mRNA and protein in human osteocytes³² using different antibodies.⁵² Osteocytes have multiple functions and regulate different processes in bone homeostasis.54 After six months of healing, osteocytes present in the new vital bone in our biopsies consistently expressed OPN. OPN expression in osteocytes was inversely correlated with the count of osteoblasts (CD56positive cells) per mm² (rho coefficient = -0.828, p = 0.042, Spearman test). Araújo et al. detected OPN expression on ABB particles in dogs during the early stages of healing.¹³ According to their study, after the migration of polymorphonuclear cells to the ABB surface, these cells are replaced by TRAP-positive cells (osteoclasts), which will remove material from the surface of the xenograft.¹³ Subsequently, osteoblasts will attach to the remodeled surface and bone apposition will begin. However, OPN behavior is likely different during the late stage of healing. In the present samples, osteoblasts were rarely observed in the ABB particles, and a significant inverse relationship was found between OPN expression and CD56 expression (rho coefficient = -828, p = 0.04, Spearman test). This higher expression with fewer osteoblasts and the positive correlation between OPN and CD68 expression (rho coefficient = 0.583, *p* = 0.009; rho coefficient = 0.938 p = 0.006; Spearman test, respectively) indicate that OPN expression on ABB particles promotes resorption at six months of healing. In support of this hypothesis, it is known that OPN can both activate osteoclasts for bone matrix resorption and induce their migration in an $\alpha_{\nu}\beta_{3}$ integrin-dependent manner.^{55, 56} Nakamura et al. demonstrated that $\alpha_{\nu}\beta_{3}$ integrin plays a key role in osteoclast migration, which is essential for efficient osteoclastic bone resorption.57 Perrotti et al. reported that multinucleated cells generated on ABB were positive for the $\alpha_{\nu}\beta_{3}$ subunit of vitronectin, 58 the main integrin-mediating osteoclast cell attachment to the bone matrix;59 hence, this mechanism may be functional on ABB particles. OPN is a ligand for the $\alpha_{\nu}\beta_{3}$ integrin through an RGD sequence.⁶⁰ The osteoclast cell membrane must be sealed to the substrate by means of cell surface

receptors and proteins of the integrin family before beginning its proteolytic activity. The binding of OPN to the $\alpha_{v}\beta_{3}$ integrin in the sealing zone or podosomes appears essential to the reorganization of the actin cytoskeleton for osteoclast motility.⁶¹ Additionally, osteoclast adhesion and osteoclast migration are mediated by phosphorylated OPN,⁶² and this biological event is regulated by endogenous TRAP.63 Interestingly, TRAP and OPN expressions showed similar patterns on ABB particle surfaces at six months of healing in our samples, indicating the close relationship between OPN expression and the osteoclastic resorption of ABB. Questions have been raised about this resorption of the particles,^{64, 13} but this proposition is supported by our histological findings on these particles of bone-remodeling units with multinucleated cells in different stages of differentiation (CD68 positive and TRAP positive) that promote these phenomena. In our view, these results may in part explain OPN-mediated ABB resorption during the late stage of graft healing.

Our findings support the proposal that osteoclasts are the source of OPN in bone cement lines during remodeling.³² The detection of OPN expression within lacunae represents clear evidence of the secretion of OPN by osteoclasts, because exogenously added OPN has no access to these sites.²³

Conclusion

Immunohistochemical expression of OPN is related to bone remodeling and maturation changes in maxillary sinus lift with ABB xenograft.

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Competing interests

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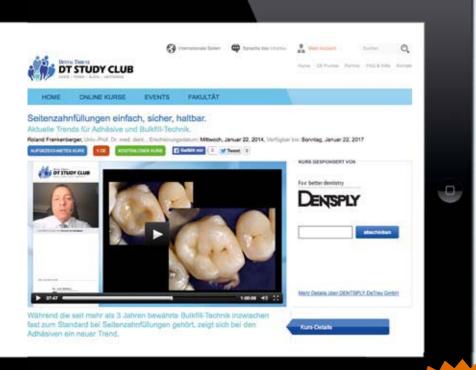
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Evaluation of the effect of supervised plaque control

after the surgical removal of partially erupted mandibular third molars on the periodontal condition distal to second molars affected by localized periodontal disease:

A randomized blind clinical study

Abstract

Objective

The objective of this study was to evaluate the effect of supervised plaque control on the periodontal condition distal to the second molars after the extraction of partially erupted mandibular third molars.

Materials and methods

All of the 33 patients had a probing pocket depth (PPD) of 7.4 mm (S.D. \pm 1.5) and bone loss of 4.4 mm (S.D. \pm 2.0) distal to the second molars. After the surgical extraction of the third molars and subgingival debridement of the distal site of the second molar, the patients were randomly assigned to a test group or a control group. The test group received oral hygiene information and professional dental hygienist treatment one month after the extraction. The control group did not receive any specific information or treatment.

Results

At six months, the percentage reduction of plaque at the distal sites of the second molars from the baseline value was 69% and 47% in the test and control groups, respectively. The PPD reduction was 3.4 mm and 3.5 mm in the test and control groups, respectively. These values were statistically significant compared with baseline (p < 0.001). The radiographic measurements found a bone gain of 0.7 mm and 0.8 mm in the test and control groups, respectively.

Conclusion

The removal of the third molar improved access for self-performed plaque control. This, together with subgingival debridement, improved the periodontal status at the second molars.

Keywords

Extraction, third molar, semi-impacted tooth, local periodontitis, plaque control.

Introduction

Population studies have suggested that the visible presence of a third molar increases the risk of periodontal inflammatory disease at second molars^{1–3} adjacent to both symptomatic and asymptomatic third molars.^{4, 5} This was also the case in young subjects (18–40 years of age) with low severity of periodontal disease in the overall dentition.^{1,3,5}

In young subjects, when the early stages of periodontal pathology are detected in the third molar region, the removal of third molars may improve the periodontal status at the distal sites of second molars.^{6,7} Studies also indicate that the removal of third molars in younger individuals compared with older subjects decreases the time needed for the extraction and decreases the risk of complications. The age of 25 appears to be critical, after which complications increase more rapidly.⁸

In a retrospective study of 215 patients, Kugelberg et al. found that two years after the surgical removal of impacted mandibular third molars, 43.3% of the cases exhibited a probing pocket depth (PPD) of > 7 mm and 32.1% showed intrabony defects of > 4 mm distal to the mandibular second molars.⁹ The postoperative plaque control score indicated that in most of the participants the level of plaque control at the distal surface of the second molar was not optimal. Leung et al. showed that a regime of strict plaque control prevented residual pockets at periodontally involved second molars six months after the removal of the third molar.¹⁰ Kan et al. investigated the periodontal condition distal to mandibular second molars 6-36 months after routine surgical extraction of adjacent impacted third molars in 158 subjects under 40 years of age.¹¹ Three possible risk indicators were associated with localized increased PPD at the distal surface of the mandibular second molar: third molar mesioangular impaction; pre-extraction signs of bone loss; and inadequate post-extraction local plaque control.¹¹

The aim of the current study was to evaluate the effect of supervised plaque control after the extraction of partially erupted mandibular third molars on the periodontal condition distal to the second molars.

Materials & methods

Patient recruitment

The subjects involved in this study were selected from consecutive patients referred to the department of oral surgery at Södra Älvsborg Hos-

pital (Borås, Sweden) for extraction of mandibular third molars. The protocol of the study was approved by the Central Ethical Review Board at the University of Gothenburg (Sweden). The patients who met the inclusion criteria were informed about the diagnosis and treatment plan. They were also informed of the purpose of the study and gave their consent for participation.

In order to be included in the study, the patients had to be 18 years of age or older, have a partially erupted third molar in need of extraction, present with bone loss distal to the adjacent second molar of > 2 mm (as measured from available radiographs) and a PPD of \geq 6 mm, but otherwise be healthy from a periodontal perspective (i.e., no bone loss of > 1 mm and no PPD of \geq 5 mm at the residual dentition; **Fig. 1a**). Patients with medical conditions that could compromise healing at the extraction site were excluded.

Clinical examination

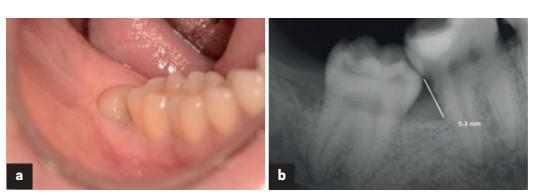
The following clinical variables were recorded at the baseline examination by one examiner (ASP) at the distal surface of the second molars:

Plaque index (PI): The presence or absence of plaque was determined after staining with disclosing solution (Rondell Blue, Nordenta, Enköping, Sweden) at the distal sites of the second molars.

Bleeding/suppuration on probing (BoP/Sup): The presence or absence of bleeding/suppuration up to 15 s after probing was determined.

PPD: Pocket depth was measured in millimeters with a manual PCP-15 periodontal probe (Hu-Friedy, Leimen, Germany) to the nearest millimeter at the distobuccal, distal and distolingual surfaces of the second molars.





Figs. 1a & b

Partially erupted third molar in a patient without periodontal disease except distal to the second molar **(a)**. The bone-level measurement before extraction of the third molar **(b)**. **Radiographic bone loss:** The presence of bone loss distal to the second molar of > 2 mm was assessed on available digital bitewing or periapical radiographs **(Fig. 1b)**.

Treatment

Surgical phase

All of the patients received an analgesic prior to surgery (1 g Alvedon, AstraZeneca, Mölndal, Sweden). The treatment was performed under aseptic conditions. After local anesthetic had been administered, a mucoperiosteal incision was placed using a #15 Bard-Parker blade according to the technique described by Nordenram.¹² Bone removal and sectioning of the third molar were performed with a low-speed rotary instrument under constant irrigation with sterile saline. After tooth extraction, the granulation tissue and follicular remnants were removed from the extraction alveolus. Correction of the anatomical architecture of the bone was performed under saline irrigation. The distal surface of the second molar was carefully scaled with hand instruments. After saline irrigation, the flap was repositioned in order to cover the alveolus and sutured with two (occasionally three) sutures (VICRYL, Ethicon, Somerville, N.J., U.S.).

After the surgery, the patients were randomly assigned to a test group or a control group by opening closed envelopes containing the group assignment.

Postoperative adverse events

Two patients came to the clinic before the suture removal because of postoperative pain. At this point, the extraction alveoli were rinsed with sterile saline and a prescription for stronger analgesics was given, but there was no need for the prescription of antibiotics.

Postoperative treatment

The sutures were removed seven days after the surgery. After suture removal, the patients in the control group did not receive any specific information or treatment. However, the patients in the test group were informed about the importance of good oral hygiene, especially distal to the mandibular second molars; furthermore, they were instructed on how to use a special toothbrush (Compact Tuft, Tepe Munhygienprodukter, Malmö, Sweden) to clean distal to the second molars.

At the one-month examination, the patients in the test group were recalled by a dental hygienist at the Department of Periodontology, who was not aware of the aim of the study. The patients received supra- and subgingival scaling and oral hygiene reinstruction and motivation if needed. Plaque and gingival bleeding at the distal sites of the mandibular second molars were also recorded in the following way:

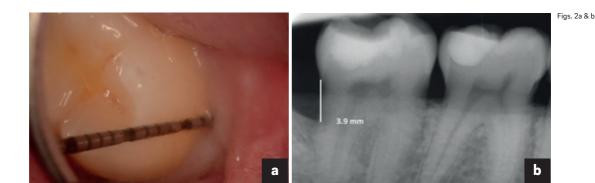
PI: The presence or absence of plaque was determined in the same manner as at the baseline examination.

Gingival bleeding index (GI): The presence or absence of bleeding was determined after running the probe in the gingival sulcus distal to the second molars.¹³

Six-month re-evaluation

At six months, all of the patients were recalled for a control visit. This visit was performed by a periodontist (GS), who was not aware of the group assignment. At this time, the following parameters were recorded:

PI: The presence or absence of plaque was determined in the same manner as at the baseline examination.



Six-month control. Clinically healthy gingival condition distal to the second molar, with a PPD of 3 mm (a). Bone-level measurements (b). suppuration was determined in the same manner as at the baseline examination.

PPD: Pocket depth was measured in the same jects per group. manner as at the baseline examination (Fig. 2a).

A radiograph, aiming to control the area distal to the mandibular second molars after extraction, was also taken at this appointment using the paralleling technique.14

Clinical criteria for healthy or diseased sites after treatment

At the re-evaluations, the surface distal to the second molars was considered healthy if there was a PPD of \leq 4 mm without BoP/Sup. The presence of periodontal disease was determined based on a PPD of \geq 5 mm with BoP/Sup.

> Baseline and six-month radiographic measurements

The radiographs were evaluated by a second periodontist (AT), who was not aware of the study design. The bone loss distal to the second molar at baseline and at the six-month examination was measured. Thus, the distance between the cemento-enamel junction and the most coronal level along the root surfaces at which the periodontal space was considered to have a normal width¹⁵ was measured using a program for digital radiographic images (Planmeca Romexis, Helsinki, Finland) with 10× magnifying power and a precision of 0.1 mm (Fig. 2b). The presence or absence of an alveolar bone defect (i.e., a bony defect 2 mm wide and 2 mm deep) was also recorded.

Data analyses

Each mandibular second molar was regarded as an independent observation. The Wilcoxon signed-rank test and Mann–Whitney U test were applied to test the difference in PPD and radiographic bone loss within and between the two groups at baseline and the six-month examination. The Fisher exact probability test was applied to assess differences in treatment outcome in the test and control groups. A p-value of < 0.05 was considered to be statistically significant.

Sample size calculation

Based on an anticipated difference in mean PPD of 1.0 mm between the test and control groups

BoP/Sup: The presence or absence of bleeding/ and calculated standard deviation of 1.1 mm from previous studies,^{10, 11} Type I error and 80% power, the calculated sample size was 20 sub-

Fig. 3

Flowchart of the study.

Dropout

During the study period, four patients in the test group and three in the control group dropped out from the study; one moved and the others did not attend the six-month examination (Fig. 3).

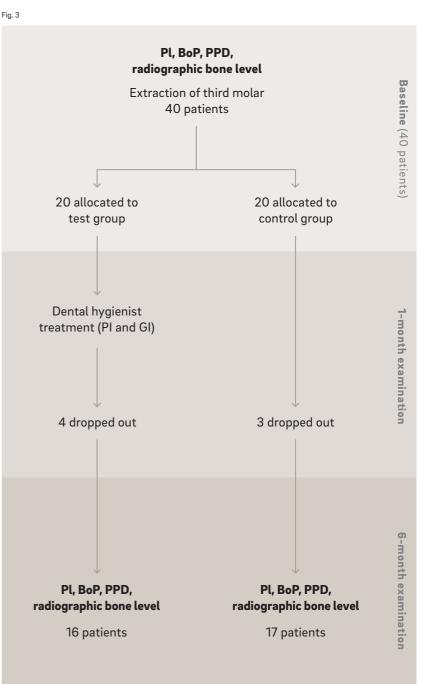


Table 1

Clinical features of patients and teeth included in the study.

		Test	Control	All
Number of patie	nts	16 (5 female) 17 (9 female)		33 (14 female)
Age		21–48 19–36 (mean 30.1, S.D. ± 8.9) (mean 24.8, S.D. ± 5.0)		19–48 (mean 27.3, S.D. ± 7.8)
Smokers		1 2		3
Number of teeth #48		9	9 11	
Number of teeth	#38	7	6	13
Number of molars	Horizontal	8	6	14
according to	Mesio-angular	8	10	18
angulation	Vertical	0	1	1
	Disto-angular	0	0	0

Results

Baseline examination

The 33 subjects (14 females and 19 males) who completed the study had a mean age of 27.4 (S.D. \pm 7.8; range: 19–48). Sixteen subjects were allocated to the test group and 17 to the control group. No difference in age was noted between the test and control groups. Only three were smokers, one in the test group and two in the control group (**Table 1**).

A total of 33 mandibular third molars (20 on the right and 13 on the left side) were examined. The majority had a mesio-angular or horizontal position **(Table 1)**. The presence of plaque was noted at all distal sites of the second molars, and 29 of these sites had BoP **(Tables 2a & b)**. The mean PPD at the distal sites of the second molars, based on the deepest value measured at three points (distobuccal, distal and distolingual), was 7.4 mm (S.D. \pm 1.5), with no difference between the test and control groups **(Table 2c)**.

The radiographic measurements at the baseline examination showed that 26 molars had bone loss of up to one-third of the root length, and 7 molars between one-third and two-thirds, while bone loss exceeding two-thirds of the root length was not recorded in any molars **(Table 2e)**. The mean (± S.D.)/median bone loss was 4.9 (2.4)/3.6 mm for the test group and 4.5 (0.9)/4.2 mm for the control group, and no statistically significant difference was noted in this respect between the two groups **(Table 2d)**.

One-month examination

At one month after the extraction of the third molars, only 4 of the 16 (25%) distal sites of the second molars presented with plaque **(Table 2a)** and 5 showed bleeding after running the probe in the gingival sulcus.

Six-month examination

At six months after extraction, 5 out of the 16 (31%) distal sites of the second molars presented with plaque in the test group, compared with 9 out of 17 (53%) in the control group, with a reduction of 69% and 47%, respectively, from the baseline value (Table 2a). The presence of BoP was recorded at 6 out of 16 (38%) in the test group, compared with 8 out of 17 (47%) in the control group (Table 2b). The mean PPD measured at the distal sites of the second molars was 4.1 mm (S.D. ± 1.1) in the test group and 3.8 mm (S.D. ± 1.4) in the control group. None of these measurements were statistically significantly different between the two groups (Table 2c). The PPD reduction with respect to the baseline value was 3.4 mm in the test group and 3.5 mm in the control group. Both values were statistically significantly different from the baseline values (p < 0.001). No difference in the healing pattern was observed between the test and control groups with respect to the presence of a PPD of < 5 mm without BoP/Sup. Only one pocket distal to the second molar with a PPD of 6 mm with bleeding was recorded in a patient in the control group.

Table 2t

Plaque	Baseline	1 month	6 months
Test (16)	16	4	5
Control (17)	17	0	9
All (33)	33	0	14

BoP	Baseline	6 months		
Test (16)	14	6		
Control (17)	15	8		
All (33)	29	14		

Table 2a

PPD	Baseline	6 months	
Test (16)	7.5 (1.5)/7	4.1 (1.1)/4	
Control (17)	7.3 (1.5)/7.5	3.8 (1.4)/4.5	
All (33)	7.4 (1.5)/7	4.0 (1.2)/4	

Bone loss	Baseline	6 months
Test (16)	4.9 (2.4)/3.6	3.5 (1.6)/2.9
Control (17)	4.5 (0.9)/4.2	3.1 (1.4)/3.3
All (33)	4.4 (1.6)/4.1	3.2 (1.5)/3.3

Table 2c

		Baseline		6 months		
	Bone loss ≤ 1/3	Bone loss 1/3–2/3	Bone loss > 2/3	Bone loss ≤ 1/3	Bone loss 1/3–2/3	Bone loss > 2/3
Test (16)	13	3	0	15	1	0
Control (17)	13	4	0	16	1	0
All (33)	26	7	0	31	2	0

Table 2d

Table 2e

The radiographic measurements found no bone loss in either group. The bone level (mean/ median) at six months was 3.5/2.9 mm in the test group and 3.1/3.3 mm in the control group, with a gain of 0.7 mm in the test group and 0.8 mm in the control group with respect to the baseline value **(Table 2d; Figs. 4a & b)**.

Discussion

The results of the present study showed that in subjects presenting with localized periodontal disease distal to mandibular second molars the periodontal condition improved at six months after extraction of the adjacent partially erupted third molars and subgingival plaque debridement. All of the distal sites of the second molars showed a clinically significant reduction in PPD and the radiographic measurements indicated bone gain distal to the second molars for both the test and control groups.

The presence of periodontal disease at the second molars adjacent to third molars in subjects with low severity of periodontal disease in

the overall dentition has been reported in other studies.^{1, 3, 5} None of the patients included in our study had signs of periodontal attachment or bone loss at the dentition except distal to the second molar. However, considering the young age of the sample (mean age of 27.4, S.D. \pm 7.8) and the radiographic mean bone loss distal to the second molar of 4.9 mm (S.D. \pm 2.4), the annual rate of bone loss distal to the second molar (if calculated from the age of 17) was approximately 0.4 mm/year. This rate is comparable to the annual bone loss (> 0.2 mm/year) in subjects with rapid disease progression described in longitudinal epidemiological studies.¹⁶⁻¹⁸

When early stages of periodontal pathology are detected, the removal of third molars may improve the periodontal status at the distal sites of second molars.^{6, 7} In our study, both the test and control groups showed relatively good plaque control distal to the second molars after the removal of the third molars. This may be related to easier access for self-performed plaque control distal to the second molars once the third molars had been extracted. The test

Tables 2a-e

Clinical and radiographic measurements at baseline and at the one- and six-month examinations.

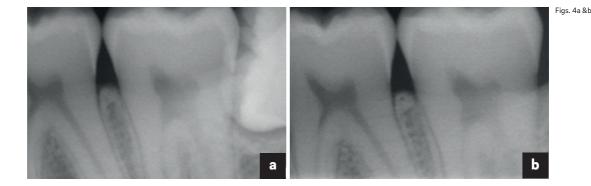
Table 2a: Plaque present at the distal sites of second molars. Table 2b: BoP present at the distal sites of second molars. Table 2c: Mean (± S.D.)/median PPD (mm) at the distal sites of second molars.

Table 2d: Mean

(± S.D.)/median radiographic bone level (mm) at the distal sites of second molars. **Table 2e:** Number of second molars with distal bone loss with respect to root length.

Figs. 4a & b

Radiographic images before and six months after the extraction of tooth #38. The bone healing distal to tooth #37 is noticeable.



group, who received the dental hygienist treatment at one month after the extraction, presented with a lower number of sites with plaque and sulcular gingival bleeding compared with the control group, but the differences did not reach statistical significance.

At the six-month evaluation, both groups had a clinically relevant PPD reduction distal to the second molars, and only one patient (in the control group) presented with a PPD of 6 mm. Thus, no additional surgical periodontal treatment was needed, except in one patient. In this respect, it should be underlined that, after the extraction of the third molars, meticulous debridement distal to the second molars was performed, together with removal of the granulation tissue. In a literature review, Aloy-Prósper et al. also concluded that debridement of the distal radicular surface of the second molars, together with oral hygiene control, reduced PPD values after the extraction of third molars.¹⁹ Leung et al., in their clinical study, concluded that plaque control prevented residual pockets at periodontally involved second molars six months after the removal of the adjacent third molar.¹⁰

In our study, no bone loss distal to the second molars was recorded. In a study evaluating the adjunctive effect of guided tissue regeneration in conjunction with surgical removal of an impacted third molar, Karapataki et al. concluded that an intrabony defect distal to the second molars would depend on the existing amount of periodontal ligament of the second molar and whether this was affected by periodontal disease before surgery.²⁰ Thus, undiagnosed periodontal lesions and the presence of bacteria on the root surface of second molars might affect wound healing in the area and develop into a persistent intrabony defect. later time.²¹ In our study, the periodontal condition distal to the second molars in all of the patients (except one in the control group) at the six-month evaluation did not require additional periodontal surgical treatment.

Kan et al. investigated the periodontal condition distal to mandibular second molars 6-36 months after routine surgical extraction of adjacent impacted third molars in 158 subjects under 40 years of age.¹¹ Three possible risk indicators were associated with localized increased PPD: third molar mesio-angular impaction; pre-extraction signs of bone loss; and inadequate post-extraction local plaque control.11

In our study, the majority of the patients (76%) were under 30 years of age, without compromised general condition, only three were smokers and none had periodontal disease, except at the distal sites of their second molars. Furthermore, 79% of the subjects had bone loss distal to the second molars not exceeding one-third of the root length and no patient presented with bone loss exceeding twothirds of the root length. All of these factors could have had a positive effect on the healing pattern. The moderate bone loss distal to the second molars at baseline could also have had a positive effect on the soft-tissue healing, preventing concavity in the gingiva, which could have been a retaining factor for plaque.

In the interpretation of similar studies, it is important to distinguish between those reporting results on totally impacted and on partially erupted third molars. Moss et al. reported results from 7,000 subjects (mean age of 62) and found that the PPD at the first or second molars was significantly higher when partially erupted third molars were present, compared with totally impacted third molars.² Similarly, These defects require surgical treatment at a in 52- to 74-year-old patients in the Dental

Atherosclerosis Risk in Communities Study, the presence of visible third molars was associated with a 50% increased probability of a PPD of > 5 mm at adjacent second molars.²² This finding has also been confirmed in a group of 5,831 young adults (18-34 years old) in the U.S. Third National Health and Nutrition Examination Survey, where the presence of visible third molars was associated with twice the probability of a PPD of > 5 mm at the adjacent second molars.¹

Regarding postoperative events, only two patients in our study came to the clinic before suture removal because of postoperative pain. At this time, the extraction alveoli were rinsed with sterile saline and a prescription for stronger analgesics was given, but there was no need for any antibiotic prescription. This confirms the findings that the removal of third molars in younger subjects compared with older subjects decreases the risk of complications; the age of 25 appears to be critical, after which complications increase more rapidly.⁸ It Ålvsborg County (Borås, Sweden). should be underlined that in our study the removal of third molars was performed by an experienced dentist in this area of dentistry (ASP), who meticulously removed the plaque and calculus accumulated at the distal sites of the second molars.

Conclusion

In the presence of localized periodontal disease distal to second molars, early diagnosis, extraction of the third molar and debridement at the distal site of the second molar were an effective treatment of localized periodontal disease, because no additional surgical periodontal treatment was needed at the six-month follow-up.

Competing interests

The authors declare that they have no competing interests.

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Abstract

Objective

The objective of this study was to validate the use of a novel method to elaborate 3-D data on dimensional changes to the alveolar process after one year of healing at implants placed immediately into extraction sockets.

Materials and methods

Ten consecutive subjects were recruited and included in the test. Impressions were taken using polyvinyl siloxane before tooth extraction and one year after implant placement, and gypsum casts were obtained.

The two casts were digitalized using a laboratory laser scanner and imported into two different analysis software programs for 2-D and 3-D analyses. In order to analyze global errors of the 3-D procedure, a contralateral control site was included.

Results

The 2-D analysis indicated a tendency to higher horizontal resorption of the alveolar process in the central regions compared with the mesial and distal regions. Similar results were observed at the lingual/palatal aspect and in the global horizontal variation.

The 3-D analysis found that, when the absolute values were taken into account, the larger the region of interest, the higher the volume loss, with a positive linear correlation between the two variables (R2 = 0.9346; y = 0.126x).

The global volume loss in percentage was $12.7 \pm 3.1\%$, of which $5.9 \pm 1.9\%$ was at the buccal and $6.8 \pm 2.2\%$ at the lingual/palatal aspects. The difference between the two aspects was not statistically significant. Small variations in volume at the control sites were also observed that represented the errors included in the 3-D analysis.

Conclusion

The 2-D method can be very useful for understanding changes at a localized point. The 3-D method proposed is faster, more accurate at expressing the volume loss and correlated to the dimensions of the analyzed region. The use of this method is consequently highly recommended.

Keywords

Implant dentistry, bone healing, extraction socket, Type I placement, immediate implant, IPIES (implants placed immediately into extraction sockets).

Introduction

A recent systematic review of the literature regarding dimensional changes to the hard and soft tissue after tooth extraction was evaluated.1 A vertical hard-tissue loss of 11-22% after six months of healing was found. When the combined hard- and soft-tissue dimensional changes were considered, a variation of +0.1 to -0.9 mm after six months and of +0.4 to -0.8 mm after 12 months was found. A horizontal dimensional reduction of the hard tissue of between 29% and 63% was observed six to seven months after tooth extraction. When the combined hard- and soft-tissue dimensional changes were considered, a loss of 1.3 mm after three months and of 5.1 mm after 12 months was found. Moreover, the reduction was more rapid during the first three to six months, followed by a minor gradual reduction in dimensions thereafter.

In that review, the methods of measurement of the dimensional variation between the time of extraction and the subsequent period of reanalysis were also reported. For the hard tissue, radiographs, computed tomography scans, cone beam computed tomography scans, or reentry surgical procedures that included stents or other references were used for the analysis of the dimensional changes. For the combined hard- and soft-tissue dimensional changes, the casts mainly were analyzed.

Dimensional changes to the alveolar process may be analyzed using digitalized images (meshes) obtained by various 3-D digital methods: on casts, using laser scanners and structured-light 3-D scanners,² or chairside using 3-D intra-oral photogrammetric systems.³ The reproducibility of these methods has been shown to be high and their use for analyzing dimensional variations of the alveolar process has been recommended.⁴⁻⁶ Many of the recent studies that have used 3-D systems to analyze dimensional variations of the alveolar process, however, lost substantial information in transforming 3-D data to 2-D measurements.^{7,8}

Volumetric data instead were reported in a clinical study in which augmentation procedures were used at implants placed in edentulous ridges reduced in volume.⁹ In the study, a grid was superimposed on the images so that both the global difference in volume before and after treatment and the differences in specific areas were reported.

2-D variations of the hard tissue around implants placed immediately into extraction sockets have been reported in clinical studies,^{10, 11} and, in an animal study, combined hard- and soft-tissue 2-D changes have been analyzed.¹² However, there is a lack of studies that report volumetric data on combined hardand soft-tissue variation at implants placed immediately into extraction sockets in humans using a 3-D system. Hence, the aim of the present study was to validate the use of a novel method to elaborate 3-D data on dimensional changes to the alveolar process after one year of follow-up at implants placed immediately into extraction sockets.

Materials & methods

The research protocol was approved by the ethics committee of Azienda Ospedaliera di Padova, Department of Neurosciences, University of Padua (protocol #2629P; 10 April 2012).

Patient selection

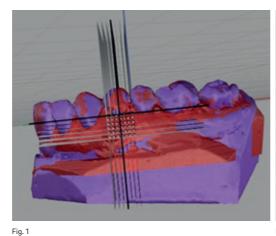
In order to be recruited for the study, the patients had to meet the following inclusion criteria: willing to participate for the duration of the study and to provide informed consent, at least 18 years of age, in good general health, presence of a tooth to be extracted, willing to accept the immediate placement of an implant into the extraction socket, and presence of adjacent teeth both mesially and distally. The following exclusion criteria were adopted: pregnancy or untreated dental disease. Smoking status was recorded, but was not considered a contraindication to treatment. Patients were advised that smoking is associated with an increased risk of implant failure.

Ten consecutive subjects were recruited. Written consent was obtained from the patients. All patients received a careful dental and periodontal examination, followed by oral hygiene instructions and dental and periodontal treatment, when necessary. All treatments and follow-ups were carried out in one clinic in Italy between September 2012 and September 2014.

An impression using polyvinyl siloxane (Sky Putty and Sky Light, Sweden & Martina, Due Carrare, Italy) was taken before tooth extraction (Time 0 = T0) and a gypsum cast was obtained (ORTOTYPO 4, LASCOD, Sesto Fiorentino, Italy).

Figs. 1 & 2

The two meshes (red TO and purple T1) superimposed together were cut seven times vertically and six times horizontally using a Python script for Rhinoceros.



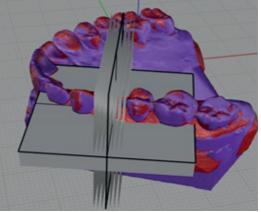
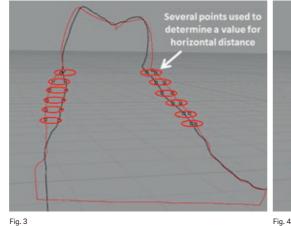


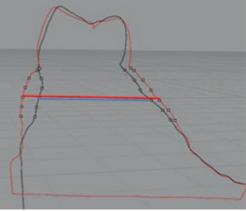
Fig. 3

A vertical 2-D cut was taken in the middle. In red is the section of the first mesh (TO) and in black the second mesh at (T1). The distance between two points was taken for the measurements in absolute values.

Fig. 4

The width of the alveolar process was also measured. The red line represents the width at TO and the blue line at T1. Fig. 2





Subsequently, local anesthesia was administered and the tooth was extracted. An implant was immediately placed into the extraction socket and no filler material or membrane was used. Implants with a ZirTi surface (Premium TG, Sweden & Martina, Due Carrare, Italy) were placed. A cover screw was placed on top of the implant and resorbable sutures were provided. No temporary prosthesis was seated. Antibiotics (amoxicillin 875 mg and clavulanic acid 125 mg b.i.d. for six days) and analgesics if needed were prescribed and the patients were enrolled in a maintenance follow-up. A porcelain-fused-tometal crown was provided to the patients approximately three months after placement. Another impression was taken 12 months after implant placement (Time 1 = T1).

2-D digital analysis

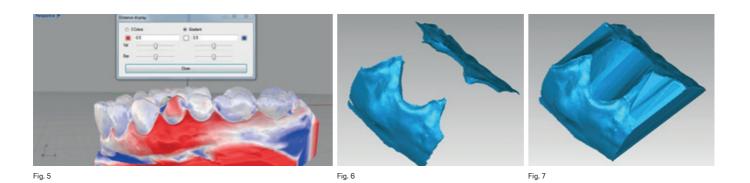
The casts obtained from the first and second impressions were digitalized using a 3-D laser scanner (Dental Wings 7Series, Montreal,

Canada). The meshes (digital models) generated in this manner were imported into 3-D elaborating mesh software (Geomagic Studio and Geomagic Qualify, Geomagic, Berlin, Germany) and cleaned of defects.

The meshes were transformed from a surface to a solid. Subsequently, teeth surfaces that coincided on the meshes obtained from both casts were selected and the two digital models were superimposed, accepting values of average convergence distance of < 0.1 mm.

The 2-D analysis was performed using the occlusal plane as the reference plane.⁷ From this reference, a perpendicular plane in the lingual– or palatal–vestibular direction (cross-section) was created and the two meshes superimposed were cut **(Figs. 1& 2)**.

The grid used to section the meshes was made by taking the middle point of the vestibular marginal gingiva of the tooth to be extracted as the reference point (0) and creating vertical and horizontal planes starting from that point:



Vertically: Seven vertical planes located at +3, +2, +1, 0, -1, -2 and -3 mm from the mesial (+3) to the distal aspect (-3);

Horizontally: Six horizontal planes at 0 (vestibular marginal gingiva), -1, -2, -3, -4 and -5 mm from the most coronal (0) to the most apical (-5).

The occlusal plane, the cutting procedure and the distance analysis were performed with automated Python scripts for Rhinoceros software (Robert McNeel & Associates, Seattle, Wash., U.S.) to reduce human error during elaboration. A total of 42 points for horizontal variation for each side was tested: 42 at the buccal aspect and 42 at the lingual/palatal aspect. The vertical variation was measured at seven points at the buccal aspect and seven points at the lingual/palatal aspect. Using the measures of the alveolar process at T0 and at T1, the dimensional variations (Δ) were expressed in absolute (**Fig. 3**) and relative (**Fig. 4**) values (in respect of the total alveolar width).

3-D digital analysis

The 3-D analysis was performed by subtracting the volume of the second mesh (T1) from that of the first mesh (T0), generating a resulting volume that represented the difference between the two meshes (Boolean difference). Consequently, the software automatically defined the limits of the volume loss. The region of interest (ROI) was manually delimited mesially and distally using as limits a plane crossing through the middle of the crown of the two adjacent teeth. The limits of the ROI were decided on because the main volume changes were included in that region, as indicated by the mesh-to-mesh deviation (Δ ; **Fig. 5**) performed with the Rhino Open Projects for Rhinoceros plug-in.

Using the Geomagic software, the two meshes (TO and T1) were further cleaned of the teeth and of apical imperfections derived from

the technical procedures applied to obtain the casts. Finally, the delimited areas were elaborated by closing the holes and obtaining a solid that represented the buccal and lingual/palatal volume changes (**Fig. 6**). The file containing the data on the solid was exported in STL format and imported into Rhinoceros for volumetric analysis.

In order to obtain standardized data, the solid was further elaborated using the Geomagic software. Only the outer surfaces were maintained, while the rest of the solid was eliminated.

The two outer surfaces were combined together with bridges and, after closing the gaps (between bridges), another solid was generated that represented the global volume of the alveolar process delimited into the ROI (V-ROI; **Fig. 7**) before volume changes (i.e., at TO). Similar procedures were applied to the corresponding contralateral control site to obtain volume changes between TO and T1.

Volumetric analysis

The average convergence distance represents the misfit between the two meshes. The differences in volume (Δ) between the meshes of the two periods at the extraction sites were calculated as total amount (V-tot), as well as for the buccal (V-b) and lingual/palatal (V-l) aspects separately, and expressed in mm³.

In order to reduce the variability associated with the use of absolute measurements in mm³ due to the dimensional variability of patients' arches, the relative percentage of loss was also calculated in relation to the V-ROI at TO. Percentages of the total amount (V-tot%) and of the buccal (V-b%) and lingual/palatal (V-l%) aspects were obtained.

At the control sites, the same methodology for measurements was applied for both absolute and relative (percentage) values, and the results

Fig. 5

The mesh-to-mesh deviation utility in Rhino Open Projects. The red area represents the volume loss. The area most affected by resorption corresponds to about the vertical middle line of the two adjacent teeth.

Fig. 6

Palatal and buccal volumes, isolated from the rest of the model during 3-D elaboration. They represent the volume loss in the ROI and can be measured as absolute values in mm³.

Fig. 7

ROI volume of the first mesh, which represents the volume of the alveolar process at TO, expressed in mm³. were used to define the global errors of the procedure due to the Boolean method, superimposition, impression taking, gypsum casting and 3-D scanning.

Data analysis

Mean values and standard deviations were calculated for the 2-D data, while mean values and standard deviations, as well as the 25^{th} , 50^{th} (median) and 75^{th} percentiles, were calculated for the 3-D data. Differences in the volumetric variation (Δ) between the implant and the contralateral sites were analyzed using the Wilcoxon signed-rank test. The level of significance was set at $\alpha = 0.05$. In case of normal distribution, a *t*-test was also performed.

Results

2-D analysis

At the buccal aspect **(Table 1)**, a tendency to higher horizontal resorption of the alveolar process was seen in the central regions where tooth extraction was performed compared with the mesial and distal regions. Moreover, the resorption had a tendency to be higher at the coronal aspects compared with the apical. The horizontal resorption varied between 3% and 25%, depending on the interception point from which it was analyzed, the highest variation being in the central/coronal regions, and the lowest being at the mesiodistal/apical regions. Similar results were observed at the lingual/palatal aspect **(Table 2)** and in the global horizontal variation **(Table 3)**.

The vertical resorption of the alveolar process analyzed on the seven vertical cutting planes was higher in the mesial and distal regions compared with the central regions at the buccal aspect. A tendency to higher resorption was seen in the central regions at the lingual/ palatal aspect **(Table 4)**.

3-D analysis

When the absolute values were taken into account **(Table 5)**, it was observed that the larger the ROI, the higher the volume loss, with a positive linear correlation between the two variables ($R^2 = 0.9346$; y = 0.126x). The volume loss was 69.7 ± 39.1 mm³ and 74.3 ± 29.8 mm³ at the buccal and lingual/palatal aspects, respec-

tively, and a global volume loss of 144.1 ± 61.2 mm³ was observed. The global volume loss in percentage was $12.7 \pm 3.1\%$, showing a lower variability of the results between sites compared with the absolute values **(Table 5)**. The loss was $5.9 \pm 1.9\%$ at the buccal and $6.8 \pm 2.2\%$ at the lingual/palatal aspects, the difference not being statistically significant. Small variations in volume at the control sites were also observed that represented the errors included in the 3-D analysis.

Discussion

2-D analysis

The 2-D analysis demonstrated a reduction of the dimensions at both the buccal and lingual/palatal aspects. However, the analysis of each intersection point and the comparison of all of the patients were very demanding. Moreover, the variability per intersection point was very large, making drawing conclusions using this method difficult. It is, of course, possible to select just one intersection point and compare it with the lingual/palatal aspect or with that of other patients. However, to perform a complete analysis of the phenomenon, 42 intersection points (such as those that composed the grid) were analyzed.

2-D analysis offers advantages for investigation of defect shape and for analysis of local defects. However, limits to consider include the use of 2-D numbers to express 3-D aspects, the lack of information about the size of the area affected by the resorption, and the huge amount of data that must be recorded and that require a great deal of time to analyze.

Moreover, great variability in resorption exists, depending on where the volume loss is investigated. In the present study, a horizontal mean global volume loss of 3.8–43.9% in the analyzed area made it impossible to summarize the phenomenon with a unique number that expresses the volume loss. For the vertical loss in the 2-D analysis, the results have to be reported in millimeters, since it does not seem to be appropriate to report data in percentages because of the lack of a reference dimension.

3-D analysis

The 3-D analysis showed that shrinkage of the volume of the alveolar process occurred at both the

VP-2

-19.7 (9.5)

-11.8 (6.6)

-15.9

-10.8

-6.9

-4.7

VP-3

-17.9 (13.6)

-8.5 (4.6)

-12.2

-8.5

-5.7

-4.0

Table 1

2-D analysis. Mean values and standard deviations of the horizontal reduction of the buccal aspect of the alveolar process at the 42 intersection points in %. HP = Horizontal plane; VP = Vertical plane.

Table 2

2-D analysis. Mean values and standard deviations of the horizontal reduction of the lingual/palatal aspect of the alveolar process at the 42 intersection points in %. HP = Horizontal plane; VP = Vertical plane.

Table 3

2-D analysis. Global horizontal reduction of the alveolar process in % represented by the sum of the mean values of the buccal and lingual/palatal reduction at the 42 intersection points. HP = Horizontal plane: VP = Vertical plane.

buccal and lingual/palatal aspects after tooth extraction and immediate placement of an implant in the extraction socket. A global volume loss of 12.7% was observed, being 5.9% at the buccal and 6.8% at the lingual/palatal aspects. The difference was not statistically significant. These outcomes differ from those reported on 2-D variations of the alveolar process¹³ or of the bony crest.^{10,11} In those studies, however, a single reference point was used, while the global volume of the ROI was analyzed in the present study. Moreover, the studies on bony crest variation did not include measurements of soft-tissue dimensions. It has to be considered that the procedure used in the present study allowed for the use of 2-D data too regarding a single intersection point or single plane, and this may have permitted a more complete analysis.

The 3-D method can be affected by various errors related to the impression, model fabrication, 3-D scanning (reverse engineering phase), mesh creation, 3-D elaboration and superimposition. In the present study, the dimensional variations between the two periods (TO and T1) at the contralateral sites were also analyzed. Small variations were found, most likely due to the errors included in the method. The differences between the implant sites and the contralateral sites were highly statistically significant. This, in turn, meant that these errors did not affect the data that this 3-D method produced and the volume differences found were not due to the case or to errors, but to the biological phenomenon of resorption.

In the present study, a positive linear correlation between the global volume of the ROI and the volume loss was found. This means that the larger the jaw, the larger the resorption. This observation makes it senseless to investigate volume loss with a superimposed standardized grid for analysis. In fact, in the present study, a

HP -2	-4.1 (3.1)	-6.0 (3.7)	-7.6 (4.6)	-8.7 (4.8)	-8.1 (4.5)	-6.8 (3.9)	-5.2 (3.0)
HP -3	-2.7 (2.0)	-3.3 (2.3)	-4.3 (2.8)	-4.8 (2.7)	-4.6 (2.4)	-4.1 (2.1)	-3.5 (2.0)
HP -4	-1.7 (1.4)	-2.0 (1.8)	-2.7 (2.0)	-2.9 (1.8)	-2.9 (1.8)	-2.8 (1.8)	-2.7 (1.7)
HP -5	-1.2 (1.3)	-1.1 (1.4)	-1.5 (1.7)	-2.0 (1.9)	-2.2 (1.6)	-2.1 (1.5)	-2.1 (1.5)
	VP3	VP2	VP1	VP0	VP-1	VP-2	VP-3
HP 0	-24.2	-32.2	-43.9	-40.9	-40.9	-37.8	-30.4
HP -1							

Table 1

Table 2

Table 3

HP 0

HP -1

HP -2

HP -3

HP -4

HP -5

VP3

-9.4 (3.9)

-7.6 (5.6)

-13.2

-9.4

-6.2

-3.8

VP2

-14.7 (4.2)

-11.1 (5.6)

-17.0

-11.3

-7.6

-4.4

	VP3	VP2	VP1	VPO	VP-1	VP-2	VP-3
HP 0	-14.8 (7.1)	-17.5 (4.6)	-24.0 (24.0)	-16.8 (17.8)	-20.0 (15.9)	-18.0 (10.5)	-12.5 (5.7)
HP -1	-12.7 (6.5)	-15.7 (7.0)	-17.6 (8.2)	-15.9 (7.5)	-15.7 (6.1)	-12.5 (3.3)	-9.5 (4.4)
HP -2	-9.1 (5.6)	-11.0 (5.8)	-12.2 (5.1)	-11.9 (4.1)	-10.6 (3.4)	-9.2 (3.8)	-7.0 (3.6)
HP -3	-6.7 (5.1)	-8.1 (5.5)	-8.8 (5.0)	-8.9 (4.7)	-8.3 (4.3)	-6.7 (3.7)	-5.0 (2.9)
HP -4	-4.6 (4.7)	-5.7 (5.3)	-5.9 (5.0)	-5.6 (4.4)	-5.1 (3.7)	-4.1 (2.9)	-3.0 (2.3)
HP -5	-2.6 (3.7)	-3.3 (4.3)	-3.6 (4.2)	-3.4 (4.2)	-3.1 (3.9)	-2.6 (3.1)	-2.0 (2.4)

VP0

-24.1 (14.1)

-17.7 (12.7)

-20.6

-13.7

-8.5

-5.4

VP-1

-20.9 (8.4)

-15.4 (10.1)

-18.7

-12.9

-7.9

-5.3

VP1

-19.9 (7.8)

-14.6 (9.2)

-19.8

-13.1

-8.6

-5.2

Table 4

2-D analysis. Mean values and standard deviations of the vertical reduction of the buccal and lingual/palatal aspects of the alveolar process at the most coronal of the seven intersection points in mm.

Vertical plane	Buccal aspect	Lingual/palatal aspect	
3	-1.4 (0.6)	-1.1 (0.5)	
2	-1.2 (0.4)	-1.2 (0.6)	
1	-0.8 (0.5)	-1.5 (0.6)	
0	-0.5 (0.7)	-1.3 (1.2)	
-1	-0.6 (0.9)	-1.5 (0.8)	
-2	-0.9 (0.6)	-1.1 (1.6)	
-3	-1.2 (1.0)	-1.0 (1.6)	

Table 5

Table 4

		ΔV-b mm ³	ΔV-l mm ³	ΔV-tot mm ³	V-ROI mm ³	ΔV-b%	Δ V-l%	ΔV-tot%
Test implant site	Mean (S.D.)	69.7 (39.1)	74.3 (29.8)	144.1 (61.2)	1202.9 (524.4)	5.9 (1.9)	6.8 (2.2)	12.7 (3.1)
	Median (25 th ; 75 th percentiles)	63.8* (34.4; 105.4)	75.5* (49.6; 88.8)	134.3* (106.0; 174.5)	1288.5 (895.3; 1557.8)	6.4* (5.4; 7.1)	6.7* (5.6; 8.3)	12.9* (11.8; 13.4)
Controlatorel	Mean (S.D.)	1.98 (1.74)	3.31 (4.04)	5.29 (5.24)	1202.9 (524.4)	0.18 (0.13)	0.39 (0.42)	0.57 (0.50)
Contralateral control site	Median (25 th ; 75 th percentiles)	1.73* (0.60; 3.31)	2.67* (0.17; 3.61)	4.02* (2.23; 7.11)	1288.5 (895.3; 1557.8)	0.19* (0.11; 0.26)	0.20* (0.02; 0.77)	0.45* (0.18; 0.98)

*A *p*-value of < 0.05 between the test and control sites

Table 5

3-D analysis. Mean values, standard deviations (S.D.), medians, and 25^{th} and 75^{th} percentiles of the volume reduction of the alveolar process in absolute values in mm^3 and in % at the test and control sites.

standardized grid was used with squares of 1 mm in dimension and not a grid that was adapted in dimensions to those of the alveolar process. It has to be considered that the distance between the two adjacent teeth is not the same in different locations and in different subjects, so the area covered by a standardized grid does not include the whole ROI. Moreover, the measures taken in each intersecting plane do not represent the same position in all patients. Consequently, the grid should be adapted to the dimension of the space between the two adjacent teeth. The use of 2-D analysis may be comparable if used in the middle of the ROI be cause it is a reference plane easily detected in all models.

From a clinical perspective, the 3-D method may help clinicians to understand in a more objective manner what happens to the alveolar process after tooth extraction and the immediate placement of an implant. Differentiation between hard- and soft-tissue loss cannot be expressed by the data from this 3-D method and requires a different approach, such as surgical re-entry or radiographic assessment. The 3-D

standardized grid was used with squares of analysis used in the present study was found to 1 mm in dimension and not a grid that was be fast, accurate and noninvasive.

Conclusion

The 2-D method can be very useful for understanding changes at a localized point. The 3-D method proposed is faster, more accurate at expressing the volume loss and correlated to the dimensions of the analyzed region. The use of this method is consequently highly recommended.

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Competing interests

The authors declare that they have no competing interests related to this study.

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Influence of smoking and oral hygiene

on success of implants placed after direct sinus lift

Abstract

Objective

The objective of this study was to evaluate the influence of smoking and oral hygiene on the success and periimplant marginal bone loss of implants placed in one-stage and two-stage direct sinus lift procedures.

Materials and methods

A retrospective clinical study of patients who underwent direct sinus lift and implant placement was conducted. Forty-six patients with 58 direct sinus lifts were included and a total of 102 implants were placed. Cigarette consumption was quantified and the level of oral hygiene determined at the time of surgery using a simplified calculus and plaque index. Bone loss and implant success (according to Buser's criteria) were monitored after 12 months of prosthetic loading.

Results

The success rate for implants placed after direct sinus lift was 93.1% at 12 months. There was a higher success rate in nonsmokers (94.2%) than in smokers (90.9%), with a mean bone loss of 0.52 mm (range: 0.21–0.84 mm) in nonsmokers and 0.60 mm (0.24–0.92 mm) in smokers at the 12-month follow-up. The success rate in patients with poor oral hygiene was lower (81.8%) than in patients with good (95.5%) or regular hygiene (92.3%). Furthermore, there was a mean bone loss of 0.51 mm (0.21–0.82 mm) in patients with good oral hygiene, 0.57 mm (0.24–0.82) with regular hygiene and 0.66 mm with poor hygiene (0.32–0.92 mm). There was no statistically significant relationship (p > 0.05) between bone loss or implant success and smoking or oral hygiene.

Conclusion

Within its limitations, the present investigation suggests that smoking and poor oral hygiene may negatively influence the outcome of implants placed both in one-stage and two-stage direct sinus lift procedures. However, differences were in no case statistically significant and studies with larger sample sizes should be conducted to corroborate or refute these findings.

Keywords

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Sinus lift, oral hygiene, smoking, bone loss.

Introduction

Placing implants in the posterior maxilla can be a complex procedure when there is atrophy of the alveolar ridge and maxillary sinus pneumatization. In some cases, these anatomical limitations may be overcome using sinus lift procedures.¹ The success rates of implants placed after sinus lift are similar to those of implants placed in mature bone.² However, the residual alveolar bone height appears to influence implant survival. Rios et al. conducted a systematic review and divided the outcomes into two groups according to residual bone height: $\leq 4 \text{ mm in Group 1 and } > 4 \text{ mm in Group 2.}^3$ The implant survival rate was 96% (range: 80–100%) for Group 1 and 99% (range: 97–100%) for Group 2.³

In addition to bone atrophy, factors such as smoking and poor oral hygiene have been suggested to increase the risk of implant failure in the posterior maxilla.⁴ Several studies have addressed the association between smoking and the outcome of implants placed using conventional techniques;^{5–7} however, few studies have addressed the influence of smoking on the success of implants placed after direct maxillary sinus lift. In all of the published studies, higher tobacco consumption yielded higher complication and/or implant failure rates;^{8–15,1,16–19} however, this effect was not always statistically significant **(Table 1)**.^{6,12–14}

The influence of oral hygiene has frequently been considered in implant studies. In some studies, poor hygiene was associated with higher periimplant marginal bone loss.²⁰ Contrarily, other studies did not find this relationship.²¹⁻²³ However, evidence relating patient oral hygiene to the outcome of implants placed after direct sinus lift procedures is scarce. Only one study was found, and it reported a statistically significantly higher implant failure rate in patients with poor oral hygiene.¹¹

The objective of this study was to evaluate the influence of smoking and oral hygiene on the success and periimplant marginal bone loss of implants placed in one-stage and two-stage direct sinus lift procedures.

Materials & methods

The study was approved by the University of Valencia ethics committee (#H1410262226693). All patients gave written informed consent before surgery, in accordance with the principles of the Declaration of Helsinki.

Study sample

A retrospective clinical study was performed between September 2009 and June 2012 of patients treated with dental implants placed in one-stage (simultaneous) and two-stage (delayed) direct sinus lift procedures. A minimum follow-up period of 12 months after implant loading was requested. Patients who failed to attend scheduled follow-up visits were excluded.

Surgical procedures

All of the procedures were performed by two expert surgeons, professors at the Oral Surgery Unit, Department of Stomatology, University of Valencia, under local anesthesia with 4% articaine and 1:100,000 epinephrine (Laboratorios Inibsa, Lliçà de Vall, Spain). Full-thickness flaps were raised. A window in the sinus lateral wall was made with round tungsten carbide burs and finalized with ultrasonic tips. The sinus membrane was detached with curettes and elevated using a bone graft material. A xenograft (Geistlich Bio-Oss, Geistlich Pharma, Wolhusen, Switzerland) was used as the only bone graft material (1.5-2 g). The sinus window was covered with a resorbable membrane (Geistlich Bio-Gide, Geistlich Pharma, Wolhusen, Switzerland). The implants used in this study were TSA implants with an Avantblast surface (Phibo Dental Solutions, Sentmenat, Spain). Implants were placed in the same surgery if the residual bone height was 4-6 mm, or delayed by six months if the height was < 4 mm.

All of the patients were prescribed the same postoperative medication: amoxicillin and clavulanic acid (Augmentin, GlaxoSmithKline, Madrid, Spain) 500 mg/8 h for seven days, ibuprofen (Bexistar, Laboratorio Barcino, Barcelona, Spain) 600 mg/8 h for three days, and a 0.12% chlorhexidine mouthrinse (GUM, Sunstar Americas, Chicago, Ill., U.S.) t.i.d. for seven days.

Data collection

Patient oral hygiene was evaluated using the Simplified Oral Hygiene Index (OHI-S).²⁴ This was obtained by measuring the presence of debris and calculus on the buccal surfaces of the maxillary right central incisor, mandibular left central incisor and maxillary first molars, as well as on the lingual surfaces of the mandibular first molars. The criteria for classifying debris were as follows: no debris, no stains (0); soft debris covering less than one-third of the tooth surface (1); soft debris covering more than one-third, but less than twothirds of the exposed tooth surface (2); and soft debris covering more than two-thirds of the exposed tooth surface (3). The criteria for classifying calculus were as follows: no calculus (0); supragingival calculus covering less than onethird of the exposed tooth surface (1); supragingival calculus covering more than one-third, but less than two-thirds of the exposed tooth surface (2); and supragingival calculus covering more than two-thirds of the exposed tooth surface (3). The OHI-S was obtained from the combination of the two subindices. The grading scale was 0-1.2 (good oral hygiene), 1.3-3 (regular oral hygiene), or 3.1-6 (poor oral hygiene). Each patient was classified as having good oral hygiene, regular oral hygiene or poor oral hygiene.

The implant success rate was recorded according to the clinical and radiographic criteria of Buser et al.²⁵ Implants were classified as successful if they fulfilled all of the criteria (absence of clinically detectable implant mobility, absence of pain or any subjective sensation, absence of recurrent periimplant infection, and absence of continuous radiolucency around the implant after 12 months of loading) and as failed if any criterion was not met.

Radiographic examination was performed with an X-Mind intra-oral system (ACTEON MÉDICO-DENTAL IBÉRICA, Sentmenat, Spain) and an RVG intra-oral digital receptor (RVG 5100, Carestream Dental, Atlanta, Ga., U.S.). In order to reproduce the patient alignments, the Rinn XCP system (DENTSPLY, Des Plaines, Ill., U.S.) was used with a bite registration material in the area in which the parallelometer was fixed. Marginal implant bone loss was measured in millimeters using the RVG software. For measurement purposes, two visible and easily locatable reference points were selected at the junction point between the implant and prosthetic restoration. A straight line was traced between these two reference points

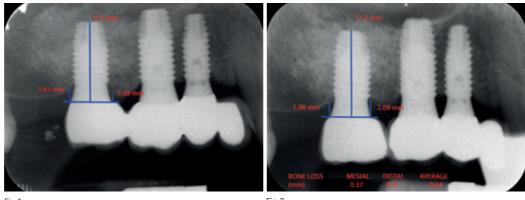


Fig. 1

Fig. 2

Fig. 1

Radiographic assessment of bone level at implant loading.

Fig. 2

Radiographic assessment of bone level 12 months after loading.

and was considered to represent zero height. In order to determine bone loss, a perpendicular line was traced mesial and distal to the implant from zero height to contact with the bone (**Fig. 1**). The difference between the value recorded at the time of implant loading and after one year of loading was used to calculate bone loss mesial and distal to the implant. The largest value, either mesial or distal, was used as the bone loss value for that implant (**Fig. 2**).²⁶

Smoking and oral hygiene were recorded at the time of surgery. A patient who smoked > 1 ciga-rette/day was considered a smoker following the definition by Wallace.²⁷ Bone loss and success were recorded at 12 months of prosthetic loading.

Statistical analysis

A descriptive analysis was performed of the study variables, with their corresponding frequency distributions and measures of central tendency and dispersion. Statistical comparisons between the groups were conducted using the chi-squared test and Student's *t*-test. The SPSS for Windows statistical software package (Version 15.0; SPSS, Chicago, Ill., U.S.) was used throughout. Statistical significance was considered for p < 0.05.

Results

Fifty patients treated with direct sinus lift and implants were monitored during the study period. Four patients failed to attend scheduled follow-up visits and were thus excluded. The final sample consisted of 46 patients (16 men and 30 women) with a mean age of 49 (range: 29–69 years). These patients underwent 58 direct maxillary sinus lift procedures and received a total of 102 implants in the grafted sites: 50 were placed simultaneously with the sinus lift procedure and 52 were placed six

and was considered to represent zero height. In months thereafter. Implant lengths and diameters order to determine bone loss, a perpendicular line are detailed in **Table 2**.

Seven implants failed, all prior to loading, yielding an overall implant success rate of 93.1% at 12 months of loading. Five of these implants had been placed simultaneously and two implants six months after the grafting procedure. The survival was 90.0% for implants placed simultaneously and 96.2% for delayed implants. Overall, the mean periimplant marginal bone loss was 0.58 mm (range: 0.24–0.95 mm). Implants placed simultaneously had a mean bone loss of 0.62 mm (range: 0.21–0.97 mm) and implants placed in a second procedure of 0.54 mm (range: 0.27–0.93 mm; **Table 3**).

With respect to smoking, 69 implants were placed in nonsmokers and 33 in smokers. Nonsmokers presented a higher implant success rate at 12 months (94.2%) and lower mean bone loss (0.52 mm; range: 0.21–0.84 mm) than smokers (90.9% and 0.60 mm; range: 0.24–0.92 mm; **Table 4**). However, these differences were not statistically significant.

In relation to oral hygiene, 47 of the 102 implants were placed in patients with good oral hygiene, 42 with regular and 13 with poor hygiene. In patients with poor oral hygiene, the success rate at 12 months was lower (81.8%), compared with patients with regular (92.3%) or good hygiene (95.5%). Mean bone loss at 12 months was 0.51 mm (range: 0.21-0.82 mm) in patients with good oral hygiene, 0.57 mm (range: 0.24-0.82 mm) in patients with regular hygiene, and 0.66 mm in those with poor hygiene (range: 0.32-0.92 mm; Table 5). The observed differences were in no case statistically significant. The survival rate of implants placed in patients with poor oral hygiene was lower than in patients with regular or good hygiene. These differences were close to statistical significance (p = 0.058).

Table 1

Study			Relationship between smoking and implant success (p-value)	Relationship between oral hygiene and implant success (p-value)	
Blomqvist et al. ⁸	49	314	0.977	NS	
Jensen et al. ⁹	1007	2997	< 0.05*	NS	
Kan et al. ¹⁰	60	228	0.027*	NS	
Kan et al. ¹¹	60	228	0.027*	< 0.05*	
Levin et al. ¹²	56	— (143 DSL)	0.06	NS	
Beaumont et al. ¹³	45	— (59 DSL)	> 0.05	NS	
Peleg et al. ¹⁴	731	2132	0.394	NS	
Barone et al. ¹⁵	70	287	< 0.05*	NS	
Huynh-Ba et al.1	57	116	0.025*	NS	
Lin et al. ¹⁶	75	155	< 0.05*	NS	
Testori et al.17	106	328	< 0.05**	NS	
Zinser et al. ¹⁸	224	1045	0.009*	NS	
Cha et al. ¹⁹	161	462	0.0003*	NS	

*Significant differences; **significant differences only for > 15 cigarettes/day.

Table 2

Length (mm)	Diameter (mm)			
	3.6	4.2	5.5	
10.0	1	8	13	
11.5	10	48	3	
13.0	0	19	0	

Table 3

Implant placement	No. of implants	No. failed	12 months after loading Success rate (%) Mean bone loss (mm)	
Immediate	50	5	90.0	0.62
Delayed	52	2	96.2	0.54
Total	102	7	93.1	0.58
			p > 0.05	p > 0.05

Table 4

Smoking status	No. of implants	12 months after loading		
-		Success rate (%)	Mean bone loss (mm)	
Nonsmokers	69	94.2	0.52	
Smokers	33	90.9	0.60	
		p > 0.05	p > 0.05	

Table 5

Oral hygiene	No. of implants	12 months after loading		
		Success rate (%)	Mean bone loss (mm)	
Good	47	95.5	0.51	
Regular	42	92.3	0.57	
Poor	13	81.8	0.66	
		p > 0.05	p > 0.05	

Table 1

Effect of smoking and oral hygiene on success of implants placed after direct sinus lift (DSL = direct sinus lift; NS: not studied).

Table 2

Number of implants placed according to length and diameter.

Table 3

Number of implants placed and failed, success rate and mean bone loss according to immediate or delayed placement of implants.

Table 4

Number of implants, success rate and mean bone loss in relation to smoking.

Table 5

Number of implants, success rate and mean bone loss in relation to oral hygiene.

Discussion

Direct maxillary sinus lift is a predictable procedure. Pjetursson et al. performed a systematic review to assess the survival of implants after sinus year implant survival of 90.1% (95% confidence inlift.²⁸ Meta-analysis indicated an estimated an-

nual failure rate of 3.48% (95% confidence interval: 2.48-4.88%), which translated into a threeterval: 86.4–92.8%).²⁸ These results are similar to those obtained in the present study: a success rate of 93.1% for 102 implants placed after 58 direct sinus lifts.

The mean bone loss at 12 months was 0.62 mm for simultaneously placed implants and 0.54 mm for those placed in a second stage. No statistically significant differences were observed. These results were similar to those of Felice et al.: one year after loading, one-stage-treated implants lost an average of 1.01 mm of periimplant bone and two-stage sites about 0.93 mm.²⁹ Similarly, after one year of follow-up, Jodia et al.³⁰ reported a marginal bone loss of between 0.68 and 1.22 mm for simultaneously placed implants, and Kahnberg and Vannas-Löfqvist³¹ of 0.8 mm for implants placed in a delayed mode.

In the literature, smoking has often been associated with a higher failure rate for conventionally placed dental implants,^{32-35, 7} worse osseointegration, as well as more frequent periimplantitis, bone loss and bleeding.^{36, 37} However, in studies published on sinus lift, there is no unanimity regarding the effect of smoking on treatment outcomes. In five of the reviewed studies (Table 1), statistically significant differences were found, observing a higher success rate in nonsmokers than in smokers.^{9, 10, 11, 15, 1} In one study, only smoking > 15 cigarettes/day and a residual ridge height of < 4 mm were significantly associated with reduced implant survival.¹⁷ In other studies,^{8,} ¹²⁻¹⁴ no statistically significant relationship was found between smoking and implant success, although failure rates were higher among smokers. Moreover, Levin et al. observed relevant complications in one-third of the smokers, compared with only 7.7% of the nonsmokers.¹²

A recent systematic review evaluated the effects of tobacco smoking on the survival rate of dental implants placed in areas of maxillary sinus lift. Eight studies, three prospective and five retrospective, were included. Smoking was associated with increased implant failure rates in most individual studies and in the overall meta-analysis. However, when only prospective studies were considered, no significant differences in implant failure were observed between smokers and nonsmokers.³⁸ Similar results were obtained in this study: the implant failure rate and bone loss were slightly higher in smokers, but with the available sample size these differences were not statistically significant.

The literature clearly demonstrates the negative response of the periimplant mucosa to plaque accumulation;³⁹⁻⁴¹ however, there is disagreement regarding the influence of oral hygiene on the success of conventionally placed implants. Mombelli et al.,²¹ Smith and Zarb,²² and Baelum and Ellegaard argue that hygiene did not influence implant outcomes (success and bone loss) in the short term.²³ However, Lindquist et al. observed a higher bone loss in patients with poor oral hygiene.⁵ The influence of hygiene on the success of implants placed after direct sinus lift has been more rarely studied. Kan et al.¹¹ evaluated oral hygiene according to the modified plaque index as described by Mombelli et al.²¹ and reported a failure rate of 1.4% in patients with good oral hygiene, 13.9% with fair hygiene and 60% with poor oral hygiene; the differences between the groups were statistically significant.¹¹ In our study, a lower implant success rate was found in patients with poor hygiene (81.8%), compared with patients with regular and good hygiene (92.3% and 95.5%, respectively). The differences did not reach statistical significance, but the comparison between poor hygiene and the other two categories tended to significance (p = 0.058). In fact, a difference of over 10% with such a predictable treatment technique may be considered of clinical relevance, and the lack of statistical significance is probably related to the small number of patients with poor oral hygiene.

Conclusion

Within its limitations, the present investigation suggests that smoking and poor oral hygiene may negatively influence the outcome of implants placed both in one-stage and two-stage direct sinus lift procedures. However, the differences were in no case statistically significant, and prospective studies with larger sample sizes and longer follow-up are necessary to corroborate or refute these findings.

Competing interests

The authors declare that they have no conflict of interests related to this study.

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Authors will have the opportunity to add more information regarding their article, as well as post videos, present a webinar and blog on the DT Science website.

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