Luiz A. Lima
Anita M. Fuchs-Wehrle
Niklaus P. Lang
Christoph H.F. Hämmerle
Edson Liberti
Eduardo Pompeu
José H. Todescan

Surface characteristics of implants influence their bone integration after simultaneous placement of implant and GBR membrane

Author's affiliations:
Luiz A. Lima, José H. Todescan, Division of Periodontics, Dental Branch, University of São Paulo, São Paulo, Brazil
Anita M. Fuchs-Wehrle, Niklaus P. Lang, Department of Periodontology and Fixed Prosthodontics, University of Bern, Switzerland
Christoph H.F. Hämmerle, Department of Fixed Prosthodontics, University of Zurich, Switzerland
Edson Liberti, Department of Anatomy, Biomedical Science Institute, University of São Paulo, São Paulo, Brazil
Eduardo Pompeu, Laboratory Animal Housing Center, School of Medicine, University of São Paulo, São Paulo, Brazil

Correspondence to:
Dr Luiz Lima
University of São Paulo Dental Branch
Av. Nove de Julho,
5866 Zip code: 01406-200 São Paulo,
S.P. Brazil
Tel.: 55 11 30626188
Fax: 55 11 30833430
e-mail: lapalima@usp.br
A.M. Fuchs-Wehrle
University of Berne, Department of Periodontology and Fixed Prosthodontics
Freiburgstr. 7
3010 Berne, Switzerland
e-mail address: fuxsmile@hotmail.com

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Abstract: The purpose of this study was to evaluate the influence of titanium surface characteristics on bone integration of implants, and to describe the pattern of peri-implant tissue healing after simultaneous implant placement and guided bone regeneration. In four healthy mongrel dogs mandibular premolars were extracted. Two weeks following full mouth prophylaxis and 4 months after extractions, simultaneous membrane and implant surgeries were performed. Efforts were made to produce bony defects with dimensions of $7 \times 7 \times 7$ mm. Into these, 24 standard ITI® implants ($\varnothing = 4.1$ mm; length $= 8$ mm) with either a titanium plasma-sprayed (TPS) or a machined surface (MS) were placed. Although implants were inserted 4 mm into cancellous bone, difficulties in achieving optimal primary stability were encountered. All dogs were maintained on a soft diet. Chlorhexidine rinses were performed three times a week. Full mouth prophylaxis was performed every 2 weeks. In the case of membrane exposure, the membranes were removed prematurely (4–6 or 14–15 weeks after surgery). Two dogs were sacrificed at 16 weeks and two at 24 weeks after surgery. Nondecalcified histologic sections were processed and histometric analyses were carried out. When membranes were removed after 4–6 weeks, a vertical bone growth (VB) of 45–61% of the original defect was noted. After membrane removal at 14–15 weeks, similar VB was observed. However, if membranes were left *in situ* for 24 weeks, VB was between 79% and 96%. In this group of sites, the VB was 66% at 16 weeks and 86% at 24 weeks. Osseointegration in the regenerated bone area ranged from 12% to 32% for the TPS and from 0.0% to 3.6% for the MS implants at 16 and 24 weeks combined. Osseointegration in the pristine host bone area ranged from 16% to 35% for the TPS and from 0.0% to 11% for the MS sites at 16 and 24 weeks. In conclusion, the fraction of implant-bone integration was much higher in the pristine bone compared to that in the regenerated bone. TPS surfaces positively influenced the fraction of osseointegration in comparison to MS surfaces for both regenerated and pristine bone. Furthermore, early membrane removal negatively affected the fraction of bone defect fill.

In the last two decades, oral implants have been shown to be well incorporated into hard and soft tissues with high predictability and have been documented to have a high rate of survival after 15 years or more for instance (Adell et al. 1981; Babbush et al. 1986). In controlled longitudinal studies of titanium plasma-sprayed (TPS) implant surfaces, a success rate reaching 98% at 1 year (Buser et al. 1990b), 97% at 5 years (Mericske-Stern et al. 1994), and 81% at 10 years (Leimola-Virtanen et al. 1995) has been demonstrated. With such favorable clinical results and a histological documentation of tissue integration (Buser et al. 1991, 1992; Listgarten et al. 1991), oral implants have become a reliable device to be included in dental treatment planning.

For long-term implant predictability, recipient sites with sufficient alveolar bone

volume are a prerequisite (Adell et al. 1981). However, after tooth extractions the pattern for alveolar bone resorption may modify the height and width of the residual ridges. This is especially the case when tooth loss is caused by maxillofacial trauma, severe periodontal involvement, or after loss of the buccal bony plate following traumatic tooth extraction. Alveolar ridge atrophy and anatomic aberrations may jeopardize ideal implant installation, particularly in relation to positioning and angulation. Unfavorably positioned implants may subsequently affect the emergence profile, crown shape, embrasure space, physiologic bucco-lingual relationships, esthetics and implant-supported prosthesis function (Mecall & Rosenfeld 1991).

The principle of guided bone regeneration (GBR) allows for bone augmentation, making it possible to prepare sites for implants in areas otherwise impossible to utilize. Animal (Dahlin et al. 1988; Schenk et al. 1994; Buser et al. 1995b; Hämmerle et al. 1997) and human studies (Buser et al. 1993, 1994, 1995a, 1996; Brägger et al. 1997; Hämmerle et al. 1998a, 1998b) demonstrated positive histological proof of efficacy and predictable clinical outcomes.

Experimental studies yielded significantly higher removal torques for implants with a rough TPS surface (Wilke et al. 1990), and a higher percentage of bone-to-implant contact (Buser et al. 1991) when compared to smooth surface titanium implants (electropolished or sandblasted).

Surface roughness also favored *in vitro* (Bowers et al. 1992; Martin et al. 1995) and *in vivo* (Cochran et al. 1998) osteoblast adhesion, differentiation, and extracellular matrix production, resulting in a higher percentage of bone-to-implant contact. Whether or not a rough (TPS) surface will influence osseointegration in regenerated bone, as has been shown for pristine bone (Buser et al. 1991), has not yet been established. Also, the healing pattern of peri-implant tissues following bone augmentation by using the GBR principle simultaneously to implant installation has not yet been described.

It is well established that a knowledge of the biological mechanisms and temporal sequencing of wound-healing events involved in bone formation after GBR are prerequisites to understand the healing steps that take place during regeneration and maturation of bone tissue (for a review see Hämmerle & Karring 1998c). Thus, the description of the healing pattern of peri-implant tissues after GBR simultaneous to implant placement would contribute to this knowledge.

The purpose of this study was therefore to evaluate the influence of titanium surface characteristics on implant–bone integration after simultaneous placement of implant and GBR membrane.

Material and Methods

Surgical procedures and preparation of defects

Four healthy, female mongrel dogs in the age range of 3–5 years were used. They were intraorally examined and received a full mouth prophylaxis. After 2–3 weeks, all mandibular premolars were extracted, thus creating a 41–45-mm-long mandibular edentulous area. All surgical procedures were performed under general anesthesia accomplished by sedation with Xylazine at 2% (20 mg/kg) (Rompum, Laboratórios Bayer, São Paulo, Brazil), administered intramuscularly, and intravenous anesthesia with Thiopental (12.5 mg/kg) (Thionembutal, Abbot Laboratories, Chicago, IL, USA), for the duration of the surgical procedure.

Two weeks following full mouth prophylaxis and 4 months after the tooth extractions, simultaneous bone augmentation and implant installation surgeries were performed. The dogs received prophylactic antibiotic therapy of 5 mg/kg of Enrofloxacin 10% (Baytril, Laboratórios Bayer, São Paulo, Brazil) given once a day, perioperatively and for 7 days postoperatively. They were maintained on soft diet throughout the study period.

A semilunar split-thickness incision of the buccal mucosa was made from the first molar to the canine tooth approximately 4 mm apical to the mucogingival line. From the incision, the mucosa was dissected supraperiosteally to the mucogingival line, where the periosteum was cut. Subsequently, a combined split-thickness/full-thickness flap was elevated, exposing the mandibular alveolar ridge. The buccal periosteal flap was also elevated to allow access to the buccal aspect of the alveolar ridge.

Three rectangular, saddle-like through-and-through defects were created surgically and bilaterally into the alveolar process of the edentulous area using diamond disks, burs, and chisels. Low-speed (<800 r.p.m.) and copious irrigation with sterile saline was used during preparation of the bony defects. Efforts were made to standardize the size of the defects with approximately $7 \times 7 \times 7$ mm. Consequently, each animal received a total of six defects.

Treatment groups, implant design, and surfaces

Into the prepared defects of the jawbone, a total of 24 solid-screw ($\varnothing = 4.1$ mm; length = 8 mm) ITI® implants (Institut Straumann, Waldenburg, Switzerland) with either a TPS or a machined surface MS were placed (Fig. 1). Each dog received three TPS (roughness of 6 μm) and three MS roughness of 0.7 μm) implants. They were

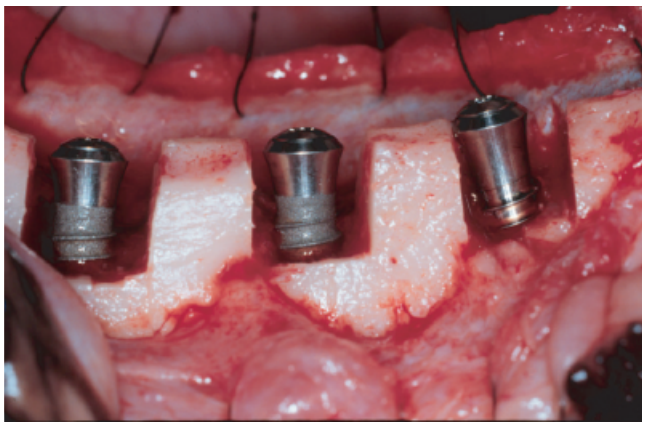

Fig. 1. MS and TPS implants were placed into experimental bony defects.

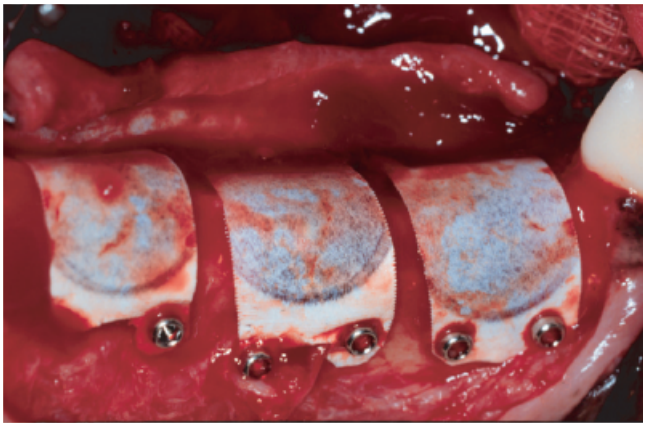

Fig. 2. Membranes were trimmed to shape and placed to cover the implants and the defects. Miniscrews secured the membranes in place.

placed according to standard surgical procedures for implant installation described by Sutter (1996) and covered with small closure screws. Although the implants were inserted with a sink depth of 4 mm into cancellous bone, difficulties in achieving optimal primary stability were encountered. The top of the implants was placed from 0 to 1 mm coronal to the level of the mesial bony crests. A UNC periodontal probe (Hu-Friedy, Chicago, IL, USA) was used to measure the distance from the top of the implant to the bottom of the defects. The measurement was used to double-check defect standardization. Each defect was covered with an e-PTFE membrane (GTAM Oval-9, WL Gore & Associates, Flagstaff, AZ, USA). Each membrane was trimmed to shape and draped over the alveolar ridge to cover the defects completely and extend beyond the defect margins by 2 mm (Fig. 2). This resulted in a wound space defined by the saddle-like defect and the membrane. The membranes were stabilized on the buccal aspects with two stainless-steel miniscrews (Memfix-System®, Institut Straumann, Waldenburg, Switzerland). Intravenously aspirated blood was injected underneath the membranes with a syringe to fill up the defect and to ensure the formation of a blood clot in the defects. Care was taken not to touch the implant surfaces. Subsequently, primary wound closure was achieved with vertical mattress and interrupted e-PTFE sutures (Gore-Tex® sutures, WL Gore & Associates, Flagstaff, AZ, USA).

Anti-inflammatory and analgesic (1 mg/kg of Ketoprofeno, Ketofen 1%, Rhone Merieux, São Paulo, Brazil) medications were given intramuscularly for 5 days following surgery to reduce postoperative swelling and pain. Full mouth prophylaxis with hand and ultrasonic instrumentation were performed every 2 weeks. In addition, chlorhexidine irrigations were performed three times a week. Sutures were removed 10–15 days after bone augmentation surgery. In case of exposure, the membranes were removed prematurely.

Histologic processing and evaluation

Sixteen weeks after surgery, two dogs were sacrificed by induction of deep anesthesia followed by an intravenous overdose of Thiopental (Thionembutal, Abbot Laboratories, Chicago, IL, USA). After exsanguination, the heads of the animals were perfused with a 10% formalin solution. The mandibles were cut distal to the canine and mesial to the first molar. Two-block specimens were harvested per dog with intact soft tissues. Twenty-four weeks after surgery, the two remaining dogs were sacrificed according to the same procedures.

The harvested block sections were labeled and placed in a fixative of 10% formalin. The specimens were rinsed in running tap water, trimmed, and dehydrated in a graded series of increasing ethanol concentrations. They were then embedded in methylmethacrylate resin. The specimens were cut in an oro-buccal direction. The specimens adjacent were cut in a mesio-distal direction. Undecalcified sections, about 500 µm, thick, were obtained using a slow-speed diamond saw (VARICUT®VC-50; Leco, Munich, Germany) with coolant. Subsequently, the sections were glued with acrylic cement to opaque Plexiglas, ground and polished to a final thickness of about 80–100 µm (Knuth-Rotor-3; Struers, Rødrove/Copenhagen, Denmark), and surface-stained with toluidine blue (Schenk et al. 1984).

From each specimen the central, buccolingual and mesio-distal sections were selected for quantitative analyses of different tissue components by applying standard morphometric techniques (Weibel 1980; Gundersen et al. 1988). Measurements were carried out directly under the light microscope at a magnification of × 80, using an optically superimposed eyepiece test grid composed of 10 cycloid lines per millimeter (Schenk & Olah 1980; Weibel 1980). The specimens were histologically examined, described, and photographed. Linear histometric measurements were made at buccal–lingual and mesial–distal sections to evaluate the following parameters: (Fig. 3)

1. *Defect depth* (*DD*): measured from the implant shoulder to the level of pristine host bone closest to the implant surface in an apical direction.
2. *Vertical bone growth* (*VB*): measured from the bottom of the original defect to the most coronal portion of newly formed bone.
3. *Osseointegration in regenerated bone* (*ORB*): measured by the number of test lines overlaying the profiles of bone-to-implant contact within regenerated bone area, namely, from the base of

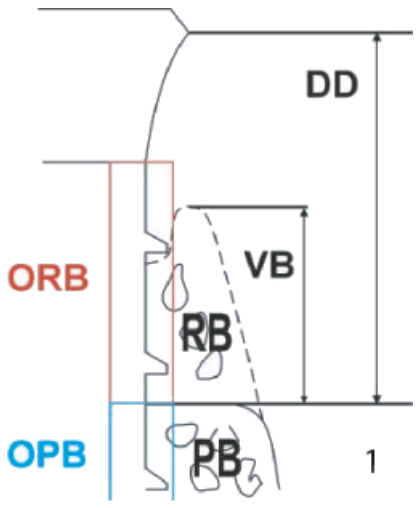

Fig. 3. Schematic drawing depicting assessed histometric parameters. DD: defect depth; VB: vertical bone growth; RB: regenerated bone; PB: pristine bone; ORB: osseointegration in regenerated bone; OPB: osseointegration in pristine host bone.

the defect to the lower level of the polished neck of the implant (2.8 mm below the implant shoulder).

4. *Osseointegration in pristine host bone* (*OPB*): measured by the number of test lines overlaying the profiles of bone-to-implant contact within pristine host bone area, namely, apical to the base of the original defect. Means and standard deviations were calculated for the four experimental groups.

Results

All dogs recovered well from the surgical interventions. Ten membranes were prematurely removed due to membrane exposure. After membrane removal, at least 8 weeks passed before the dogs were sacrificed.

Descriptive histology

Implants that had their membranes prematurely removed showed well-developed peri-implant tissues. A keratinized stratified squamous epithelium lined the external slope of the peri-implant mucosa. A nonkeratinized barrier epithelium could be observed facing the implant. Below, a low vascularized, dense connective tissue, with collagen fibers running parallel to the implant surface, was observed. Some blood vessels were seen near the crest of the bone. Variable amounts of newly formed bone were visible.

Buccal–lingual sections showed intense new bone formation at the crest of the bone, emerging from cortical bone (Fig. 4A). Remodeling below the inferior wall of the defect documented clearly that this region was participating in the healing process; reversal lines and lamellar bone formation were seen. Newly formed bone appeared more heavily stained, vessel rich, highly cellular, and less organized than pristine host bone. A lower density of mineralized tissue was observed when compared to mature bone. It is interesting to note that, at 16 weeks, the newly formed bone

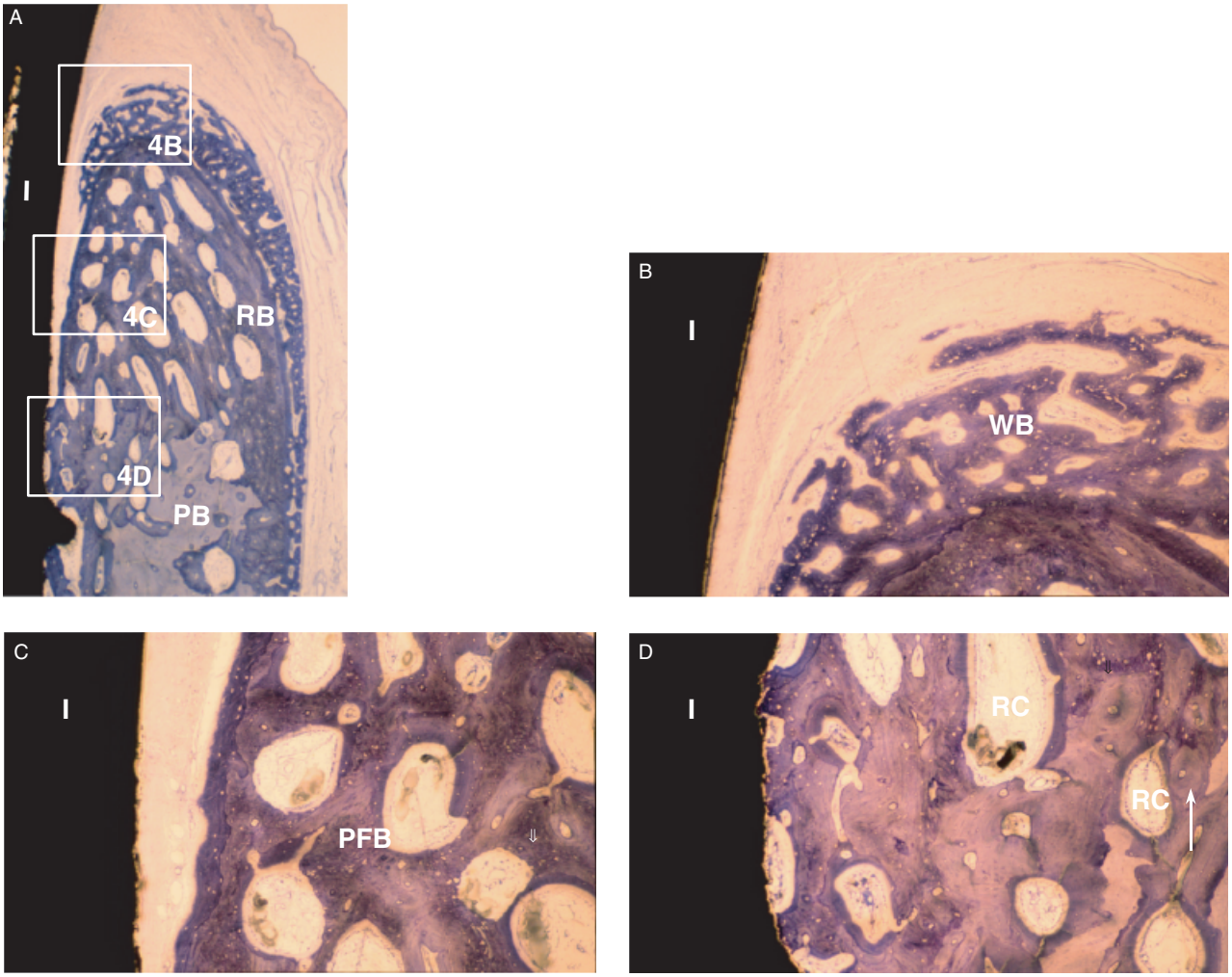

Fig. 4. Bucco-lingual section: healing pattern of the regenerated bone at 16 weeks after premature membrane removal (at 4–6 weeks). (A) Although a reasonable amount of newly formed bone may be seen, no bone-to-implant contact can be observed at the polished neck of the TPS implant. Regenerated bone (RB) displays a maturation gradient, being more mature closer to the inferior wall of the defect and more immature at its coronal portion. PB: pristine bone. (B) Woven bone (WB) formation may be seen at the crest of the newly formed bone. It is comprised of a highly cellular tissue, with large osteocytes lacunae, and bone struts and ridges contouring blood vessels forming an irregular structure. (C) Note the initial organization of concentric lamellae. Continuous bone deposition ensures the thickening of bone trabeculae, i.e., reinforcement of woven bone (arrow) by parallel-fibered bone (PFB). A continuous layer of osteoblasts surrounds well-vascularized intertrabecular spaces. (D) At the apical portion of the defect, higher bone density, with primary and secondary osteons (arrow) structure, may be observed. Note regenerated bone in contact with the implant surface. I: implant; RC: resorption canal. Toluidine blue stain, original magnification × 18 (A) and x80 (B–D).

displayed different levels of tissue maturity (Fig. 4B–D), being more mature closer to the wall of the defect (Fig. 4D) and more immature at its coronal portion (Fig. 4B).

At mesial–distal sections, a reduction of the original bone height could be observed in a few specimens, while bone formation up to the top of the implant could be noted in others. Trabecular bone depicted a disorganized lamellar formation. The most apical part of the defect showed newly formed bone reaching the implant surface (Fig. 5a). The same was not detected at the most coronal portion of the defectal, although, most of the times, newly formed bone had filled more than half the way to reach the implant surface. Dense connective tissue, with a great amount of collagen fibers running parallel to the implant surface, was seen interposed between the implant surface and the newly formed bone. The structural organization of the newly formed bone showed clearly that it originated from the walls of the previous defect. The maturation gradient observed at 16 weeks, in regenerated bone, was not recognizable anymore. A more advanced maturation level was noted at 24 weeks, although not yet similar to pristine bone (Fig. 5A and B). No differences among these descriptive parameters were encountered when TPS and MS were compared.

However, osseointegration showed significant differences for each implant surface. In regenerated bone, bone-to-implant contact was frequently observed at the most apical portion of the defect. TPS implants revealed a higher amount of bone-to-implant contact when compared to MS. Even though regenerated bone was seen high up to the top of the implant and occasionally even above it, no bone-to-implant contact was ever observed at the polished neck of an implant. Interposed between the regenerated bone and the polished implant surface, a dense connective tissue was visible. In the area of pristine bone, results revealed, again, a greater bone-to-implant contact for the TPS than for the MS implants.

Implants that had their membranes left *in situ* exhibited epithelium and connective tissue covering the membranes. The oral epithelium, a keratinized stratified squamous epithelium, displayed projections into the connective tissue. Below, a dense connective tissue showed collagen fibers running parallel to the membrane surface.

No inflammatory response was noted around the membranes, endorsing the biocompatibility of the e-PTFE membranes. At the border of the membrane, connective tissue integration was evident, showing fibroblasts and vessels penetrating its pores. Good tissue integration could also be observed with underlying bone. Mineralized bone tissue could be detected inside membrane porosities and, occasionally, reaching the external surface.

Beneath the membrane and lateral to the implant surface, a dense connective tissue was observed filling variable amounts of the preserved space. Differently to the supracrestal connective tissue displayed at the implants that had the membranes prematurely removed, these disclosed a highly vascularized tissue. Variable amounts of newly formed bone could also be noted. A dense connective tissue with collagen fibers running parallel to the implant surface was visible interposed between the implant and the regenerated bone. Similar to what was observed before, the regenerated bone tissue showed more maturation closer to the walls of the defect than at the coronal portions, at 16 weeks. Again, bone formation originated from the walls of the defect. Regenerated bone exhibited highly vascularized, profuse cells, low mineralized tissue density and was more heavily stained than pristine bone (Figs 6A and B, 7A and B).

Osseointegration at regenerated and pristine host bone revealed similar bone-to-implant contacts when the membranes had been prematurely removed. Mesial–distal sections displayed bone formation starting from the walls of the previous defects, favoring bone filling of the apical portion of the defect. Some sections revealed a connective tissue component between the implant surface and the coronal portion of the regenerated bone, while others displayed osseointegration throughout the regenerated bone below the implant collar. Often, osseointegration was observed below the polished neck of the implant.

Direct bone deposition onto the implant surface was observed for both TPS and MS, without any other detectable tissue components interposed at the light microscopy

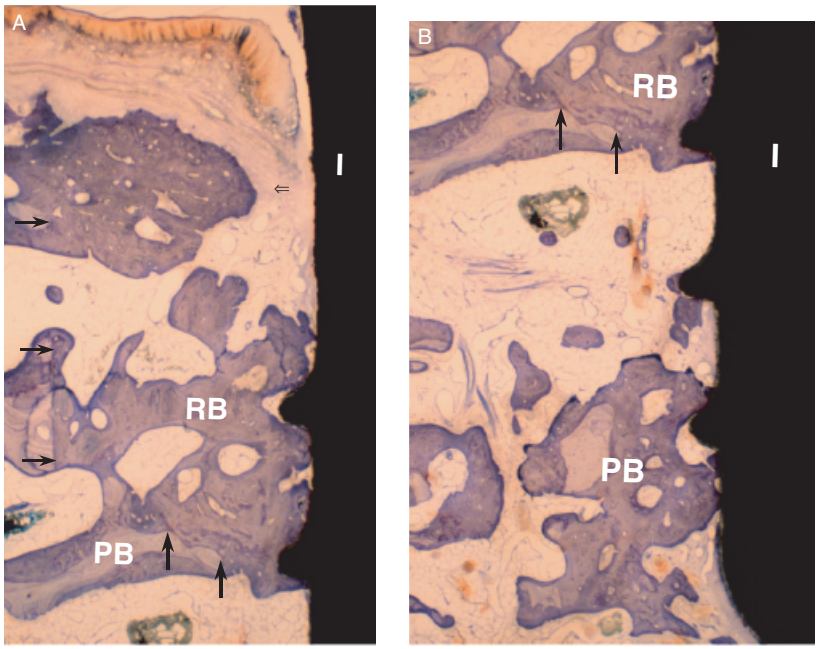

Fig. 5. Mesio-distal section: healing pattern of the regenerated bone at 24 weeks after premature membrane removal (at 15–16 weeks). (A) Cortical regenerated bone is predominantly structured by primary and secondary osteons, although tiny portions of woven bone may still be encountered. Trabecular bone shows newly formed packets of lamellar bone; RB filled the apical part of the defect reaching contact with the TPS implant surface. Newly formed bone occupied most of the coronal portion of the defect. However, a connective tissue interposed with the implant surface (arrow); (B) Note that the implant surface is in contact with a well-vascularized bone marrow and newly formed trabeculae. Newly formed bone connects pristine bone trabeculae between themselves and to the implant surface. I: implant; PB: pristine bone; RB: regenerated bone; arrows: pristine bone defect wall. Toluidine blue stain, original magnification × 18 (A and B).

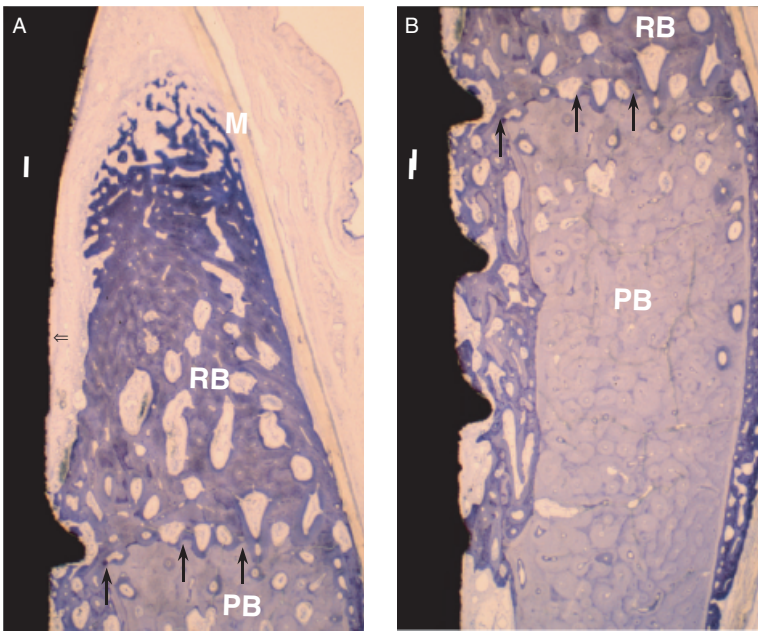

Fig. 6. Bucco-lingual section: healing pattern of peri-implant tissues with membrane *in situ*, at 16 weeks. (A) Note maturation gradient for the regenerated bone. Newly formed bone fills the space enclosed by the membrane (M), the implant (TPS surface), and the inferior wall of the defect. A highly vascularized connective tissue is seen separating the regenerated bone and the implant surface, except at the apical portion of the defect. (B) Apical to the inferior wall of the defect, newly formed bone has been growing to reach the TPS surface, ensuing osseointegration. I: implant; PB: pristine bone; RB: regenerated bone; arrows; pristine bone defect wall. Toluidine blue stain, original magnification × 18 (A and B).

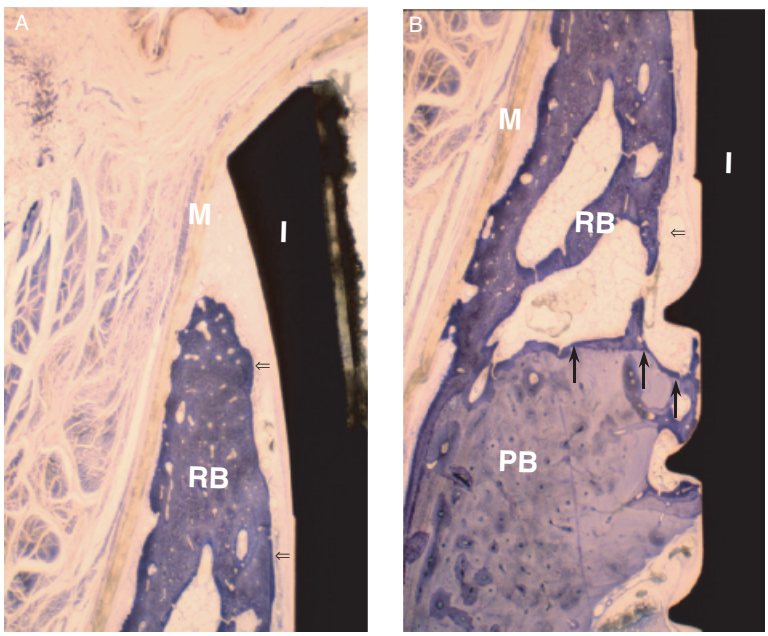

Fig. 7. Bucco-lingual section: healing pattern of peri-implant tissues with membrane *in situ*, at 24 weeks. (A) Regenerated bone displays higher density than at 16 weeks. It is mainly constructed by concentric lamellae deposition, yet small portions of woven bone may be depicted. (B) Note bone-to-implant contact with pristine as well as with regenerated bone, showing osseointegration to the MS implant surface. I: implant; PB: pristine bone; RB: regenerated bone; M: membrane; arrows: pristine bone defect wall. Toluidine blue stain, original magnification × 18 (A and B).

level. The TPS has microprotrusions and microconcavities in which mineralized bone tissue penetrated. However, the smooth surface, MS, revealed a well-delineated bone contour that reflected the implant surface profile (Fig. 8A and B).

Histomorphometric analysis

Fourteen implants were available, and the remaining sections were excluded of histomorphometric analysis because of variabilities of membrane removal. Among the implants, selected for analysis, six had the membranes removed after 4 to 6 weeks, 4 had the membranes removed after 14–15 weeks, and four had the membranes left *in situ* for the entire observation period. Buccal–oral and mesial–distal sections were analyzed. These allowed the calculation of average measurements from buccal, oral, mesial, and distal aspects of each implant.

Mean defect depths (DD) ranged from 5.3 (SD 0.1) to 6.1 (SD 1.3) mm (Table 1), reflecting the standardization of the experimentally created defects. Table 2 depicts VB. The percentage of bone defect fill demonstrated that more new bone formation could be observed when the membranes were left *in situ* as compared to a premature removal. VB ranged from 45.8% (SD 17.5%) to 61.8% (SD 12.9%) with a 4–6 week membrane removal. Similar results were observed with a membrane removal after 14–15 weeks (ranging from 46.4%, SD 7.2% to 57.5%, SD 29.9%). However, if membranes were left *in situ* for the entire period of observation, VB was between 79.1% (SD 46.9%) at 16 weeks (TPS) and 96.2% (SD 21.0%) at 24 weeks (MS). In this group of sites, when the membrane was used as the reference for the top of the defect, VB was 66.7% (SD 37.1%) (at 16 weeks for TPS) and 86.0% (SD 11.9%) (at 24 weeks for MS) (Table 3).

Osseointegration of the implants in regenerated bone (ORB) is shown as the percentage of bone-to-implant contact fraction in the area of regenerated bone. Bone-to-implant contact fraction ranged from 12% (SD 15%) to 32% (SD 7%) for the TPS and from 0.0% (SD 0%) to 3.6% (SD 7%) for the MS, at 16 and 24 weeks, respectively (Table 3).

The percentage of bone-to-implant contact in the area of pristine host bone (OPB) demonstrated greater values for TPS than for MS (Table 4). These values ranged from 16.3% (SD 12.7%) to 35.8% (SD 12.0%) for the TPS and from 0.0% (SD 0.0%) to 11.2% (SD 7.7%) for the MS, at 16 and 24 weeks, respectively. When membranes were left *in situ* for the entire observation period, OPB was 8.3% (SD 7.0%) for MS

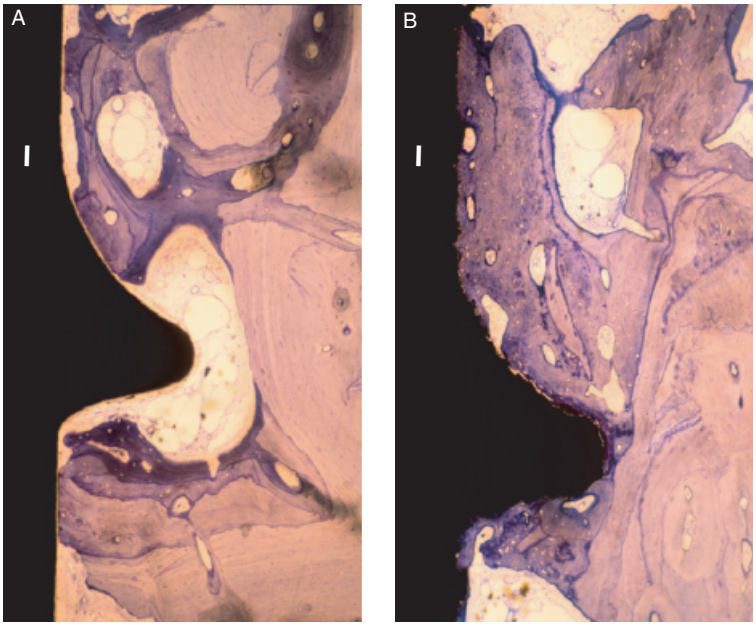

Fig. 8. Bucco-lingual sections: MS and TPS osseointegration interface after 24 weeks. (A) The bone–implant interface was not always ideally preserved for MS implants. Small gaps separated bone from the implant surface, but perfect contour similarity was frequently observed between them. This MS implant shows osseointegration in pristine and in regenerated bone; (B) Mineralized bone is in intimate contact with the TPS surface microprotrusions and microconcavities. I: implant. Toluidine blue stain, original magnification × 80 (A and B).

***Table 1*. Histomorphometric measurements of Defect depth (DD) in an apical direction from the implant shoulder to the level of the pristine host bone closest to the implant surface.**

Membrane Status	Implant Surface	Healing Period (weeks)	N	Maximum height (mm)		
				Mean	SD	range
4–6 weeks	TPS	16	3	5.6	0.7	5.2–6.0
removal	MS	16	3	6.1	1.3	5.1–7.2
14–15 weeks	TPS	24	2	5.3	0.1	5.2–5.5
removal	MS	24	2	5.4	1.6	4.1–6.7
Membrane	TPS	16	2	5.8	0.7	5.0–6.7
"in situ"	MS	24	2	5.6	0.4	5.3–6.0

N = number of specimens

***Table 2*. Vertical bone growth (VB) measured from the bottom of the original defect to the most coronal portion of newly formed bone, whether in contact with implant surface or not.**

Membrane Status	Implant Surface	Healing Period (weeks)	N	Reference mark					
				Implant shoulder			Membrane		
				Maximum height (%)			Maximum height (%)		
				Mean	SD	range	Mean	SD	range
4–6 weeks	TPS	16	3	61.8	12.9	57.0–69.7			
removal	MS	16	3	45.8	17.5	37.2–61.0			
14–15 weeks	TPS	24	2	46.4	7.2	46.1–46.8			
removal	MS	24	2	57.5	29.9	32.4–82.7			
Membrane	TPS	16	2	79.1	46.9	69.4–88.9	66.7	37.1	59.3–74.0
"in situ"	MS	24	2	96.2	21.0	93.4–99.0	86.0	11.9	83.6–88.4

N = number of specimens

after 24 weeks and 30.2% (SD 13.8%) for TPS after 16 weeks.

Discussion

The purpose of this study was to describe the pattern of peri-implant tissue healing after simultaneous implant and membrane placement in dogs. The descriptive results of the present study after premature membrane removal corroborate, to a great extent, the findings of previous studies regarding soft tissue healing (Berglundh et al. 1991, 1994; Buser et al. 1992; Listgarten et al. 1992). However, a great amount of vessels coronally to the newly formed bony crest close to the implant surface was also demonstrated. This finding may be explained by the fact that, at 24 weeks, the regenerated bone may still exhibit haversian remodeling, and may need adequate vascularization (Schenk 1994). A great amount of vessels was frequently observed between regenerated bone and the implant surface (Figs. 4–7).

Thick supracrestal collagenous fiber bundles, directed from the gingival margin to the bony crest and contouring it, as well as collagenous fibers dispersed parallel to the implant surface were recognized, which is also in agreement with earlier studies (Berglundh et al. 1991, 1994; Buser et al. 1992; Listgarten et al. 1992). No collagenous fibers inserting perpendicularly into the porous TPS surface were identified, and this is also in accordance with earlier studies (Schroeder et al. 1981).

At 16 weeks, regenerated bone originating from the pristine bone showed a maturation gradient, being more matured closer to the inferior wall of the defect than at its coronal portion. This outcome was noticed not only in specimens in which the membrane had been removed prematurely but also in specimens in which the membranes were left *in situ* for the entire observation period (Figs. 4 and 6). This effect is in accordance with the reality that bone growth is incremental (Schenk 1994).

Schenk et al. (1994) reported that haversian remodeling started at 16 weeks after a GBR procedure in dogs. The results of the present study endorsed these findings and revealed that remodeling started near the walls of the defect. After 24 weeks, remodeling was quite advanced, not dis-

Table 3. **Percentage of implant-bone contact in the regenerated bone (ORB) measured from the base of the defect to the border between the polished neck portion of the implant and the TPS or the MS surface, respectively (i.e. 2.8 mm below the implant shoulder).**

Membrane Status	Implant Surface	Healing Period (weeks)	N	Bone to implant contact (%)		
				Mean	SD	range
4–6 weeks	TPS	16	3	20.2	24.6	2.3–38.0
removal	MS	16	3	0.0	0.0	0.0–0.0
14–15 weeks	TPS	24	2	32.5	7.1	30.2–34.8
removal	MS	24	2	3.6	7.3	2.3–5.0
Membrane	TPS	16	2	12.5	15.4	0.0–25.1
"in situ"	MS	24	2	2.5	4.6	0.0–5.0

N = number of specimens

Table 4. **Percentage of implant-bone contact in pristine host bone (OPB) measured in the area apical to the base of the original defect.**

Membrane Status	Implant Surface	Healing Period (weeks)	N	Bone to implant contact (%)		
				Mean	SD	range
4–6 weeks	TPS	16	3	16.3	12.7	8.7–23.8
removal	MS	16	3	0.0	0.0	0.0–0.0
14–15 weeks	TPS	24	2	35.8	12.0	27.4–44.2
removal	MS	24	2	11.2	7.7	9.7–12.7
Membrane	TPS	16	2	30.2	13.8	21.0–39.4
"in situ"	MS	24	2	8.3	7.0	6.9–9.7

N = number of specimens

playing a maturation gradient anymore. Nonetheless, regenerated bone was not fully developed when compared to pristine bone (Figs. 4 and 5).

Premature membrane removal did not prevent osseointegration in dogs, as depicted in pristine or in regenerated bone (Figs. 4 and 5). The observation that new bone growth started from the walls of the previous defects is in accordance with former studies (Dahlin et al. 1988; Zablotsky et al. 1991; Schenk et al. 1994; Hämmerle et al. 1997).

Regarding vertical bone growth, this study has shown that premature membrane removal negatively affected its values. VB was reduced by 39%–50% of the figures found for sites where membranes were left *in situ* for 24 weeks. Smaller amounts of regenerated bone have been reported in earlier studies after bacterial contamination of the membrane and with a premature membrane removal (Buser et al. 1990a; Warrer et al. 1991; Buser et al. 1994; Kohal et al. 1998).

In humans, only 41.6% of vertical bone growth was found after premature membrane removal (Simion et al. 1994). In the present study, VB ranged from 45.8% to 61.8% if membranes were removed prematurely. But when the membranes were left *in situ*, VB increased from 79% at 16 weeks to 96% at 24 weeks. In this group of sites, when the membrane was used as the reference for the top of the defect, VB increased from 66.7% at 16 weeks to 86.0% at 24 weeks. This outcome suggests that a significant quantity of ongoing bone formation, amounting roughly to 30% or more, takes place between 16 and 24 weeks.

Important factors related to the superficial configuration of the implants are its microstructure (measured in μm) and its ultrastructure (measured in nm). Recent studies have shown that surface roughness favored *in vitro* (Bowers et al. 1992; Martin et al. 1995) and *in vivo* (Cochran et al. 1998) osteoblast adhesion, differentiation, and extracellular matrix production, resulting in more bone-to-implant contact, which was more rapidly achieved. Cells seem to be susceptible to surface microtopography and capable of using this morphology for orientation and migration (Brunette 1988, Chehroudi et al. 1989; 1990). Furthermore, the chemical composition of the implant surface plays a fundamental role in tissue response. Damen et al. (1991) demonstrated, *in vitro*, that titanium oxide may act as a nucleating substrate for calcium phosphate crystals.

Moreover, it has been difficult to establish comparable parameters between studies because of the deficiency of adequate perception of cellular and tissue reactions to infinite possible variations of the surface characteristics. The same macroscopic design was used. The objective was to control the macroscopic variables and to be able to compare the influence of the titanium surface characteristics within the conditions established for the present study.

The results of the present study have shown that the percentage of implant–bone contact in regenerated bone was higher for the TPS surface (12.5%–32.5%) than for the MS (0.0%–3.6%) implants. Similar results were observed for osseointegration in pristine bone (TPS, 16.3%–35.8%; MS, 0.0%–11.2%). This outcome is in agreement with previously reported results in pristine bone (Buser et al. 1991). Table 3 shows an increase of bone-to-implant contact from 16 to 24 weeks, for MS (0.0%–3.6%) and for TPS (20.2%–32.5%). These results corroborate those which reported an increased osseointegration over time (Johansson & Albrektsson 1987, 1991).

After GBR membrane placement simultaneously to machined surface implant placement, Kohal et al. (1998) reported 20.9% bone-to-implant contact in regenerated bone and 37.3% in pristine bone. The same procedure performed by Palmer et al. (1998), in humans, resulted in 6% bone-to-implant contact in regenerated bone and 20%–25% in pristine bone. After loading, osseointegration increased to 22% in regenerated bone and to 28–52% in pristine bone. A possible explanation for the lower percentages of osseointegration observed in the present study is the fact that most of the cortical bone had been removed from contact with the implants during creation of the experimental defects. Moreover, most of the studies evaluated bone-to-implant contact using the best threads measured in buccal–lingual sections, which mostly means measurements in cortical bone. The results of the present study originated from buccal, lingual, mesial, and distal measurements of the whole

implant surface, measured in cortical and cancellous bone.

Osseointegration was frequently observed only at the most apical part of the defect, even when regenerated bone reached up to the top of the implant. These outcomes might be explained by the limited capacity of bone tissue to repair gaps of 1 mm in one single step, known as critical bone jumping distance (Schenk 1994), and/or by the effect reported by Schwartz et al. (1996) that the cellular response to a specific surface is influenced by cell maturation state. The present study demonstrated a maturation gradient in regenerated bone. Carlsson et al. (1988) reported a distance of 0.35 mm from pristine bone to be the limit for lamellar bone deposition to the implant surface within a period of 12 weeks of healing.

It is important to note that complete bone defect fill did not determine the end stage of healing. Regenerated bone may appear to have adequate bone density but needs maturity characteristics, which provide satisfactory functional capabilities. Haversian remodeling of regenerated cortical bone and lamellar deposition of cancellous bone furnish adequate functional structure (Schenk 1994).

Additional studies should be carried out to further evaluate the importance of the gap between the implant surface and the defect bony walls on the outcome after placement of membranes simultaneously to implant placement.

Conclusion

Peri-implant tissues heal adequately after premature membrane removal, although the end stage of healing is not reached at 24 weeks in dogs. Premature membrane removal does not prevent implant integration in the bone but it does negatively affect the fraction of bone defect fill.

The percentage of implant–bone contact was much higher in the pristine bone compared to the regenerated bone. TPS surfaces positively influenced the fraction of bone-to-implant contact in comparison to MS surfaces for both regenerated and pristine bone.

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Résumé

Le but de cette étude a été d'évaluer l'influence des caractéristiques de surface du titane sur l'intégration osseuse d'implants et de décrire la guérison tissulaire paroïmplantaire après un placement implantaire simultané à la régénération osseuse guidée (GBR). Chez quatre chiens batârds sains les prémolaires inférieures ont été avulsées. Deux semaines suivant une prophylaxie de l'entiereté de la bouche et quatre mois après les avulsions, une chirurgie avec implant plus membrane a été effectuée. Des efforts ont été fournis pour produire des lésions osseuse avec des dimensions de 7x7x7 mm. Dans ces dernières, 24 implants ITI® standards (diamètre 4,1 mm, longueur 8 mm) avec soit une surface plasma-spray en titane (TPS) soit usinée (MS) ont été placés. Bien que les implants ont été insérés 4 mm dans l'os spongieux des difficultés pour obtenir une stabilité primaire optimale ont été rencontrées. Tous les chiens ont été maintenus dans un régime de nourriture molle. Des rinçages à la chlorhexidine ont été effectués trois fois par semaine. Une prophylaxie a été faite toute les deux semaines. En cas d'exposition des membranes, ces dernières ont été enlevées prématurément (4 à 6 ou 15 à 16 semaines après la chirurgie). Deux chiens ont été tués après seize semaines et deux autres 24 semaines après la chirurgie. Des coupes histologiques non-décalcifiées et des analyses histométriques ont été effectuées. Lorsque les membranes avaient été enlevées après 4 à 6 semaines, une croissance osseuse verticale (VB) de 45 à 61 % de la lésion originale était notée. Lorsqu'elles avaient été retirées après 14 à 15 semaines, un VB semblable était observé. Cependant, si les membranes étaient restées *in situ* pendant 24 semaines, VB était de 79 à 96 %. Dans ce groupe de sites, le VB était de 66 % à seize semaines et de 86% à 24 semaines. L'ostéoïntégration dans la zone d'os régénéré s'étalait de 12 à 32 % pour les TPS et de 0,0 à 3,6% pour les MS à 16 et 24 semaines combinées. L'ostéoïntégration dans la zone osseuse de l'hôte original s'étalait de 16 à 35 % pour les TPS et 0,0 à 11% pour les MS à 16 et 24 semaines. En conclusion, la fraction implant-intégration osseuse était beaucoup plus importante dans l'os original comparé à celle trouvée dans l'os régénéré. Les surfaces TPS influencent de manière positive la fraction d'ostéoïntégration en comparaison aux surfaces MS tant pour l'os original que pour le régénéré. De plus, l'enlèvement précoce de la membrane affecte de manière négative la fraction de remplissage osseux de la lésion.

Zusammenfassung

Die Oberflächencharakteristika eines Implantates beeinflussen bei der Sofortimplantation und gleichzeitiger Abdeckung mit einer GBR-Membran die Knochenintegration.

Das Ziel dieser Studie war es, den Einfluss der Charakteristika einer Titanoberfläche auf die Knochenintegration von Implantaten zu untersuchen, und die Abläufe bei der Heilung der periimplantären Gewebe nach gleichzeitiger Implantation und GBR zu beschreiben. Bei vier gesunden Bastardhunden extrahierte man die Prämolaren im Unterkiefer. Vier Monate nach den Extraktionen und nach zweiwöchiger guter Mundhygiene wurden gleichzeitig Implantate gesetzt und die Membranen gelegt. Man versuchte wenn möglich Knochendefekte von 7x7x7 mm Grösse zu produzieren. Dort hinein setzte man 24 ITI®-Standartimplantate (ø = 4.1mm; Länge = 8mm) mit entweder einer titanplasmabesprayten (TPS) oder maschinell gedrehten (MS) Oberfläche. Obwohl die Implanate 4 mm in spongiösen Knochen implantierte wurden, bekundete man Mühe, eine optimale Primärstabilität zu erreichen. Alle Hunde wurden auf einer weichen Diätkost gehalten. Dreimal wöchentlich führte man Chlorhexidinspülungen durch. Jede zweite Woche reinigte man professionell den ganzen Mund. Sobald es zu einer Membranexposition kam, entfernte man sie vorzeitig (4-6 oder 14-15 Wochen nach der Chirurgie). Zwei Hunde wurden 16 Wochen und die zwei anderen 24 Wochen nach der Chirurgie geopfert. Anschliessend führte man histometrische Analysen auf nichtentkalkten histologischen Schnitten durch. Wenn die Membranen nach 4-6 Wochen entfernt werden mussten, verzeichnete man ein vertikales Knochenwachstum (VB) von 45 %-61 % des ursprünglichen Defektes. Wenn die Membranen nach 14-15 Wochen entfernt werden mussten, beobachtete man ein ähnliches VB. Konnten die Membranen jedoch während 24 Wochen in situ belassen werden, so zeigte sich ein VB zwischen 79 %-96 %. In dieser Gruppe von Stellen veränderte sich der VB von 66 % in Woche 16 zu 86 % in Woche 24. Die Osseointegration in der Region des regenerierten Knochens schwankte zwischen 12 %-32 % bei den TPS- und von 0.0 %-3.6 % bei den MS-Implantaten, sowohl in der Gruppe von 16 und 24 Wochen. Die Osseointegration in der ursprünglichen Region des Wirtsknochens schwankte für beide Gruppen zwischen 16 %-35 % bei den TPS- und von 0.0 %-11 % bei den MS-Implantaten. Zusammenfassend kann man sagen, dass der Anteil der Knochenintegration von Implantaten in ursprünglichen Wirtsknochen viel grösser als im regenerierten Knochen war. Die TPS-Oberfläche beeinflusste den Anteil dieser Osseointegration verglichen mit der MS-Oberfläche sowohl im regenerierten wie auch im ursprünglichen Knochen positiv. Darüber hinaus, beeinflusste eine frühzeitige Membranentfernung die Menge der Defektauffüllung negativ.

Resumen

La intención de este estudio fue evaluar la influencia de las características de la superficie de titanio en la integración ósea de los implantes, y describir el patrón de cicatrización del tejido periimplantario tras colocación simultanea de implantes y GBR. Se extrajeron los molares mandibulares en cuatro perros mongrel sanos. Dos semanas tras una profilaxis oral completa y cuatro meses tras las extracciones, se llevaron a cabo cirugías de implante y membrana simultaneas. Se realizaron esfuerzos para producir defectos óseos de unas dimensiones de $7 \times 7 \times 7$ mm. En ellos se colocaron 24 implantes estándar ITI® ($\varnothing = 4.1$ mm; longitud = 8 mm) con superficie pulverizada de plasma de titanio (TPS) o pulida (MS). Aunque los implantes se insertaron en 4 mm de hueso esponjoso, se encontraron dificultades en la consecución de una óptima estabilidad primaria. Todos los perros se mantuvieron con una dieta blanda. Se llevaron a cabo enjuagues con clorhexidina tres veces por semana. Se llevó a cabo una profilaxis completa cada dos semanas. En caso de exposición de la membrana, estas se retiraron prematuramente (4–6 o 14–15 semanas tras la cirugía). Se sacrificaron dos perros a las 16 semanas y dos a las 24 semanas tras la cirugía. Se procesaron secciones histológicas y se realizó análisis histométrico. Cuando se retiraron las membranas tras 4–6 semanas, se observó un crecimiento vertical de hueso (VB) del 45%–61% del defecto original. Tras la retirada de la membrana a las 14–15 semanas, se observó un VB similar. De todos modos, si las membranas se quedan *in situ* durante 24 semanas, el VB fue del 79–96%. En este grupo de lugares, el VB fue del 66% a las 16 semanas y del 86% a las 24 semanas. La osteointegración en el área de hueso regenerado varió entre el 12–32% para los lugares TPS y del 0.0% al 3.6% para los MS a las 16 y 24 semanas. La osteointegración en el área de hueso original huésped varió entre 16–35% para los lugares TPS y entre 0.0–11% para los MS a las 16–24 semanas. En conclusión, la fracción de integración hueso-implante fue mucho mayor en el hueso original comparado con aquel en el hueso regenerado. Las superficies TPS influyeron positivamente en la fracción de osteointegración en comparación con la superficie MS para tanto el hueso regenerado y el original. Más aún, la retirada temprana de la membrana afectó negativamente el relleno del defecto de la fracción de hueso.

要旨

本研究は、チタン表面の性状がインプラントの骨性結合に及ぼす影響を評価し、GBRとインプラント同時埋入後の、インプラント周囲組織の治癒パターンを調べることを目的に行った。健常雑種犬４匹において、下顎前臼歯を抜去した。全顎の衛生処置を行った２週間後及び抜歯４ヶ月後に、メンブレン貼付とインプラント手術を同時に行った。寸法７ｘ７ｘ７ｍｍの骨欠損を実験的に形成し、そこに、チタン・プラズマ溶射（ＴＰＳ）表面または機械加工の表面（ＭＳ）の、標準 ITI® インプラント（直径＝４．１ｍｍ；長径８ｍｍ）２４本を埋入した。インプラントは海綿骨に４ｍｍ挿入したが、最適な初期固定を得るのは困難であった。全ての犬に柔らかい餌を与えた。クロルヘキシジンによる洗浄を１週間に３回行った。全顎の衛生処置を２週間ごとに行った。メンブレンが露出した際は、早期に（術後４－６週間または１４－１５週間）除去した。２匹の犬を術後１６週間後に、２匹は２４週間後に屠殺した。非脱灰組織切片を作成し、組織学的分析を行った。メンブレンを４－６週間で除去した群では、元の欠損の４５％－６１％の垂直的骨成長（ＶＢ）が得られた。１４－１５週間後にメンブレンを除去した群でも、類似のＶＢが観察された。しかしメンブレンを局所に２４週間留置した群では、ＶＢは７９％－９６％であった。この群では、ＶＢは１６週間後に６６％、２４週間後に８６％であった。再生骨における骨性結合は、１６週間後と２４週間後を組み合わせて、ＴＰＳの場合が１２％－３２％、ＭＳが０．０％－３．６％であった。既存骨における骨性結合は、１６週間後と２４週間後で、ＴＰＳが１６％－３５％、ＭＳが０．０％－１１％であった。結論として、インプラント－骨の結合の率は、再生骨に比べて既存骨の方がはるかに高かった。ＴＰＳ表面はＭＳ表面に比べ、再生骨でも既存骨でも骨性結合率にプラスの影響を与えた。さらに早期のメンブレン除去は、欠損部の骨再生率にマイナスの影響を及ぼした。

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