THE INTERNATIONAL JOURNAL OF DENTAL IMPLANTS & BIOMATERIALS

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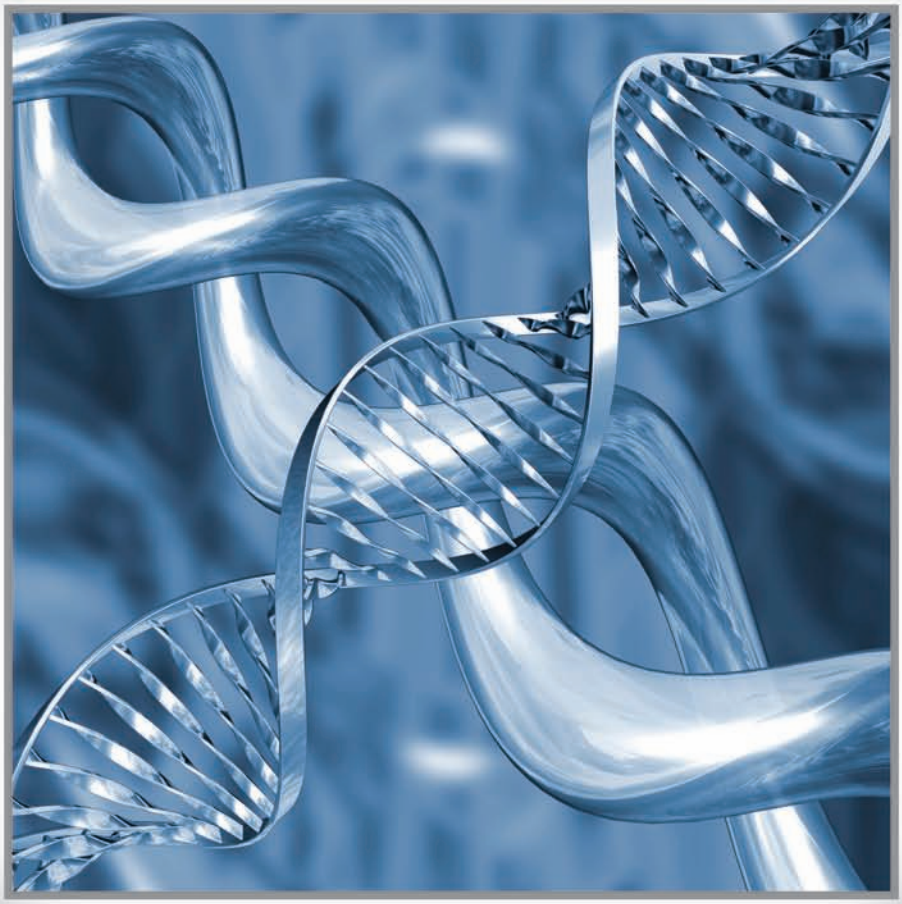

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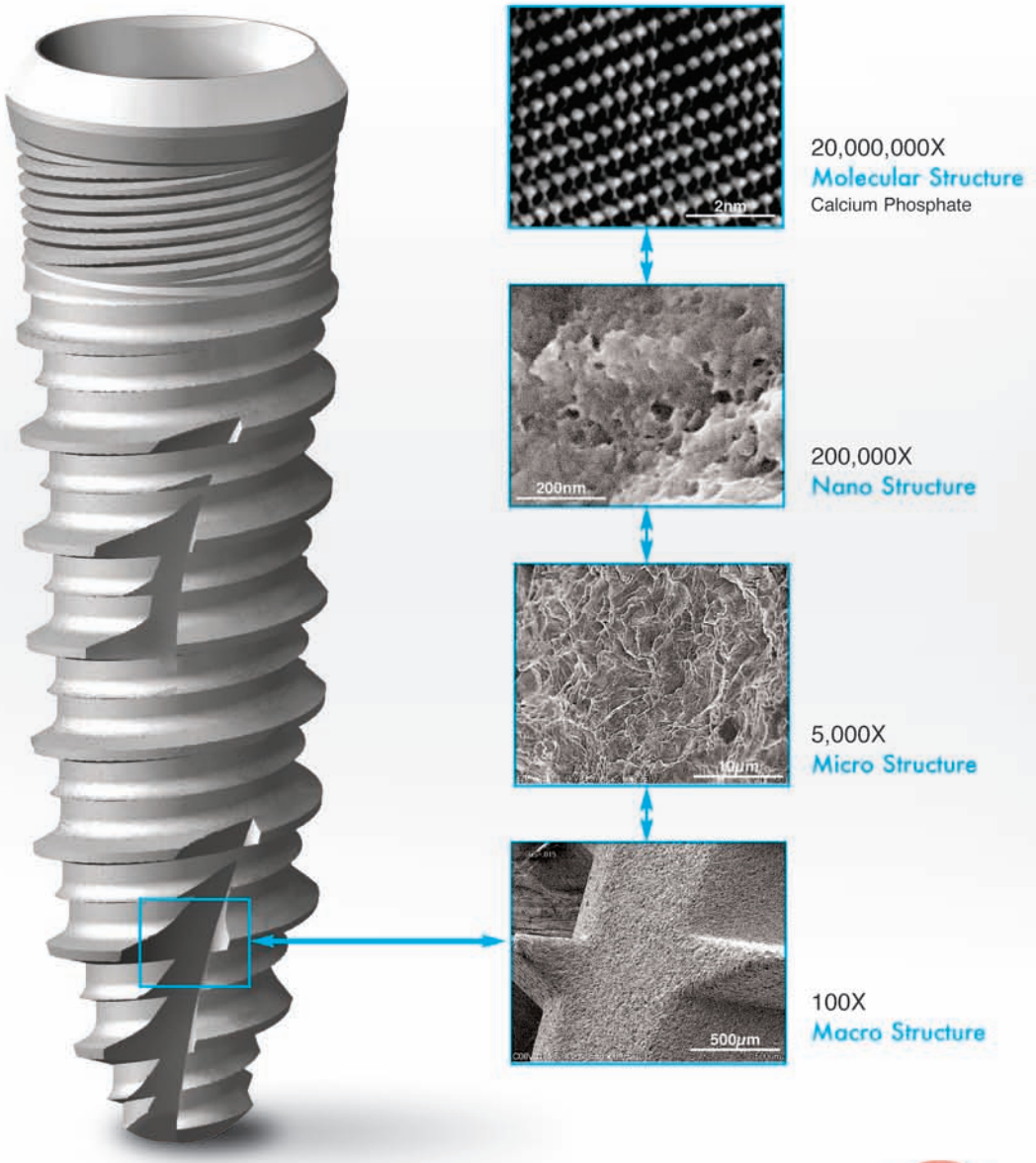

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The International Journal of Dental Implants & Biomaterials

Adriano Piattelli
EDITOR-IN-CHIEF

Arthur B. Novaes Jr.
EDITOR-IN-CHIEF

On behalf of the associate editors, the assistant editors, and the members of the editorial board, we are honored to introduce to you a new journal covering the science and application of dental implants and biomaterials: TITANIUM.

The first questions that we have to answer are: "Why a new journal in this field?" and "What is the mission of this new journal?"

In the past several years there has been a tremendous increase in the knowledge pertaining to the implant and biomaterials field. This knowledge has been reported in some scholarly journals. However, what many colleagues, who work as basic researchers or clinicians, have felt is that this increase in the output of research has not been accompanied by a corresponding increase in the space in which it could be reported. This is causing a logjam, with a conspicuous time delay between submission and publication, and leads to the printing of outdated papers.

Not only have the number of article submissions increased, but the number of countries that contribute high-quality papers is also on the rise. Excellent papers are submitted from all corners of the globe, so we have established a superb and truly international Editorial Advisory Board with representatives from all continents.

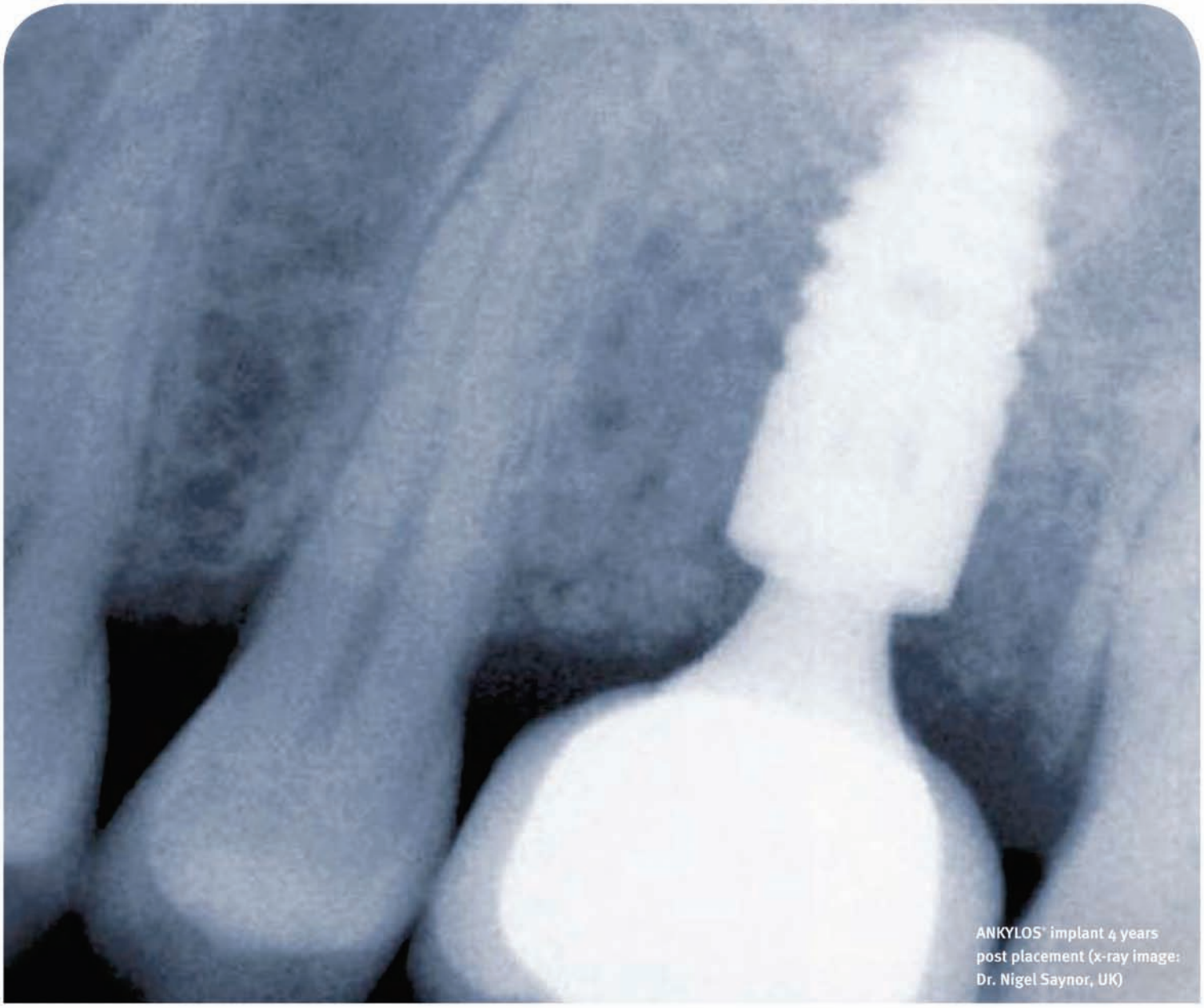
The mission of TITANIUM will be to disseminate to the readership at large, state of the art and up-to-date basic and clinical research in the field of implant dentistry and biomaterials, and to improve patient care and public health in the fastest way possible.

The first aim of the editorial board will be to accelerate the manuscript management and reviewing procedure. The web-based submission and reviewing process will certainly expedite things, but will not suffice. This goal will be attained thanks to the common interest of the editorial board in achieving excellence and providing the most current and accurate results of cutting edge research. In addition, the functional structure of the editorial board divided into groups of expertise (Biomaterials and Biomechanics, Biomaterials and Tissue Engineering, Basic Research, Clinical Research, Implant Science and Clinical Innovations) will advance the review process by focusing areas of interest. Our second goal will be to combine the best in basic and clinical research, publishing only papers that meet rigid quality standards with well designed research projects, executed according to sound scientific principles, and reporting accurate data presented in an unbiased and fair manner.

Another goal is to provide the industry with the availability of an innovative, first-class, and well distributed journal in which to adverstise. We, therefore, invite the implant industry to support our initiative.

We are excited about the launch of this premiere journal in implantology and biomaterials, and we know that the journey of TITANIUM will be one of success!

Sincerely,
Adriano Piattelli & Arthur B. Novaes Jr.
Editors-in-Chief



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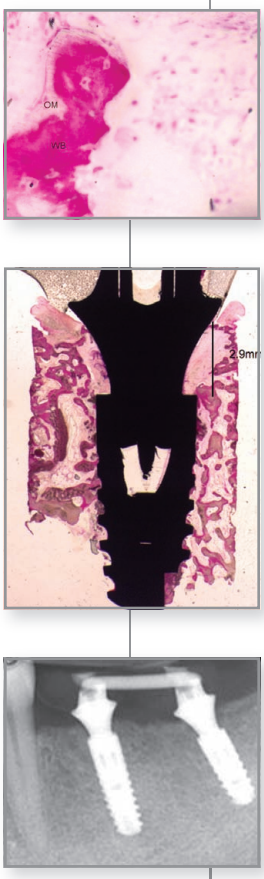

Bone Formation Around a Dental Implant With a Platform Switching and Another With a TissueCare Connection: A Histologic and Histomorphometric Evaluation in Man

Marco Degidi MD, DDS[1] / Adriano Piattelli, MD, DDS[2] / Jamil A. Shibli, DDS, MS, PhD[3] / Rita Strocchi, MD[1] / Giovanna Iezzi, DDS, PhD[1]

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Background: *Peri-implant crestal bone must be stable for aesthetic reasons. Aim of this study was a histological and histomorphometrical evaluation of an implant with a TissueCare connection (Implant A) compared to an implant with a platform switched implant-abutment connection (Implant B).*

Materials and Methods: *A 32-year-old male patient participated in this study. The patient needed a bilateral mandibular restoration. Four implants were used, restored and loaded immediately the same day of insertion. After a six week healing period two implants were retrieved with a 5 mm trephine. Before retrieval both implants were clinically osseointegrated and were not mobile.*

Results: *In Implant A, pre-existing and newly formed bone was found about 2.9 ± 0.2 mm over the implant shoulder. The coronal bone had undergone no resorption. At the level of the implant shoulder areas of new bone formation were present in tight contact with the metal surface, and osteoblasts were depositing osteoid matrix. The bone-implant contact percentage was 55 ± 1.5 %. In Implant B, on one side of the implant, a 1 mm resorption of the crestal bone was present. On the other side, on the contrary, no bone resorption had occurred and approximately 1 mm of bone was present over the implant shoulder. The bone-implant contact percentage was 65.1 ± 6.3 %.*

Conclusion: *Both implants had successfully osseointegrated with mineralized tissue on a large portion of the surface. Platform-switching or the use of an implant with a TissueCare connection could be useful in maintaining the peri-implant crestal bone.*

Key words: *conical abutment connection, crestal bone remodelling, immediate loading, microgap, platform switching, retrieved dental implants*

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INTRODUCTION

A loss of the papilla in the inter-proximal area can produce esthetic problems, phonetic problems and a lateral food impaction.[1] The peri-implant crestal bone must be stable to allow for a continuous presence of the papilla.[2-5] A resorption of the peri-implant crestal bone, frequently observed during the first year following prosthetic restoration,[3,6] up to about 1.5 to 2 mm below the implant-abutment junction (IAJ),[7-11] can induce a recession of the gingival margins, particularly in individuals with thin biotypes.[4] The preservation of the peri-implant bone appears to be of particular importance in the esthetic zone and in areas with a limited bone supply.[5] The microgap between implant and abutment and its bacterial contamination seems to play, at least, a partial role in the previously described bone remodeling.[3,12-18] The crestal bone resorption is absent when the implant is still submerged and becomes present after the implant exposure to the oral environment.[3,5] There seems to be a cause/effect relationship between the extent of peri-implant inflammation and the degree of alveolar bone loss.[13] When the IAJ is positioned deep into bone, there is an increase in the loss of vertical crestal bone height.[6,12] If a matching implant-abutment diameter is used, the inflammatory cell infiltrate (abutment ICT), located at the outer edge of the implant-abutment junction, is close to the crestal bone.[7] If, however, the horizontal relationship between the outer edge of the implant and a smaller-diameter component ("platform switching") is changed, the ICT is moved inward toward the central axis of the implant and away from the crestal bone.[3,4,7] This fact should, theoretically, lead to a lesser degree of bone resorption. Moreover, with a platform-switching a 90° step will be created, compared to what happens to implants with a matching implant-abutment diameter, where a 180° step is present; the resulting confined area may produce a restriction of the ICT to this region.[3] Furthermore, the internal repositioning of the IAJ away from the external, outer edge of the implant and neighboring bone decreases the effects of the abutment ICT on surrounding tissues.[7,19] On the contrary, another implant system shows an inbuilt TissueCare connection with a gap-free bacteria-proof tapered abutment connection with maximum mechanical stability and lack of any micromovement; this should determine a high stability of the peri-implant soft tissues.[20-23]

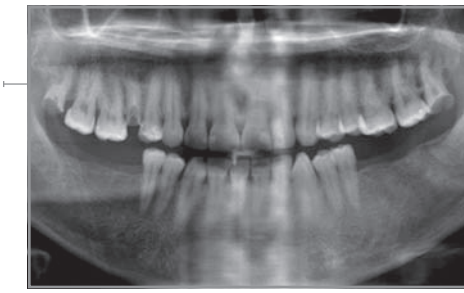

Figure 1: Pre-operative orthopantomography.

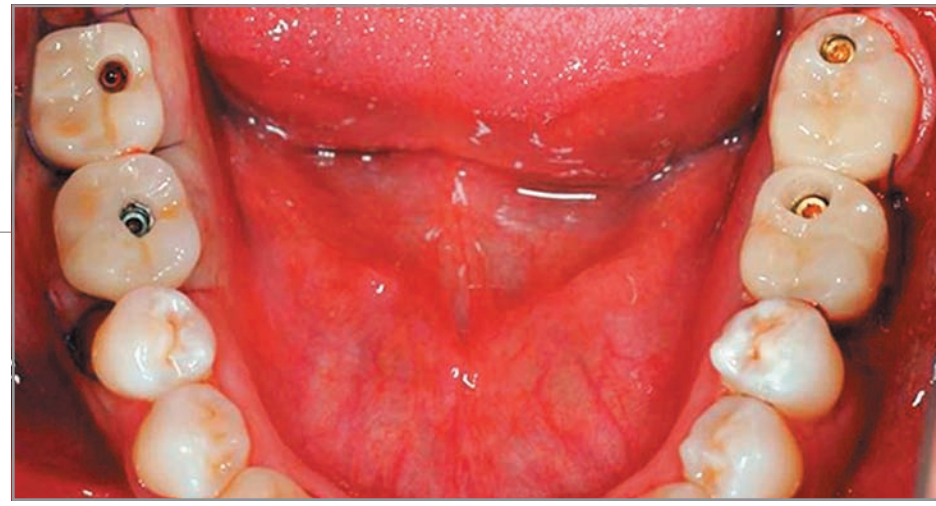

Figure 2: Immediate bilateral prosthetic restoration of the posterior mandible.

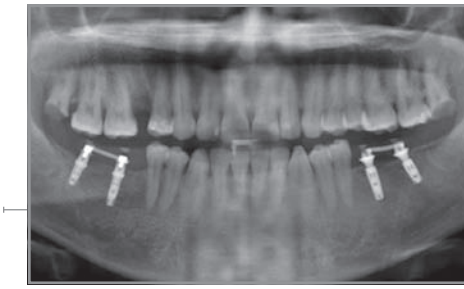

Figure 3: Post-operative orthopantomography.

In a recent paper, Hurzeler et al[3] found that one year after final restoration, the mean values of crestal bone loss were 0.22 ± 0.53 mm for implants with platform-switched abutments, while control implants showed a loss of 2.02 ± 0.49 mm. Similar results were reported by Vela-Nebot et al.,[24] who found in test implants a bone loss of 0.76 mm, while, in control implants, the bone loss was 2.53 mm.

Chou et al.,[2] in a study of over 1,500 implants, reported a total overall mean loss from implant placement to

36 months post-loading of only 0.60 mm or 0.2 mm per year, including the bone loss that can be attributed to surgical trauma.

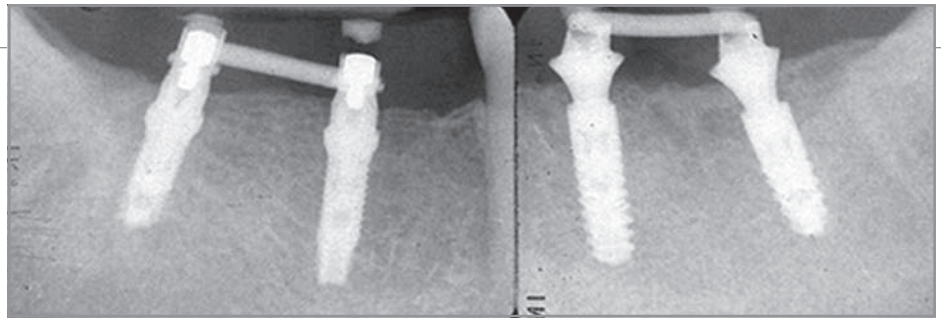

Figure 4: Post-operative periapical x-ray; Implant B on the left, Implant A on the right.

Immediate loading of dental implants was thought to produce a fibrous repair at the interface.[25-29] Several histological

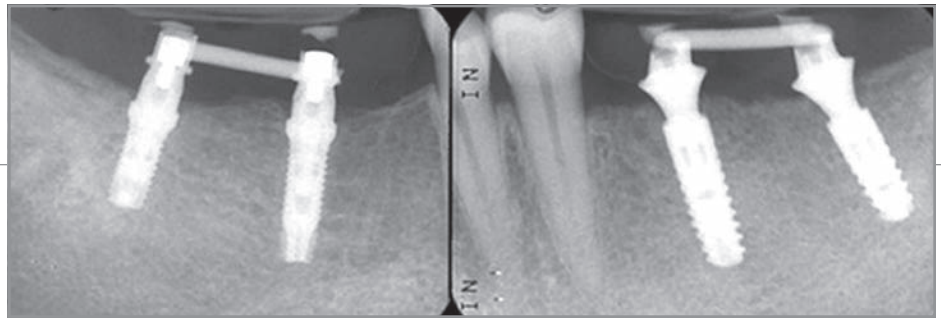

Figure 5: Six weeks follow-up.

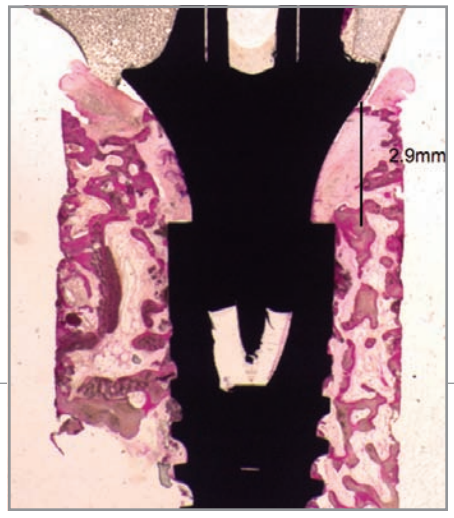

Figure 6: Implant A. Pre-existing and newly formed bone was found about 2.9 ± 0.2 mm over the implant shoulder. The coronal bone had undergone no resorption. At the level of the implant shoulder areas of new bone formation were present in tight contact with the metal surface. Acid fuchsin-toluidine blue 8X.

reports, in man and experimental animals have, on the contrary, shown mineralized tissues at the interface in early and immediately loaded implants.[30-46] Immediate loading allows immediate restoration of esthetics and functions, reduces the morbidity of a second surgical intervention, and facilitates the functional rehabilitation increasing patient acceptance and satisfaction. An analysis of human biopsies of immediately loaded implants is the best way to ascertain the quality and quantity of the peri-implant hard tissues.

The aim of the present study was a histological and histomorphometrical evaluation of an implant with a TissueCare connection (Implant A) compared to an implant with a platform switched implant-abutment connection (Implant B).

MATERIALS AND METHODS

A 32-year-old male patient participated in this study. The protocol of the study was approved by the Ethical Committee of the UnG (University of Guarulhos, Sao Paulo, Brazil) and the patient signed a written informed consent form. The patient was partially edentulous. The patient needed a bilateral posterior mandibular restoration (Fig. 1). Four implants were inserted: two implants in the right mandible (3i® implant with a Nanotitesurface, Implant Innovations, West Palm Beach, FL, USA), and two implants in the left mandible (Ankylos® plus implant, Dentsply, Friadent, Mannheim, Germany). All implants were restored with a fixed provisional screwed prosthesis the same day of the implant surgery and loaded immediately the same day of insertion, without occlusal contact (Fig. 2). The implants had been splinted (Figs. 3-4). Two of these implants, one Ankylos® plus implant (Implant A) and one 3i® implant (Implant B) were separated from the splinted implant and retrieved, together with the abutment which was never removed, with a 5 mm trephine after a six week healing period (Fig. 5). Before retrieval, both implants were clinically osseointegrated and were not mobile. Implant A had been inserted 3 mm below the alveolar crest, in D3 bone, while Implant B had been inserted 1 mm below the crest, in D2 bone .

Processing of Specimens

The implants and the surrounding tissues were stored immediately in 10% buffered formalin and processed to obtain thin ground sections with the Precise 1 Automated System.[47] (Assing, Rome, Italy) The specimens were dehydrated in an ascending series of alcohol rinses and embedded in a glycolmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim,

Germany). After polymerization, the specimens were sectioned longitudinally along the major axis of the implants with a high-precision diamond disc at about 150 µm and ground down to about 30 µm. Three slides were obtained. The slides were stained with acid fuchsin and toluidine blue. A double staining with von Kossa and acid fuchsin was done to evaluate the degree of bone mineralization. One slide, after polishing, was immersed in $AgNO_3$ for thirty minutes and exposed to sunlight. The slides were then washed under tap water, dried, immersed in basic fuchsin for five minutes, and then washed and mounted.

Histomorphometry of bone-implant contact percentage was carried out using a light microscope (Laborlux S, Leitz, Wetzlar, Germany) connected to a high resolution video camera (3CCD, JVC KY-F55B, JVC, Yokohama, Japan) and interfaced to a monitor and PC (Intel Pentium III 1200 MMX, Intel, Santa Clara, CA, USA). This optical system was associated with a digitizing pad (Matrix Vision GmbH, Oppenweiler, Germany) and a histometry software package with image capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics Inc., Immagini & Computer Snc).

RESULTS:

Implant A

Pre-existing bone quality was D3. Pre-existing and newly formed bone was found about 2.9 ± 0.2 mm over the implant shoulder (Fig. 6). The coronal bone had undergone no resorption; no osteoclasts were present in this region. The first bone to implant contact (fBIC) was 0.3 mm on one side and 0.1 mm on the other side, above the implant shoulder. In this portion of the interface, areas of bone remodelling, with bone remodelling units (BMU), were present. An absence of infrabony pockets was observed. In the coronal area, bone remodelling was present with areas of new bone formation. At the interface with the abutment it was possible to see a loose, richly vascularized, with the presence of many small sized vessels, connective tissue. This tissue was in close contact with the metal surface and no gaps were present at the interface (Figs. 7-8). At the level of the implant shoulder areas of new bone formation were present in tight contact with the metal surface, and osteoblasts were depositing osteoid matrix (Fig. 9-10). Newly formed bone trabeculae had formed in an apico-coronal direction, and in some areas these trabeculae were present in the most coronal aspect of the abutment and contacted the lower part of the prosthetic crown. Only in the most coronal portion of the abutment the connective tissue appeared to be detached from the metal surface, most likely from an artifact during the preparation of the specimen. No inflammatory cell infiltrate was found around the implant. Only a few, scattered inflammatory cells were present inside the connective tissue. In the middle and apical third, trabecular bone, with many marrow spaces and capillaries, was present. The newly formed bone was mainly located inside the thread concavities. Bone resorption was absent in the middle and apical portion of the implant perimeter; no osteoclasts were present in this area. No gaps or fibrous connective tissue were found at the bone-implant interface. No epithelial downgrowth was present. Many wide marrow spaces were present in the peri-implant bone, and few of these spaces were observed directly on the implant surface. The bone-implant contact percentage was 55 ± 1.5 %.

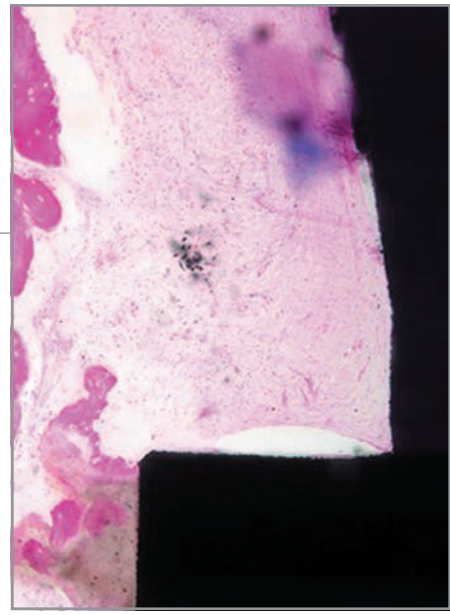

Figure 7: Implant A. Connective tissue was present around the abutment. Many small diameter blood vessels were present in this tissue. Only a few inflammatory cells were present. Acid fuchsin-toluidine blue 40X.

Figure 8: Implant A. The connective tissue was closely adherent to the abutment surface. Acid fuchsin-toluidine blue 40X.

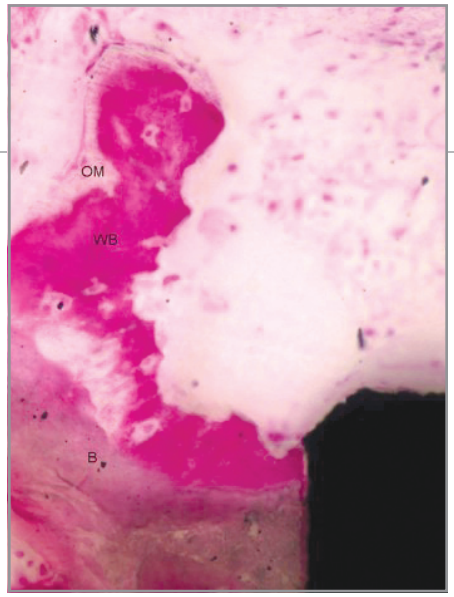

Figure 9: Implant A. Newly formed bone was present at the level of the shoulder of the implant. It was possible to observe osteoblasts depositing newly formed bone (WB) in a coronal direction. B = Pre-existing bone OM = Osteoid Matrix. Acid fuchsin-toluidine blue 100X.

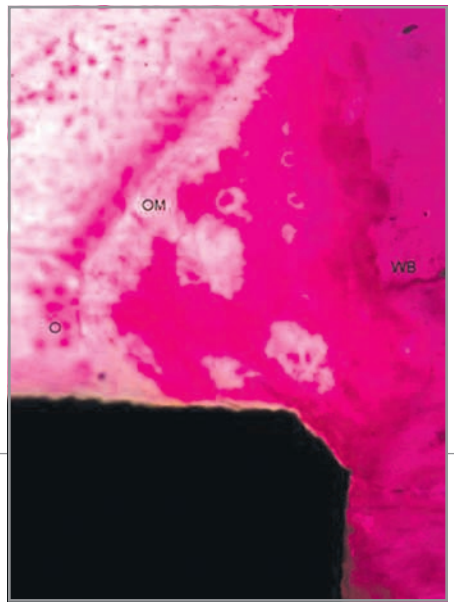

Figure 10: Implant A. Newly formed bone was present at the level and over the implant shoulder. Many osteoblasts (O) were depositing osteoid matrix (OM). WB = Newly formed bone. Acid fuchsin-toluidine blue 100X.

Implant B

The pre-existing bone quality was D2. On one side of the implant, a 1 mm resorption of the crestal bone was present and the bone was located at the same level of the implant shoulder (Fig. 11).

On one side, a gap (0.6 mm) was present, at the level of the implant shoulder, between the bone and the implant (Fig. 12). Some bone trabeculae forming toward the surface of the implant were present inside this gap. The first bone to implant contact (fBIC) was at 0.7 mm from the implant shoulder. No inflammatory cell infiltrate, osteoclasts or resorption areas were found in the area of the gap. At about 1 mm from the implant, some bone trabeculae were present 1 mm above the level of the implant shoulder. On the other side, a very small gap (0.2 mm) was present between the shoulder of the implant and the newly formed bone (Fig. 13). In this area it was possible to observe that many osteoblasts were present inside the gap depositing osteoid matrix in an apico-coronal and implantopetal direction. Overall, in this portion of the interface (near the implant shoulder) only newly formed bone was present. The first bone to implant contact (fBIC) was at 0.3 mm from the implant shoulder.

No inflammatory cell infiltrate, osteoclasts, or areas of resorption were present. Connective tissue was present at the interface with the abutment. In some portions this connective tissue was detached from the metal surface; this was due, in all probability, to an artifact during the retrieval or the processing of the specimen. The connective tissue was loose, with a few, scattered inflammatory cells; only a few small vessels were present. Newly formed bone was found inside the thread concavities. Many osteoblasts were observed depositing osteoid matrix directly on the implant surface. In some areas, newly formed, strongly-stained bone was present in close contact with the implant surface. No gaps or connective fibrous tissue were found at the interface. No epithelial downgrowth was present. The bone-implant contact percentage was 65.1 ± 6.3 %.

DISCUSSION

Immediate loading can reduce the treatment period because the soft tissues heal simultaneously with the hard tissues according to the contours of the provisional restoration.[48,49] Immediate loading has esthetic, psychological and functional advantages in eliminating second-stage surgery, in reducing the patient discomfort and the additional costs of the procedure.[48-49] A concern has been expressed about the formation of connective, fibrous tissue at the interface of dental implants subjected to early or immediate loading.[25-29] Micromotion has been reported to influence in a positive way the healing of bone fractures, and optimal healing is not achieved in total absence of micromotion.[50,51] Immediate and early loading of dental implants can then function as a mechanical stimuli for osteoblasts.[50,51]

It has been reported that when an implant-abutment interface is located at the level of the alveolar bone, a significant inflammatory cell infiltrate with resorption of the peri-crestal alveolar bone occurs.[13,24] Furthermore, the deeper the location of the interface, the greater the degree of inflammation at the level of the implant-abutment junction[13]. This fact could have relevant implications for the clinic because an implant placement in a more apical position could be required by esthetic considerations.[13] The resorption of the peri-implant bone could cause a recession with an esthetic failure.[13] The loca-

tion of the implant shoulder subcrestally avoids the metal exposure and allows to obtain an adequate vertical dimension with an esthetic emergence profile.[24] This technique is what is used with the Ankylos® implant system.[23] Contrary to what happens with other implant systems, the insertion of this type of implant deeper in the bone does not seem to produce the complications of the soft and hard tissues that have been reported in the literature.[23]

A stable internal-tapered abutment connection with absence of any micro-gap[21] or micromovement, the subcrestal insertion of the implant, the microroughness to the interface, the platform switching, and the presence of a thick layer of soft tissues in the narrowed neck of the smaller-diameter abutment[21] (TissueCare Concept) could explain these findings. This wedge-shaped collar of soft tissue, composed by thick, fibrous connective tissue with few scattered inflammatory cells, could provide an additional protection to the peri-implant bone.[21]

The results of the present study were confirmed by a clinical study. In 50% of implants the X-ray examination after one year of prosthetic loading showed crestal bone at or slightly above the level of the implant shoulder.[23] The crestal bone in the region of the implant shoulder then generally remains in place during the loading of the implants and may even increase in density.[23]

The results of the present study seem to show that a smaller dimension of the abutment compared to the diameter of the implant (platform switching) can create a zone around the circumference of the implant that helps to minimize the invasion of the biological width with a protection of the peri-implant soft and mineralized tissues and the establishment of a tissue collar that overlaps the bone-implant interface. This fact could partially explain the reduced rate of bone resorption reported for this type of implant connection, and observed in the present histological case report. The bacteria-proof seal, the lack of micromovements due to the friction grip and the minimally invasive second-stage surgery, found in the Ankylos® implant system, are also important factors in preventing the cervical bone loss.[21,22]

The present results show that a high percentage of bone contact can be obtained even in immediately loaded implants inserted in soft bone, after a very short healing period (six weeks). Immediate loading did not interfere with bone formation and did not have adverse effects on osseointegration.

In the present case report, both implants had successfully osseointegrated from a clinical point of view and were stable at retrieval time. Mineralized tissue was found on a large portion of the surface of both implants with no foreign body, inflammatory reactions or pericoronal bone resorption. This is probably partially related to the types of surface characteristics of both implants.[52-55] The difference in the BIC observed around both implants is, with all probability, related to the fact that some bone at the interface, in the apical region, was lost during the retrieval of one of the implants. The stable internal-tapered abutment connection could possibly help a soft and hard tissue stability with maximum mechanical stability at the implant-abutment interface, due to the friction grip.[21-23]

Platform-switching could then be useful to obtain and maintain the results concerning the peri-implant crestal bone,[5] and the post implant insertion bone remodeling seems to be halted

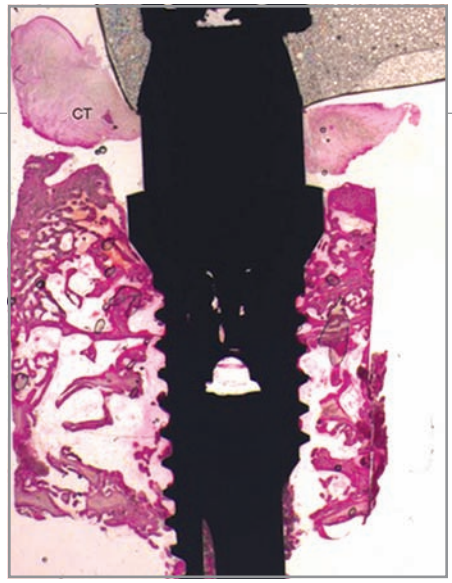

Figure 11: Implant B. On one side of the implant, a 1 mm resorption of the crestal bone was present and the bone was located at the same level of the implant shoulder. CT = Connective Tissue Acid fuchsin-toluidine blue 8X.

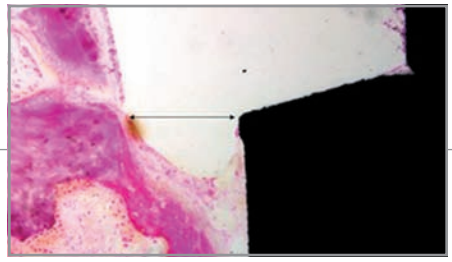

Figure 12: Implant B. A small gap (*arrow*) was present between the shoulder of the implant and the newly formed bone. Acid fuchsin-toluidine blue 40X.

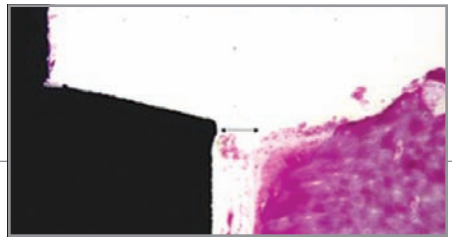

Figure 13: Implant B. A small gap (*arrow*) was present between the shoulder of the implant and the newly formed bone. In this area it was possible to observe that many osteoblasts were depositing osteoid matrix in an apico-coronal and implantopetal direction. Acid fuchsin-toluidine blue 40X.

with the use of a platform-switched abutment or with the use of an implant with conical Morse taper connection (TissueCare connection).

Both of these techniques can then be useful in reaching the ultimate aim of implantology, i.e. the creation of an optimal prosthetic restoration with a neighboring stable bone and a natural architecture of the peri-implant soft tissues.[4]

CONCLUSION:

1) The width of the alveolar crest can help in maintaining the bone crest level.

2) One implant had been inserted in a much lower position than the other (3 mm vs. 1 mm) and the fact that in the former implant no bone resporption was histologically present must be underlined.

3) Abutments should not be removed and reconnected after insertion. This fact is certainly extremely helpful in preserving crestal bone. Abrahamsson et al.[56] have shown, in an experimental study in dogs, that repeated dis- and reconnection (five times in a six month period) produced an apical shift of the bone which was found 1.5 mm apical to the implant-abutment junction. Contrary to Abrahamsson et al.[56] results, no ICT was found in our specimens.

4) The immediate loading of the implants could also be a favorable factor in preserving bone.[57-63]

5) The use of an abutment smaller than the platform could have a biomechanical advantage. A recent Finite Element Analysis study has shown that in this case the stress concentration is moved away from the cervical bone toward the center of the implant.[64]

ACKNOWLEDGMENTS

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REFERENCES

1. Novaes AB, de Oliveira R, Muglia VA, Papalexiou V, Taba M. The effects of inter-implant distances on papilla formation and crestal resorption in implants with a Morse cone connection and a platform switch: a histomorphometric study in dogs. J Periodontol 2006;77:1839-1849.
2. Chou CT, Morris HF, Ochi S, Walker L, DesRosiers D. AICRG, Part II: crestal bone loss associated with the Ankylos implant – loading to 36 months. J Oral Implantol 2004;30:134-143.
3. Hurzeler M, Fickl S, Zuhr O, Wachtel HC. Peri-implant bone level around implants with platform-switched abutments: preliminary data from a prospective study. J Oral Maxillofac Surg 2007;65 (Suppl.):33-39.
4. Calvo Guirado JL, Saez Yuguero MR, Pardo Zamora G, Munoz Barrio E. Immediate provisionalization on a new implant design for esthetic restoration and preserving crestal bone. Implant Dent 2007;16:155-164.
5. Hermann F, Lerner H, Palti A. Factors influencing the preservation of the peri-implant marginal bone. Implant Dent 2007;16:165-175.
6. Hermann JS, Buser D, Schenk RK, Cochran DL. Crestal bone changes around titanium implants. A histometric evaluation of unloaded non-submerged and submerged implants in the canine mandible. J Periodontol 2000;71:1412-1424.
7. Lazzara RJ, Porter SS. Platform switching: a new concept in implant dentistry for controlling postrestorative crestal bone levels. Int J Periodontics Restorative Dent 2006;26:9-17.
8. Cochran DL, Hermann JS, Schenk RK, Higginbottom FL, Buser D. Biologic width around titanium implants. A histometric analysis of the implanto-gingival junction around unloaded and loaded nonsubmerged implants in the canine mandible. J Periodontol 1997;68:186-98.
9. Hermann JS, Buser D, Schenk RK, Higginbottom FL, Cochran DL. Biological width around titanium implants. A physiologically formed and stable dimension over time. Clin Oral Impl Res 2000;11:1-11.
10. Hermann JS, Cochran DL, Nummikoski PV, Buser D. Crestal bone changes around titanium implants: a radiographic evaluation of unloaded nonsubmerged and submerged implants in the canine mandible. J Periodontol 1997;68:1117-1130.
11. Hermann JS, Schofield JD, Schenk RK, Buser D, Cochran DL. Influence of the size of the microgap on crestal bone changes around titanium implants. A histometric evaluation of unloaded non-submerged implants in the canine mandible. J Periodontol 2001;72:1372-1383.
12. Piattelli A, Vrespa G, Petrone G, Iezzi G, Annibali S, Scarano A. Role of the microgap between implant and abutment: a retrospective histologic evaluation in monkeys. J Periodontol 2003;74:346-352.
13. Broggini N, McManus CM, Hermann JS, Medina R, Schenk RK, Buser D, Cochral DL. Peri-implant inflammation defined by the implant-abutment interface. J Dent Res 2006;85:473-478.
14. Persson LG, Lekholm U, Leonhardt¬ A, Dahlen G, Lindhe J. Bacterial colonization on internal surfaces of Branemark system implant components. Clin Oral Impl Res 1996;7:90-95.
15. Quirynen M, Bollen CM, Eyssen H, van Steenberghe D. Microbial penetration along the implant components of the Branemark system. An in vitro study. Clin Oral Impl Res 1994;5:239-44.
16. Jansen VK, Conrads G, Richter EJ. Microbial leakage and marginal fit of the implant-abutment interface. Int J Oral Maxillofac Implants 1997;12:527-40.
17. Piattelli A, Scarano A, Paolantonio M, Assenza B, Leghissa GC, Di Bonaventura G, Catamo G, Piccolomini R. Fluids and microbial penetration in the internal part of cement-retained versus screw-retained implant-abutment connections. J Periodontol 2001;72:1146-1150.
18. Quirynen M, van Steenberghe D. Bacterial colonization of the internal part of two-stage implants. An in vivo study. Clin Oral Impl Res 1993;4:158-161.
19. Abrahamsson I, Berglundh T, Lindhe J. Soft tissue response to plaque formation at different implant systems. A comparative study in the dog. Clin Oral Impl Res 1998;9:73-79.
20. Degidi M, Iezzi G, Scarano A, Piattelli A. Immediately loaded titanium implant with a tissue stabilizing/maintaining design ("beyond platform switching") retrieved from man after 4 weeks. A histological and histomorphometrical evaluation. A case report Clin Oral Impl Res 2008;19:276-282.
21. Nentwig GN. The Ankylos implant system: concept and clinical application. J Oral Implantol 2004;30:171-177.
22. Morris HF, Ochi S, Creum P, Orenstein IH, Winkler S. AICGR, Part I: a 6-year multicentered, multidisciplinary clinical study of a new and imnnovative implant design. J Oral Implantol 2004;30:125-133.
23. Doring K, Eisenmann E, Stiller M. Functional and esthetic considerations for single-tooth Ankylos implant-crowns: 8 years of clinical performance. J Oral Implantol 2004;30:198-209.
24. Vela-Nebot X, Rodriguez-Ciurana X, Rodado-Alonso C, Segalà-Torres M. Benefits of an implant platform modification technique to reduce crestal bone resorption. Implant Dent 2006;15:313-320.
25. Adell R, Lekholm U, Rockler B, Brånemark P-I. A 15 year study of osseointegrated implants in the treatment of the edentulous jaw. Int J Oral Surg 1981;10:387-416.
26. Brånemark PI, Hansson BO, Adell R, Breine U, Lindstrom J, Hallén O, Ohman A. Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. Scand J Plast Reconstr Surg; 1977;11 (suppl 16):1-132.

27. Brunski JB. Forces on dental implants and interfacial stress transfer in Laney WR, Tolman DE (Eds.). Tissue integration in oral, orthopaedic and maxillofacial reconstruction. Chicago: Quintessence; 1992: 108-124.
28. Brunski JB. Influence of biomechanical factor at the bone-biomaterial interface in Davies JE (Ed.). The bone-biomaterial interface, Toronto: Toronto University Press; 1991:391-405.
29. Carter DR, Giori NJ. Effect of mechanical stress on tissue differentiation in the bony implant bed in Davies JE. The Bone-Biomaterial Interface Toronto: University of Toronto Press; 1991:367-379.
30. Linkow LI, Donath K, Lemons JE. Retrieval analyses of a blade implant after 231 months of clinical function. Implant Dent 1992;1:37-43.
31. Trisi P, Emanuelli M, Quaranta M, Piattelli A. A light microscopy, Scanning Electron Microscopy and Laser Scanning Microscopy Analysis of retrieved blade implants after 7 to 20 years of clinical function. J Periodontol 1993;64:374-378.
32. Piattelli A, Ruggeri A, Trisi P, Romasco N, Franchi M. A histologic and histomorphometric study of the bone reactions to non submerged unloaded and loaded single implants in monkeys. J Oral Implantol 1993;19:314-320.
33. Piattelli A, Corigliano M, Scarano A, Quaranta M. Bone reactions to early occlusal loading of two-stage titanium plasma-sprayed implants: a pilot study in monkeys. Int J Periodontics Restorative Dent 1997;17:163-169.
34. Piattelli A, Corigliano M, Scarano A, Costigliola G, Paolantonio M. Immediate loading of titanium plasma-sprayed implants: a pilot study in monkeys. J Periodontol 1998;69:321-327.
35. Piattelli A, Trisi P, Romasco N, Emanuelli M. Histological analysis of a screw implant retrieved from man: influence of early loading and primary stability. J Oral Implantol 1993;19:303-306.
36. Piattelli A, Paolantonio M, Corigliano M, Scarano A. Immediate loading of titanium plasma-sprayed screw-shaped implants in man: a clinical and histological report of two cases. J Periodontol 1997;68:591-597.
37. Ledermann PD, Schenk R, Buser D. Long-lasting osseointegration of immediately loaded bar-connected TPS screws after 12 years of function: a histologic case report of a 95-year-old patient. Int J Periodontics Restorative Dent 1999;18:553-556.
38. Piattelli A, Scarano A, Paolantonio M. Immediately loaded screw implant removed for fracture after a 15-year loading period: histological and histochemical analysis. J Oral Implantol 1997;23:75-79.
39. Romanos G, Toh CG, Siar CH, Swaminathan D, Ong AH, Donath K, Yaacob H, Nentwig GH. Peri-implant bone reactions to immediately loaded implants. An experimental study in monkeys. J Periodontol 2001;72:506-511.
40. Testori T, Szmukler-Moncler S, Francetti L, Del Fabbro M, Scarano A, Piattelli A, Weinstein RL. Immediate loading of Osseotite implants : a case report and histologic analysis after 4 months of occlusal loading. Int J Periodontics Restorative Dent 2001;21:451-459.
41. Romanos GE, Toh CG, Siar CH, Swaminathan D, Ong AH. Histological and histomorphometric evaluation of peri-implant bone subjected to immediate loading: an experimental study with Macaca Fascicularis. Int J Oral Maxillofac Implants 2002;17:44-51.
42. Siar CH, Toh CG, Romanos G, Swaminathan D, ong AH, Yaacob H, Nentwig GH. Peri-implant soft tissue integration of immediately loaded implants in the posterior macaque mandible: a histomorphometric study. J Periodontol 2003;74:571-578.
43. Rocci A, Martignoni M, Burgos PM, Gottlow J, Sennerby L. Histology of retrieved immediately and early loaded oxidized implants: light microscopic observations after 5 to 9 months of loading in the posterior mandible. Clin Impl Dent Relat Res 2003;5 (Suppl):88-98.
44. Degidi M, Scarano A, Piattelli M, Perrotti V, Piattelli A. Bone remodeling in immediately loaded and unloaded titanium implants: a histologic and histomorphometric study in man. J Oral Implantol 2005,31:18-24.
45. Traini T, Degidi M, Caputi S, Strocchi R, Di Iorio D, Piattelli A. Collagen fiber orientation in human peri-implant bone of immediately loaded titanium dental implants. J Periodontol 2005;76:83-89.
46. Traini T, Degidi M, Strocchi R, Caputi S, Piattelli A. Collagen fiber orientation near dental implants in human bone: do their organization reflect differences in loading? J Biomed Mater Res Part B: Appl Biomater 2005;74B:538-546.
47. Piattelli A, Scarano A, Quaranta M. High-precision, cost-effective system for producing thin sections of oral tissues containing dental implants. Biomaterials 1997;18:577-579.
48. Gapski R, Wang HL, Mascarenhas P, Lang NP. Critical review of immediate implant loading. Clin Oral Impl Res 2003;14:515-527.
49. Romanos GE. Present status of immediate loading of oral implants. J Oral Implantol 2004;30:189-197.
50. Meyer U, Wiesmann HP, Fillies T, Joos U. Early tissue reaction at the interface of immediately loaded dental implants. Int J Oral Maxillofac Implants 2003;18:489-499.
51. Meyer U, Joos U, Mythili J, Stamm T, Hohoff A, Fillies T, Stratmann U, Wiesmann HP. Ultrastructural characterization of the implant/bone interface of immediately loaded dental implants. Biomaterials 2004;25:1959-1967.
52. Degidi M, Scarano A, Iezzi G, Piattelli A. Histologic analysis in man of an immediately loaded implant retrieved after 8 weeks. J Oral Implantol 2005;31:247-254.
53. Di Iorio D, Traini T, Degidi M, Caputi S, Piattelli A. Blood clot organization on different implant surfaces in man: an in vitro study. J Biomed Mater Res Part B: Appl Biomater 2005;74B:636-642.
54. Iezzi G, Degidi M, Scarano A, Perrotti V, Piattelli A. Bone response around submerged unloaded implants inserted in poor bone sites: a retrospective histological and histomorphometrical study of 8 titanium implants retrieved from man. J Oral Implantol 2005;31:225-233.
55. Orsini G, Piattelli M, Scarano A, Petrone G, Kenealy J, Piattelli A, Caputi S. Randomized-controlled histological and histomorphometric evaluation of implants with nanometer-scale calcium phosphate added to the dual acid-etched surface in the human posterior maxilla. J Periodontol 2007,78:209-218.
56. Abrahamsson I, Berglundh T, Sekino S, Lindhe J. Tissue reactions to abutment shift: an experimental study in dogs. Clin Implant Dent Relat Res 2003;5:82-88.
57. De Smet E, Jaecques S, Vandamme K, Vandere Sloten J, Naert . Positive effct of early loading on implant stability in the bicortical guinea-pig model. Clin Oral Impl Res 2005;16:402-406.
58. Vandamme K, Naert I, Geris L, Vander Sloten J, Puers R, Duyck J. The effect of micromotion on the tissue response around immediately loaded roughened titanium implants in the rabbit. Eur J Oral Sci 2007;115:21-29.
59. Fritton JC, Myers ER, Wright TM, van der Meulen MCH. Loading induces site-specific increases in mineral content assessed by micrcomputed tomography of the mouse tibia. Bone 2005;36:1030-1038.
60. Vandamme K, Naert I, Geris L, Vander Sloten J, Puers R, Duyck J. Influence of controlled immediate loading and implant design on peri-implant bone formation. J Clin Periodontol 2007;34:172-181.
61. Duyck J, Vandamme K, Geris L, van Oosterwyck H, de Cooman M, Vander Sloten J, Puers R, Naert I. The influence of micromotion on the tissue differentiation around immediately turned titanium implants. Arch Oral Biol 2006;51:1-9.
62. Vandamme K, Naert I, Vander Sloten J, Puers R, Duyck J. Effect of implant surface roughness and loading on peri-implant bone formation. J Periodontol 2008;79:150-159.
63. Vandamme K, Naert I, Geris L, Vander Sloten J, Puers R, Duyck J. Histodynamics of bone tissue formation around immediately loaded cylindrical implants in the rabbit. Clin Oral Impl Res 2007;18:471-480.
64. Maeda Y, Miura J, Taki I, Sogo M. Biomechanical analysis of platform switching: is there any biomechanical rationale? Clin Oral Impl Res 2007;18:581-584.

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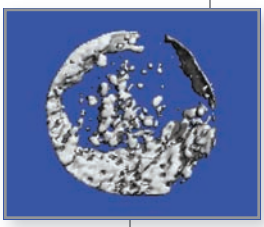

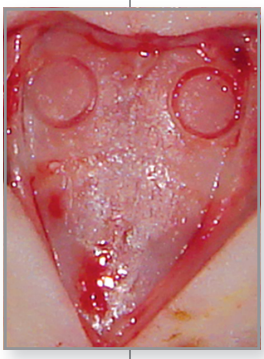

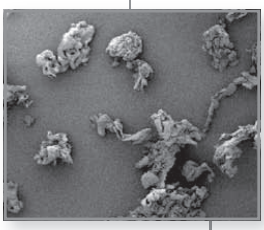

Physico/Chemical Characterization, In Vitro, and In Vivo Evaluation of Hydroxyapatite/PLGA Composite and Tricalcium Phosphate Particulate Grafting Materials

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Background: *The purpose of this study was to physico/chemically characterize and evaluate the in vitro cytotoxicity/in vivo bone regeneration of two grafting materials in a rat calvaria model.*

Materials and Methods: *Two particulate grafting materials, Hydroxyapatite (HA)/PLGA (poly [L-lactide-co-glycolide]) composite (MA1) and Tricalcium Phosphate (MA2), were characterized by SEM, TEM, XRD (Rietveld), and FTIR. The cytotoxicity was evaluated by the ISO10993-5 method. Two critical defects with 5.5 mm in diameter were created bilaterally in the calvaria of 20 Wistar rats. One defect was filled with a grafting material, and the other was the control (C, blood clot). After four and eight weeks in vivo, the amount of new bone filling the defects was evaluated using micro-computed tomography with a slice resolution of 30 μm. Statistical analysis was performed by one-way ANOVA at 95% level of significance.*

Results: *The physico/chemical analytical tools showed that MA1 was a Si- and Mg-doped Hydroxyapatite/PLGA composite, and MA2 was a ß-TCP-based powder presenting ~9% $Ca_2P_2O_7$ secondary phase. Both powders were not cytotoxic up to 50% extract concentration. The new bone volume to total defect volume ratios at four weeks were (mean ± SD) MA1=14.8±7.9%[a], MA2=16.1±7.2%[a], and C=6.5±1.6%[b]. At eight weeks, the values were MA1=22.6±7.2%[a], MA2=19.9%±4.0[a], and C=7.4±5.2%[b].*

Conclusion: *Despite substantial compositional differences, non-significant differences in the amount of new bone formation were observed. Both materials presented osseoconductive properties.*

Key words: *grafting materials, characterization, HA/PLGA, ß-TCP, in vivo, MicroCT*

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INTRODUCTION

An important aspect of the human skeleton is its ability to regenerate itself as part of a repair process. However, depending on the size of the defect, full regeneration of the lost tissue may not occur, representing challenging scenarios for the reestablishment of organ form and function.[1,2] In general, grafting procedures are necessary for improving the bone tissue ability to regenerate. For this purpose, several approaches have been attempted for defect filling and subsequent regeneration, including autogenous and xenogenous bone grafting and synthetic biomaterials.[3]

Due to its high biocompatibility, autogenous bone taken from a secondary surgical site has been widely utilized.[4,5] However, its utilization has limitations such as supply amount and unpredictable healing kinetics. Also, donor site pain and potential post-surgical infection are common complications associated with such procedure.[4] These limitations have stimulated the development of synthetic materials/matrices engineered specifically for bone replacement applications.[3,4,6]

Over the last 10 years, attention has been devoted towards the development of optimized synthetic or semi-synthetic substitutes for autogenous bone grafting.[1] Commonly utilized grafting materials include allografts such as demineralized bone matrix particles, deproteinized cancellous chips, or synthetic alloplasts such as calcium sulfate pellets and porous calcium phosphate materials.[3] Among alloplasts, calcium and phosphate-based substitutes have been demonstrated to have the ability to fill large defects while providing osseoconductive properties leading to new bone formation in large defects.[2] In addition, Ca- and P-based alloplasts may be manufactured in a variety of forms, such as bulk ceramics, powders, and cements, and thereby may be engineered for specific applications.[2]

Bioceramics have been widely studied for orthopedic and dental applications due to their good biocompatible and osseoconductive properties. It has been general consensus that the bioactivity of bioceramics relies on their ability to induce hydroxyapatite (HA) formation in the physiologic environment.[7] Commonly available resorbable bioceramics include hydroxyapatite and tricalcium phosphate powders or blends of different Ca- and P-based phases.[2]

Synthetic hydroxyapatite is a crystalline calcium- and phosphate-based bone substitute. However, because of slow in vivo resorption, hydroxyapatite-based materials have been modified by a variety of techniques in an attempt to better tailor its in vivo osseoconductive and dissolution properties.[2] Aiming to better utilize its osseoconductive properties, hydroxyapatite has also been used in blends (biphasic powders).[2] Alternative approaches also include the incorporation of biocompatible polymers on HA powder surfaces.[8]

Tricalcium phosphate has a faster dissolution rate than hydroxyapatite. The faster dissolution/resorption of ß-TCP may allow a gradual biological degradation over a period time and a progressive replacement by the natural host tissue.[9] Thus, ß-TCP is currently considered as an alternative material for bone reconstruction and is frequently used for bone repair in the form of ceramic blocks, granules, and calcium phosphate cements.[10]

The purpose of this study was to physico/chemically characterize and evaluate the in vitro cytotoxicity and the in vivo bone regeneration of a Hydroxyapatite/PLGA composite and a ß-tricalcium phosphate-based powder grafting material in a critical size defect.

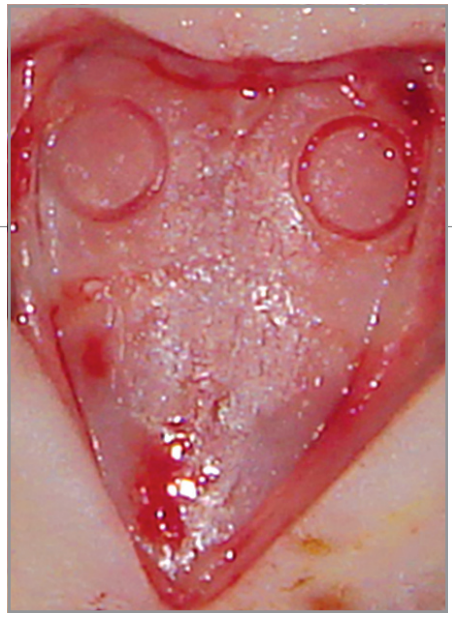

Figure 1: Skull exposure showing where the defects were made.

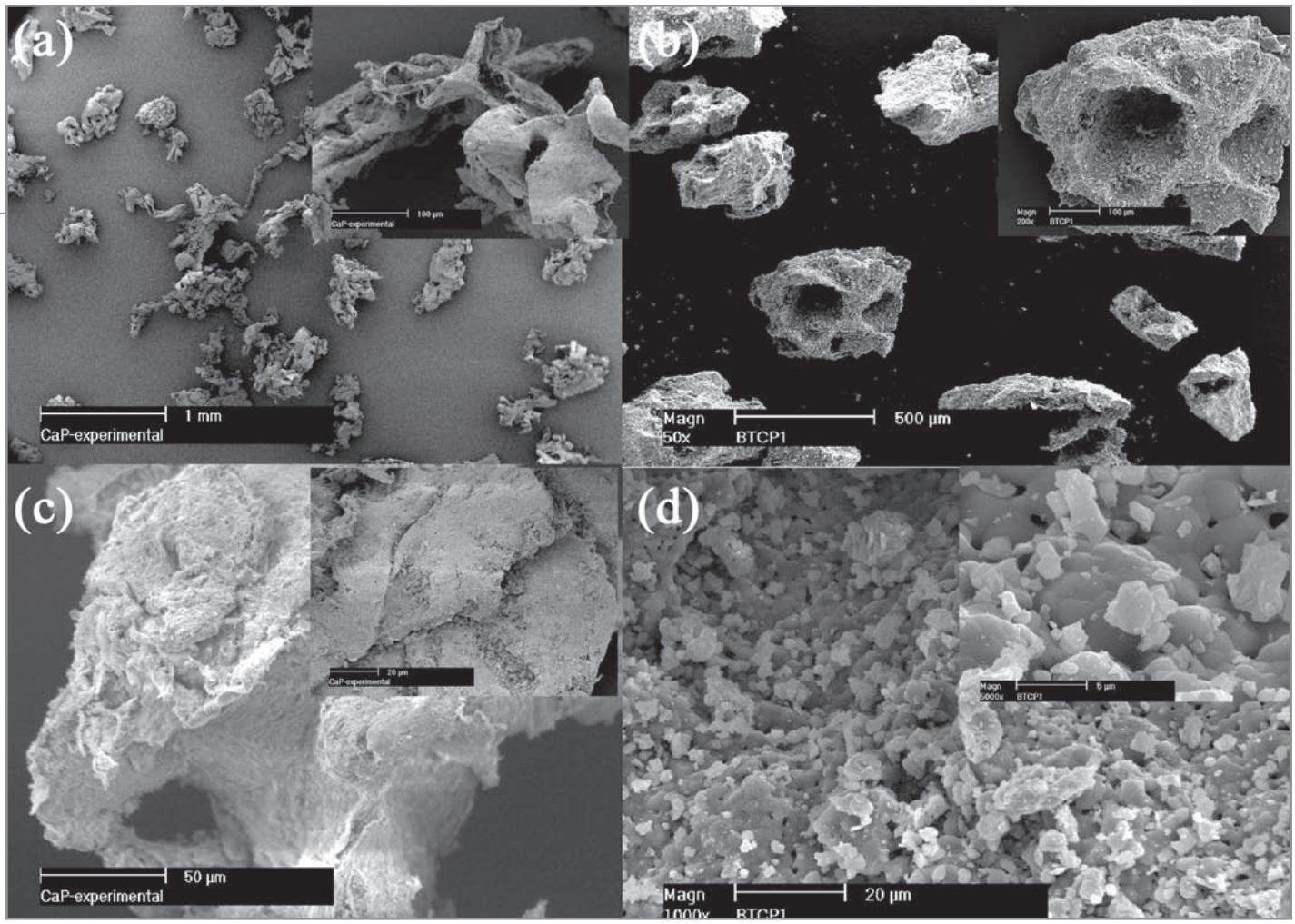

Figure 2: (a) MA1 (Hydroxyapatite/PLGA composite) powder morphology; (b) MA2 (Tricalcium Phosphate) granules morphology; (c) MA1 surface characteristic, such as roughness and porosity; (d): MA2 surface characteristic, such as roughness and porosity.

MATERIALS AND METHODS

A Hydroxyapatite (HA)/ PLGA composite particulate material– MA1 (ReOss™, Intra-Lock, Boca Raton, FL) and an FDA approved beta-tricalcium phosphate (ß-TCP) – MA2 (SynthoGraft™, Bicon LLC, Boston, MA) grafting material with particle size suitable to maxillofacial applications were evaluated. The powders were provided by the manufacturers and were characterized in the as-received form without detailed disclosure of their physico/chemical characteristics.

Physico/Chemical Analysis

Powder Morphology

For particle morphology evaluation, the powders were separated in various batches and scanning electron microscopy (SEM, Philips XL30, Eindhoven, The Netherlands) was performed at various magnifications following standard guidelines for ceramic powder imaging.

Powder Chemical Assessment

Assessment of the powder bulk composition was performed by energy dispersive spectroscopy (EDS) at various magnifications and accelerating voltages at randomly selected spots.

X-ray powder diffraction patterns (XRD) were collected in a Rigaku diffractometer (Multiflex, Tokyo, Japan), from 5° to 110° (2θ) with step interval $\Delta 2\theta$ = 0.02°, divergence slit = 1/2° and receiving slit = 0.3mm, step time = 8s, 40kV, 30ma

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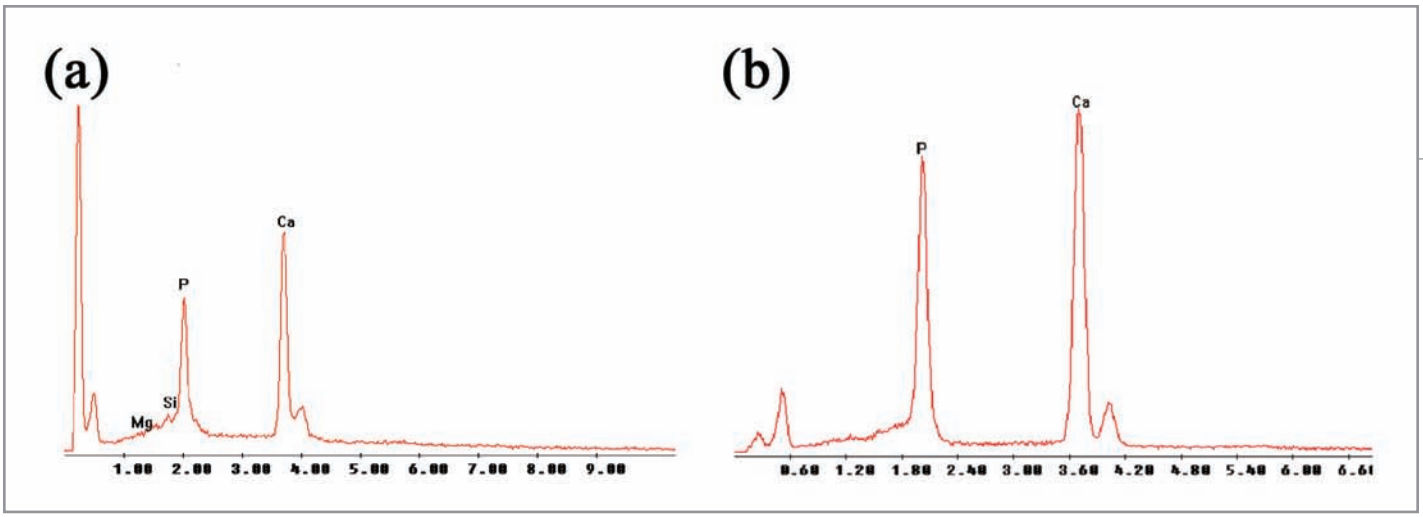


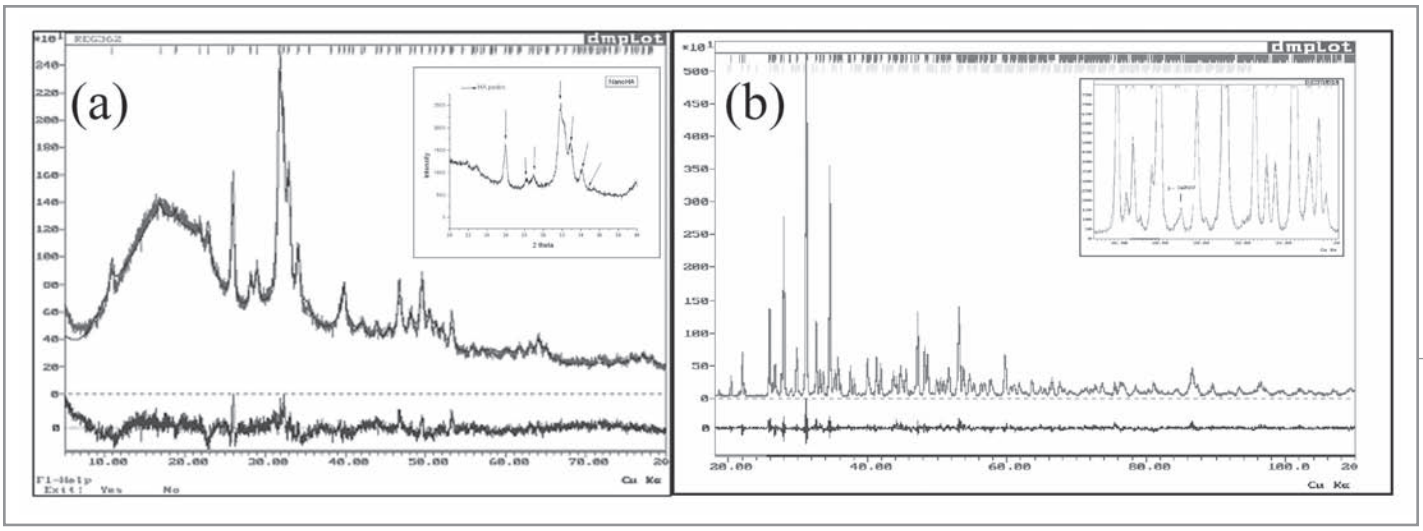


Figure 3 (Top): (a) EDS spectrum of MA1 (Hydroxyapatite/PLGA composite), and (b) EDS spectrum of MA2 (Tricalcium Phosphate).

Figure 4 (Bottom): (a) Rietveld refinement of the MA1 (Hydroxyapatite/PLGA composite) powder XRD spectrum and refinement plot results 20° to 40° 2θ spectrum showing HA peaks with broad base, typically found in non-sintered powder samples. (b) MA2 (Tricalcium Phosphate) Rietveld refinement plot results for the phases ß- $Ca_3(PO_4)_2$ and ß-$Ca_2P_2O_7$, and refinement plot results between 25° and 35° 2θ.

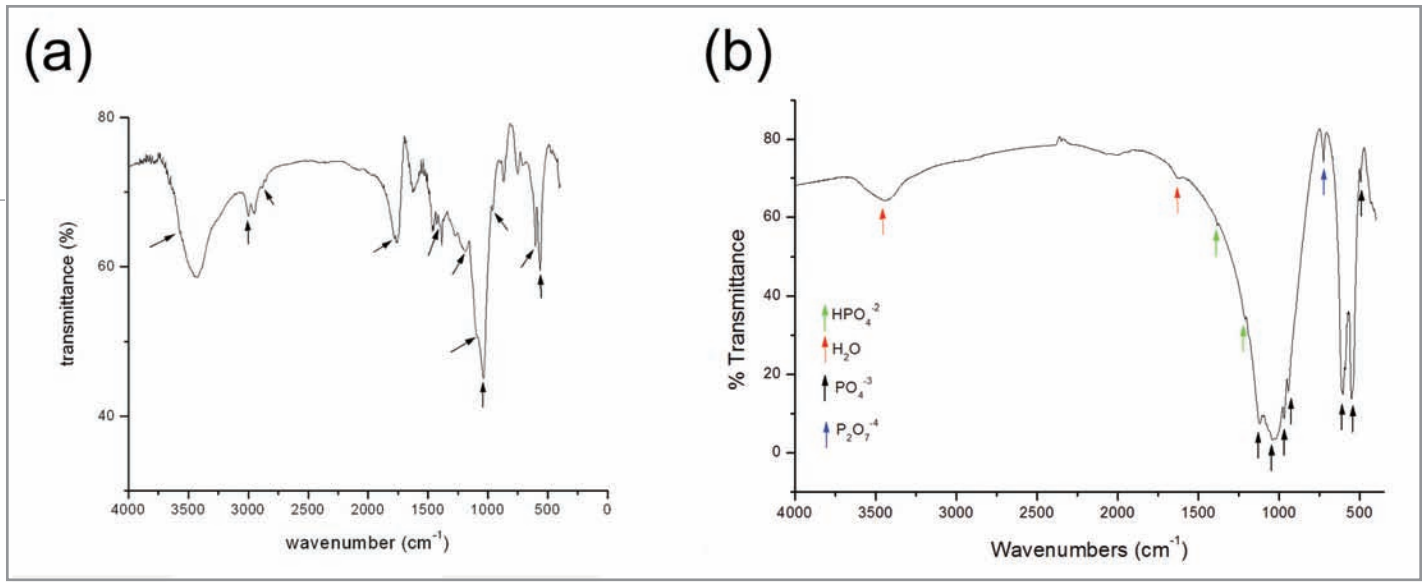
$CuK_\alpha 1$ radiation monocromatized by graphite crystal. Identification of phases was achieved by comparing the diffraction patterns obtained to the database provided by ICDD.[11]

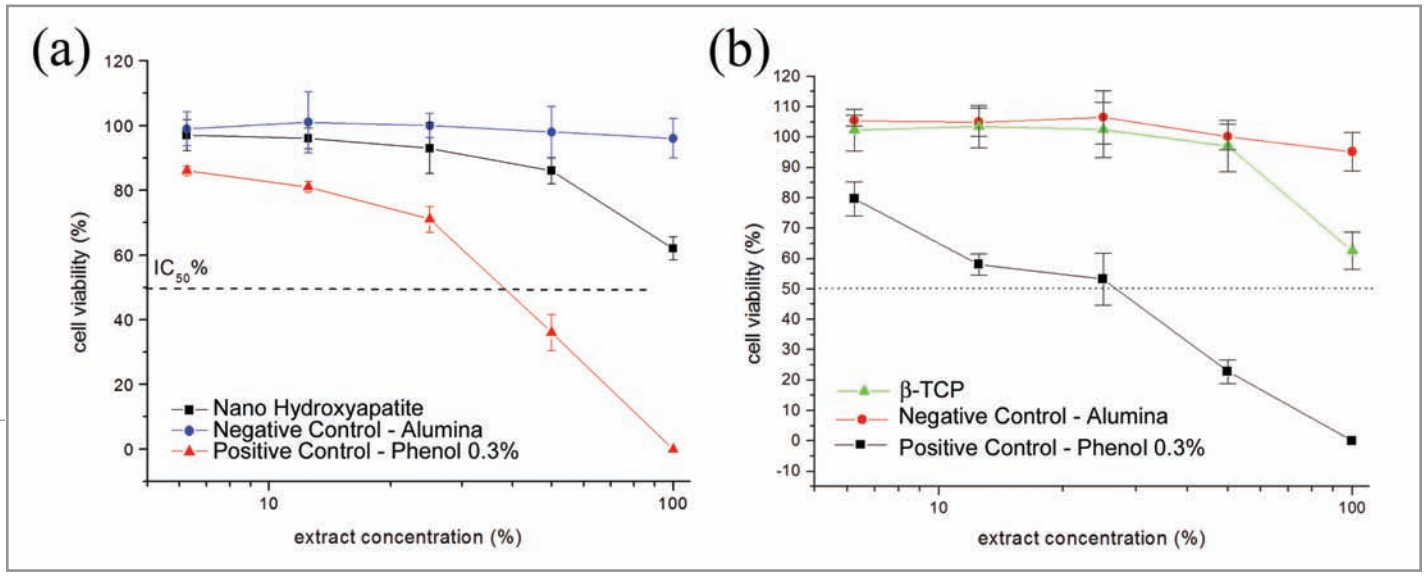
The Rietveld refinements were performed using the GSAS software Collaborative Computational Project number 14 (CCP14) in Powder and Small Molecule Single Crystal Diffraction.[12] The starting model used in the refinement was based on the theoretical structure in the Inorganic Crystal Structure Database (ICSD).[13] The peak shapes were modeled using the pseudo-Voigt function. The background, cell parameters, preferred orientation, peak asymmetry, atomic positions, site occupancy factors, and global vibrational parameters were refined. The calculated and observed patterns were fitted by least squares method until a minimum was reached. The integrated intensities and the peak heights were related to a scale factor. The fraction of each phase was determined by the equation:

$$W_i = S_i (ZMV)_i / \sum [S_j (ZMV_j)] \quad (1)$$

Where: W_i = weight fraction of the phase p; S = scale factor; Z = number of formulas units per unit cell; M = mass of the formula unit; and V = unit cell volume.

Fourier-transform infrared spectros-





copy Magna-IR 550 Spectrometer Series II (Nicolet, Madison, WI, USA) equipped with reflectance attachment was used to determine chemical groups and crystallinity and further confirm XRD results. The spectra were collected at room temperature at a nominal resolution of 4.00 and number of sample scans equal to 1,000. The FTIR spectra were recorded in the 400–4,000 cm-1 range using specular reflection.

Cytotoxicity In Vitro Test

The cytotoxicity test was carried out in order to detect toxicity levels of the as-received MA1 and MA2 powders. The employed cell line was based on the International Standards Organization ISO 10993-5 guidelines. A monolayer of cell culture of Chinese hamster ovary cells (CHO-K1 from American Type Culture Collection, ATCC) in log phase was harvested by trypsinization. The cell suspension was centrifuged and the pellets were resuspended in RPMI medium after thorough washing with sterile PBS.

One gram of each sterilized powder material was poured into 10ml glass flasks.

Figure 5 (Top): (a) FTIR spectrum for MA1 (Hydroxyapatite/PLGA composite) showing bands originating from HA, alpha-TCP phases, and PLGA component. (b) FTIR spectrum of as-received MA2 (Tricalcium Phosphate) showing bands originating from HPO_4^{-2}, H_2O, PO_4^{-3}, and $P_2O_7^{-4}$.

Figure 6 (Bottom): In vitro cytotoxicity assay employing cell culture based on the ISO 10993-5 guidelines. (a) MA1 (Hydroxyapatite/PLGA composite) cell viability level > 50% at 100% extract concentration. (b) MA2 (Tricalcium Phosphate) cell viability level > 50% at 100% extract concentration.

Four milliliters of RPMI-FCS (RPMI 1640, containing 10% fetal calf solution and 1% penicillin/streptomycin solution) culture medium was added and incubated for 48 h at 37°C under a 5% CO_2 humidified atmosphere. The supernatant was filtered through a membrane (Millipore®, Barueri, SP, Brazil), and serial dilutions (100, 50, 25, 12.5 and 6.25 vol%) were made from the extract. Dilutions were also performed for the negative and positive material controls, which were sterile alumina ceramic and 0.02 vol% phenol solutions, respectively. All concentrations were tested in quadruplicate.

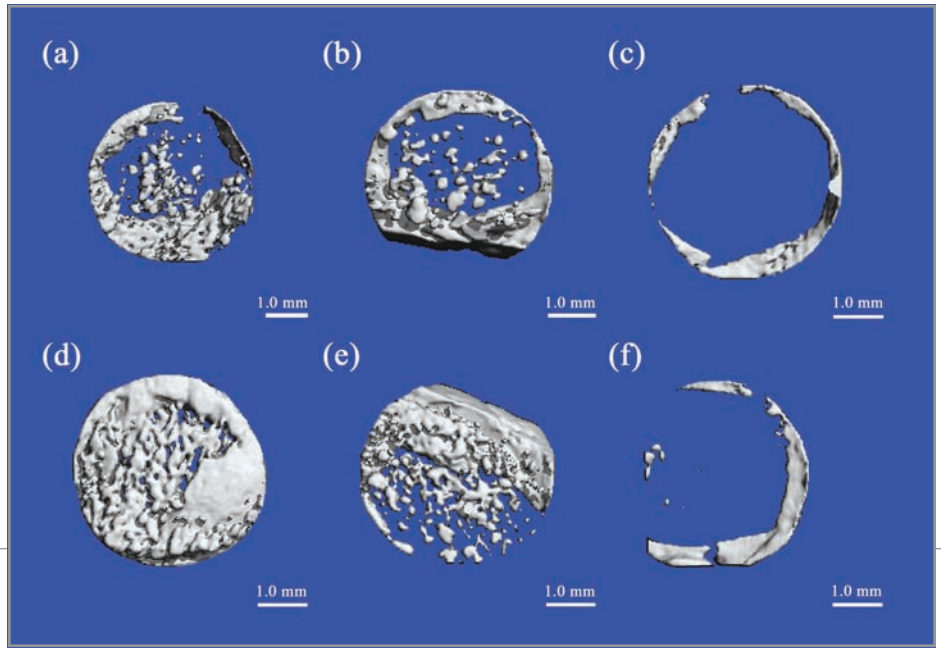


Figure 7: µCT 3D reconstruction after four weeks of healing: (a) MA1 (Hydroxyapatite/PLGA composite), (b) MA2 (Tricalcium Phosphate), (c) Control; and after eight weeks of healing: (d) MA1 (Hydroxyapatite/PLGA composite), (e) MA2 (Tricalcium Phosphate), (f) Control.

A 96-well tissue culture microtiter plate was prepared by pipetting 50 µL of the serial dilutions of each extract. The plate was brought to equilibrium at 37°C in a humidified atmosphere under 5% CO_2 while the cells were harvested for assay. Subsequently, 50 µL of the cell suspension (~3,000 cells) was dispensed into each well. The resulting volume in each well was 100 µL. Control columns of four wells were prepared with medium without cells (blank) and medium plus cells without extract (100% survival). The microplate was then incubated under a 5% CO_2 humidified atmosphere. After 72 h, 20 µl of a mixture (20:1) of 0.2% a supravital dye tetrazolium compound (MTS) and 0.09% of electron coupling reagent phenazine methosulfate (PMS) in PBS were added to the test wells. These were allowed to react for 2 h. The cytotoxicity test performed was based on the quantitative assessment of surviving viable cells upon exposure to a toxic agent, by incubation with the MTS compound and an electron coupling reagent PMS.

The MTS was bioreduced by cells into a formazan product that is soluble in cell culture medium. Following the release of this product, colorimetric analysis of the incorporated dye was performed. Incorporate dye was measured by reading the absorbance at 490 nm in a spectrophotometric microplate reader against a blank column.

In Vivo Experiment

Animals

Twenty male Wistar rats, aged 100 days with approximately 350g body mass, were used. The animals were kept in plastic cages, maintained under a 12 h light cycle (40 lux) per day and fed *ad libitum* with sterile water and food. The surgical protocol was approved by the Animal Care Committee of the University of São Paulo, São Paulo, Brazil. The animals were maintained at the IPEN animal facility.

Surgical Procedures

Two critical defects with 5.5 mm in diameter were created bilaterally in the calvaria (Fig. 1) and were allowed to heal for periods of four and eight weeks. One defect was filled with one of the grafting materials and the other was the control (C, blood clot, Table 1).

All animals were submitted to calvaria surgery under general anesthesia.

For sedation and muscle relaxation, each animal received an intraperitoneal injection (2-2-xylidine)-5,6-dyhidro-4H-1, 3-thyazyn chlorate (Rompum, Bayer, São Paulo, SP, Brazil) (5.0mg/kg). For general anesthesia, ketamine (Ketamina®, Agener, União Química Framacêutica Nacional SA, São Paulo, SP, Brazil) (60mg/Kg) in proportion 2:1 was administered. Throughout the surgical procedure, the animals were maintained at deep anesthesia for 40 minutes. Prior to shaving by a surgical blade, the frontoparietal region was scrubbed with povidone iodine 10% in aqueous solution with 1% of active iodine (PVPI).

A 15 mm long mid-sagital full-thickness incision was made with a #15 surgical blade to expose the skull. The skin, muscles, and periosteum were reflected to expose the parietal bones. By means of a surgical trephine bur of 5.5 mm diameter, a perforation crossing the parietal's bone entire diploe exposed the dura mater at the bottom of the defect under constant irrigation with sterile saline solution (Fig. 1). The defect was filled with the grafting materials according to Table 1. The control defect was allowed to fill with blood clot. The tissues were closed in layers with 4-0 Dexon sutures (Ethicon, Sommerville, NJ, USA), and 4-0 vicryl sutures (Hu-Friedy, Chicago, IL, USA).

After four and eight weeks post-surgery, euthanasia was performed with CO_2 inhalation for 10 minutes. After euthanasia, the crania were carefully dissected free of soft tissue and the skullcaps were collected and fixed in 4% buffered formalin for 24 hours. After fixation, the samples remained in 70% ethanol.

Micro Computed Tomography

The amount of bone filling the defects was examined using micro computed tomography (µCT 40, Scanco Medical, Basserdorf, Germany) with a slice resolution of 30 µm. Five hundred and seven µCT slices were imaged at the skull caps at an X-ray energy level of 70 kVp, and a current of 114 µA. Integration time was 150 ms with a total scanning time of 19.8 min (78mAs). The 3D construction of the calvaria bone was made using a template restricted to the defect margins.

Statistical Analysis

Statistical analysis was performed by one-way ANOVA at 95% level of significance and multiple comparisons were performed by Tukey's post-hoc.

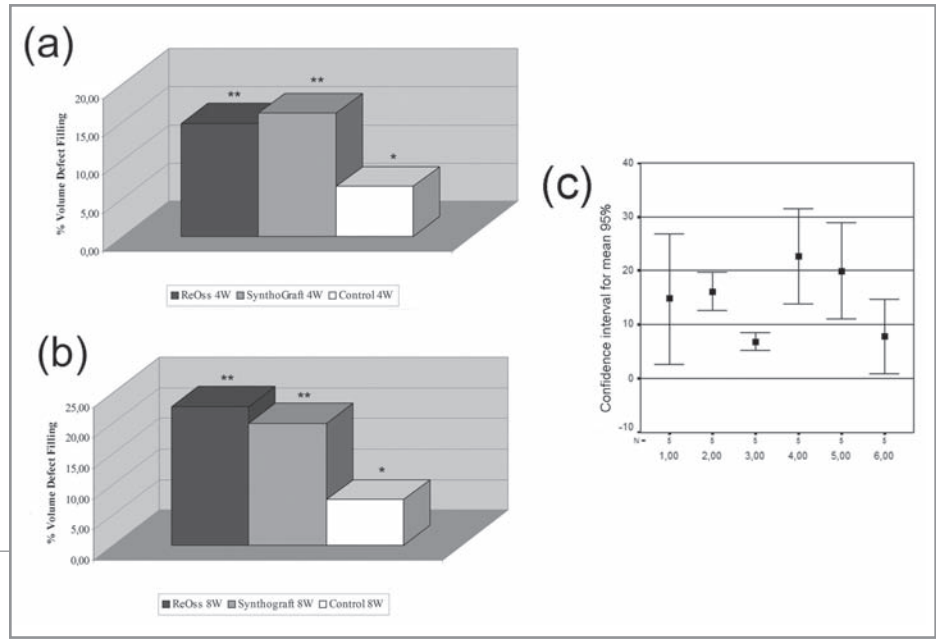


Figure 8: Mean bone fill percentages in 5.5 mm parietal defect filled with MA1 (Hydroxyapatite/PLGA composite), MA2 (Tricalcium Phosphate), and blood cloth. (a) After four weeks of healing. (b) After eight weeks of healing and (c) means ± standard deviations for the different in vivo groups (1 – ReOss 4W; 2 – SynthoGraft 4W; 3 – Control 4W; 4 – ReOss 8W; 5 – SynthoGraft 8W; 6 – Control – 8W).

RESULTS

Physico/Chemical Analysis

Powder Morphology

Irregular powder morphology was observed for the MA1 (Figs. 2a and 2c). Figures 2b and 2d depict the irregular morphology for the MA2 particles. Intragranular porosity could be observed at the ceramic bulk for the MA2 powder, revealing that the granules were subjected to a sinterization process or a thermal treatment and were subsequently milled to the final powder form. Other observations

TABLE 1: MATERIALS AND SURGICAL GROUPS DIVISION

Groups	N	Description
MA1-4 Weeks	5	5 MA1*
		5 Control (Blood Clot)
MA2-4 Weeks	5	5 MA2**
		5 Control (Blood Clot)
MA1-4 Weeks	5	5 MA1
		5 Control (Blood Clot)
MA2-4 Weeks	5	5 MA2
		5 Control (Blood Clot)

Legend: * MA1 – Hydroxyapatite PLGA composite / ** MA2 – Tricalcium Phosphate

included a range of particle size for both powders and particle porous morphology.

Powder Chemical Assessment

Within the EDS interaction/detection volume, the spectrum showed that both powders were initially free of contamination. The MA1 EDS spectrum showed the presence of Ca, P, Si, and Mg (Fig. 3a), while the MA2 spectrum showed only calcium and phosphorous peaks (Fig. 3b).

The XRD results for both powders showed peaks related to biocompatible Ca- and P-based phases. The XRD spectra presented in Figure 4a showed the absence of phases secondary to HA for the MA1 powder. During the refinement for the MA1 sample, the background was defined manually since the data was collected without a pattern and the amorphous percentage obtained was high for polynomial definition. However, further spectrum refinement was difficult due to the high background resulting from the polymeric organic content. Further investigation of the spectrum at the region ranging from 20° to 40° 2θ (Fig. 4a) showed that the peaks presented a broad base compared to dense, sintered, crystalline HA (Fig. 4a).

For the ß-TCP, the final Rietveld plot presented in Figure 4b displayed reasonable agreement between the structural model and the raw data. In general, the X-ray patterns collected for calcium phosphates have a great number of superimposed peaks. The Rietveld method resolved the peaks for the quantitative analysis even for low phase's percentages. Figure 4b depicts the most intense Bragg reflection for ß-$Ca_2P_2O_7$, according to ICDD data base 9-346, which was identified, and the quantitative analysis for this phase could be determined at approximately 9% (Table 2).

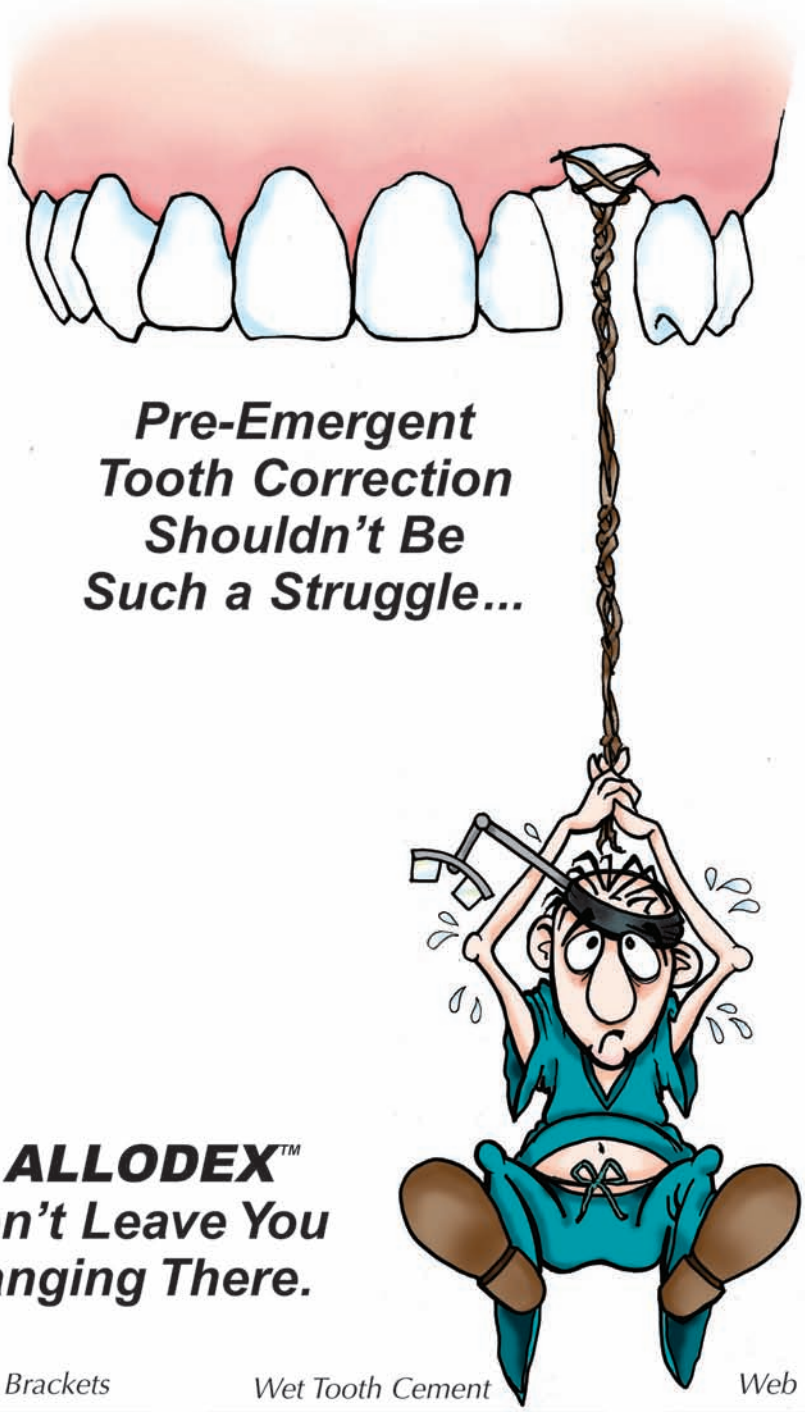
The infrared spectra further confirmed the XRD findings for both powders, and are presented in Figure 5. The FTIR spectrum of MA1 (Fig. 5a) showed bands characteristic of HA and one minor ß-TCP band, along with bands related to the PLGA polymer. The various bands chemical groups are presented as follows: Band (in cm-1), 3572 – OH-HA; 2998 – C-H-CH3; 2883 – C-H-CH2; 1400 – CO3 -Carbonate, minor; 1187 – C-O; 1087 – v3 PO4; 1039 – Si-O-Si or 1040 – PO4; 960 – v1 PO4; 601 – v4 PO4; and 563 – α-TCP.

For the MA2 powder, the absence of 460 and 740 cm^{-1} bands and of an isolated band approximately at 600 cm^{-1} characteristic of the α-TCP confirmed the XRD and Rietveld refinement findings. A characteristic of this calcium phosphate is a wide band from 900 to 1,200 cm^{-1}.[30] The band at 1,650 cm^{-1} was assigned to adsorbed H_2O. The absorption bands at 1,092; 1,044; 1,036; 960; 602; 573; and 475 cm^{-1} were assigned to the vibration in the PO_4^{3-} group. The presence of a peak at 725 cm^{-1} is characteristic of the symmetric mode v(P-O-P) $P_2O_7^{-4}$. The characteristic peak at 1,211 cm^{-1} refers to the non degenerate flat deformation of hydrogen in groups: $_3OPO\text{-}H\text{----}O\text{-}PO_3$, common in HPO_4^{-2} ions. This is related to the water molecule interaction in the crystalline net (Fig. 5b).

Cytotoxicity In Vitro Test

The cytotoxicity evaluation data obtained for the different extract concentrations of the powders, negative and positive, material controls is presented in Figure 6. These values are related to the cell viability at different extract concentrations.

The index of cytotoxicity ($IC_{50(\%)}$) is the concentration of the extract necessary to kill half of the cell population. The negative control (alumina) did not exhibit any cytotoxicity effect ($IC_{(50\%)}$ ~100). In contrast, the positive control (phenol solution 0.02 vol %) demonstrated high cytotoxicity levels ($IC_{(50\%)}$ ~0). The powders tested did not show any cytotoxicity effect up to 50% extract concentration, where its $IC_{(50\%)}$ presented comparable values relative to the negative control. At 100% extract concentration the $IC_{(50\%)}$ decreased to ~65 for both materials.



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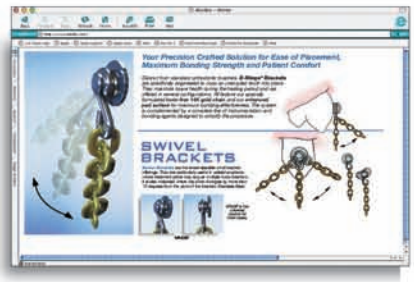

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TABLE 2: RIETVELD REFINEMENT RESULTS FOR HYDROXYAPATITE/PLGA COMPOSITE AND TRICALCIUM PHOSPHATE SAMPLES

Sample	MA1-Hydroxyapatite HA	MA-2 Tricalcium Phosphate ß-TCP	ß-$Ca_2P_2O_7$
a (Å)	9.437±0.000	10.425±0.000	6.689±0.000
b (Å)	9.437±0.000	10.425±0.000	6.689±0.000
c (Å)	6.879±0.000	37.414±0.009	24.152±0.036
V(Å^3)	530.80(1)	3521.4±0.2	1080.6±0.2
d(g.cm^3)	3.150	3.130	3.125
*R_{WP}	7.31	11.93	11.93
*S	1,82	1,51	1,51
*R_B	12,36	5.40	5.50
% mass	100.0	91.04±0.69	8.96±0.86

*R and S indexes are defined in Young & Wiles[35]

In Vivo Results

The in vivo results showed that both powder materials were biocompatible, osseoconductive, and presented the ability to provide physical support for bone in-growth for the implantation times investigated. Qualitative analysis showed that newly formed bone was in continuity with the host cortical and trabecular bone structure for both materials and times in vivo.

After four weeks of healing, MA1 and MA2 presented bone formation located primarily at the central region and the margins of the defect (Figs. 7a and 7b). After eight weeks, both materials presented higher amounts of bone regeneration throughout the critical defect (Figs. 7d and 7e). As observed at the 3D reconstructions, the materials appeared to act as bridges for the bone formation.

The new bone volume to total defect volume ratios at four weeks were (mean ± SD) MA1=14.8 ± 7.9%[a], MA2=16.1 ± 7.2%[a], and Control=6.5 ± 1.6%[b]. At eight weeks, increased values were observed for both materials, MA1=22.6 ±7.2%[a], MA2=19.9% ± 4.0[a], and Control=7.43±5.23%[b] (experimental groups non significant compared to four weeks, Fig. 8).

DISCUSSION

In agreement with previous studies testing HA/PLGA composite and TCP-based particulate materials, the in vitro and in vivo results obtained for both powders supported their biocompatible and osseoconductive physico/chemical properties suitable for bone regeneration.[14-17] The series of physico/chemical analytical results, which showed powders of Ca- and P- based composition of crystalline phases (primarily HA-based and ß-TCP-based) known to present bioactive properties without non biocompatible contaminants, supported the favorable results obtained in the in vitro cytotoxicity assessment and in vivo new bone formation.

The particle morphologies observed for both powders were representative of powder shapes and size range utilized for maxillofacial bone regeneration.[8] The irregular shapes observed for MA1 and MA2 provided both materials packability on the critical defects created in the rats' skulls. However, due to the presence of the polymer component in MA1, its placement, shaping, and initial stability was more easily achieved.

Although both ß-TCP and HA powders are considered suitable materials for bone regeneration procedures,[9,10] the optimal powder composition and morphology that will render rapid bone formation in tandem with a gradual temporal dissolution of the powder material for varied applications has been a source of speculation for a number of years.[18,19]

According to the literature,[14,20,21] due to its primary composition, the MA2

powder will present a substantially higher dissolution rate compared to MA1. In addition to the higher dissolution rate compared to pure crystalline HA powders, the presence of approximately 9% of a secondary phase on MA2 will further accelerate its initial powder dissolution.[15]

When considering the possible routes for ß-TCP synthesis:

$2\,CaHPO_4 2\,H_2O + Ca_{10}(PO_4)_6(OH)_2 \Longrightarrow 2\,Ca_3(PO_4)_2 + 2\,Ca_2P_2O_7 + 2\,CaO + 6\,H_2O$ or $Ca_{10-x}(HPO_4)x(PO_4)_{6-x}(OH)_{2-x}$ (Ca/P=1,50) ==> $2\,Ca_3(PO_4)_2 + Ca_2P_2O_7 + CaO + H_2O$, the presence of a low percentage of $Ca_2P_2O_7$ is expected.[22]

While there are concerns regarding the presence of $Ca_2P_2O_7$ resulting in a dissolution rate that is too fast for bone formation while maintaining appropriate physical integrity,[22] it has been speculated that the rapid release of Ca and P at the material surface may be beneficial for the early stages of wound healing.[22] The potential benefits may arise from the large availability of bioactive elements at the material for surface biomineralization and the stimulation of osteoclasts, and the potential phenotypic differentiation of the osteogenic cells.[22]

On the other hand, it has also been demonstrated that HA powders will present a dissolution rate that may be too slow for total powder material resorption to occur, possibly resulting in the presence of HA particles for extended periods of time after implantation.[23] For this purpose, modifications such as the interplay between its macro and micro porosity, blending HA powder with other Ca- and P-based phases of faster dissolution, elemental chemistry alteration, and application of polymeric materials have been attempted.[24,25]

Compared to ß-TCP, slower dissolution and bone remodeling is expected to occur for the MA1 powder. However, unlike commonly observed HA powders utilized for maxillofacial purposes, the Si and Mg content along with the presence of a PLGA in formulation of MA1 may result in significantly different in vivo behavior compared to a pure crystalline HA powder. Si and Mg are known to occupy the different sites of the HA lattice, and in reduced quantities have been shown to increase bioactive ceramics' osseoconductive properties[25] without substantially altering their dissolution behavior. On the other hand, the presence of PLGA, which is a blend of polylactic acid (PLA) and polyglycolic acid (PGA), will dynamically change the initial healing kinetics around the grafted material.[26,27]

The final products of the biopolymers degradation may positively affect the host to biomaterial temporal response. Immediately following implantation, the dissolution of biopolymers such as PLGA will generate the hydrolytic release of acids related to the citric acid cycle. After the hydrolysis, the degradation follows an oxidation process, transforming PLA in lactic acid and PGA in glycine and pyruvic acid. In the presence of acetyl CoA, CO_2 is released and decomposition of PLA and PGA subproducts to citrate occurs. The citrate is then incorporated at the citric acid cycle, resulting in CO_2 and H_2O, which can be excreted by urine and/or by the lungs.[27,28] Thus, through the course of the biopolymer degradation, the acidic environment generated during the degradation of the PLGA content may increase the amount of Ca and P released from the HA particles, possibly resulting in an auto-catalytic path as the MA1 particles are dissolved, exposing more PLGA from the particle microstructure.

The citotoxicity assay showed slight cytotoxic levels for both materials after the extract concentration increase. However, such slight in vitro cytotoxic at high extract concentrate values did not result in adverse effects at the tissue level since bone regeneration was observed for both materials in the rat calvaria model.

Over the last few years, µCT has been used to quantitatively investigate the 3D trabecular bone structure in physiological or pathological environment.[29] In several studies, µCT results highly correlated to those obtained with conventional histology for imaging and quantification of trabecular bone structure on human bone biopsies and animal trabecular bone.[30,31]

Although the temporal amount of newly formed bone was not significantly different among and between the materials observed, the spatial distribution of newly formed bone between groups was similar showing that, despite physico/chemical differences, both materials were biocompatible and osseoconductive.[18,19] Also, a temporal increase in the amount of bone in the critical defects increased for both materials.

At four weeks implantation time, higher amounts of bone formation were observed for MA2 compared to MA1, whereas at eight weeks, higher amounts were observed for MA1, although no significant differences in the amounts of bone formation were observed at both times in vivo. We speculate that the result obtained at four weeks was due to the higher dissolution properties presented by the ß-TCP-based powder along with the more intense initial inflammatory process due to the MA1 biopolymeric component which possibly resulted in a foreign body reaction inflammatory response.[32,33] However, while the µCT imaging provided insight concerning the critical size defect healing between MA1 and MA2, future studies evaluating the temporal tissue and cellular level events during wound healing through histology are recommended.

CONCLUSION

This study comprised the characterization, and the in vitro and in vivo biocompatibility evaluation of a hydroxyapatite/PLGA composite and a ß-TCP bone grafting particulate materials. Despite the substantial compositional differences determined by the physico/chemical characterization, non-significant differences in the amount of new bone formation were observed between materials.

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REFERENCES

1. Marins LV, Cestari TM, Sottovia AD, Granjeiro JM, Taga R. Radiographic and histological study of perennial bone defect repair in rat calvaria after treatment with blocks of porous bovine organic graft material. J Appl Oral Sci 2004;2004:62-69.
2. De Long WG, Jr., Einhorn TA, Koval K, McKee M, Smith W, Sanders R et al. Bone grafts and bone graft substitutes in orthopaedic trauma surgery. A critical analysis. J Bone Joint Surg Am 2007;89:649-658.
3. Gadzag AR, Lane JM, Glaser D, Forster RA. Alternative to autogenous bone graft: efficacy and indication. J Am Acad Orthop Surg 1995;3:1-8.
4. Summers BN, Eisenstein SM. Donor site pain from the ilium. A complication of lumbar spine fusion. J Bone Joint Surg Br 1989;71:677-680.
5. Aaboe M, Pinholt EM, Hjorting-Hansen E. Healing of experimentally created defects: a review. Br J Oral Maxillofac Surg 1995;33:312-318.
6. Devin J, Attawia M, Laurencin CT. Developmental 3-dimensional polymer for bone repair. J Biomed Sci 1996:661-669.
7. Fujibayashi S, Neo M, Kim HM, Kokubo T, Nakamura T. A comparative study between in vivo bone ingrowth and in vitro apatite formation on Na2O-CaO-SiO2 glasses. Biomaterials 2003;24:1349-1356.
8. Kim SS, Ahn KM, Park MS, Lee JH, Choi CY, Kim BS. A poly(lactide-co-glycolide)/hydroxyapatite composite scaffold with enhanced osteoconductivity. J Biomed Mater Res A 2007;80:206-215.
9. Lu J, Descamps M, Dejou J, Koubi G, Hardouin P, Lemaitre J et al. The biodegradation mechanism of calcium phosphate biomaterials in bone. J Biomed Mater Res 2002;63:408-412.
10. Descamps M, Duhoo T, Monchau F, Lu J, Hardouin P, Hornez JC et al. Manufacture of macroporous ß-tricalcium phosphate bioceramics. Journal of the European Ceramic Society 2008;28:149-157.
11. IICFD. Powder Diffraction File Database. Campus Boulevard Newtown Square, PA, U.S.A.; 1995.
12. (CCP14) GsCCPN. Powder and Small Molecule Single Crystal Diffraction; 1994.
13. Inorganic Crystal Structure Database (ICSD). U.K. ; 1995.
14. Hench LL, Wilson J. An Introduction to Bioceramics. Advanced Series in Ceramics. Singapore: World Scientific Publishing Co. Pte. Ltd; 1993.
15. Kokubo T, Kim HM, Kawashita M. Novel bioactive materials with different mechanical properties. Biomaterials 2003;24:2161-2175.
16. Jensen SS, Yeo A, Dard M, Hunziker E, Schenk R, Buser D. Evaluation of a novel biphasic calcium phosphate in standardized bone defects: a histologic and histomorphometric study in the mandibles of minipigs. Clin Oral Implants Res 2007;18:752-760.
17. Walsh WR, Vizesi F, Michael D, Auld J, Langdown A, Oliver R et al. Beta-TCP bone graft substitutes in a bilateral rabbit tibial defect model. Biomaterials 2008;29:266-271.
18. Vaccaro AR, Chiba K, Heller JG, Patel T, Thalgott JS, Truumees E et al. Bone grafting alternatives in spinal surgery. Spine J 2002;2:206-215.
19. Meynet J. Osteotomie tibiale de valgisation par ouverture interne: place des substituts osseux. Ann Orthopediques de L'Ouest 1998;30: 171-173.
20. Fulmer MT, Ison IC, Hankermayer CR, Constantz BR, Ross J. Measurements of the solubilities and dissolution rates of several hydroxyapatites. Biomaterials 2002;23:751-755.
21. Chen ZF, Darvell BW, Leung VW. Hydroxyapatite solubility in simple inorganic solutions. Arch Oral Biol 2004;49:359-367.
22. Gaasbeek RD, Toonen HG, van Heerwaarden RJ, Buma P. Mechanism of bone incorporation of beta-TCP bone substitute in open wedge tibial osteotomy in patients. Biomaterials 2005;26:6713-6719.
23. Lopes MA, Santos JD, Monteiro FJ, Ohtsuki C, Osaka A, Kaneko S et al. Osteocompatibility and in vivo evaluation of glass reinforced hydroxyapatite composite. Bioceramics 1999;12:421-424.
24. Kumta PN, Sfeir C, Lee DH, Olton D, Choi D. Nanostructured calcium phosphates for biomedical applications: novel synthesis and characterization. Acta Biomater 2005;1:65-83.
25. Kim SR, Lee JH, Kim YT, Riu DH, Jung SJ, Lee YJ et al. Synthesis of Si, Mg substituted hydroxyapatites and their sintering behaviors. Biomaterials 2003;24:1389-1398.
26. Middleton JC, Tipton AJ. Synthetic biodegradable polymers as orthopedic devices. Biomaterials 2000;21:2335-2346.
27. Peltoniemi H, Ashammakhi N, Kontio R, Waris T, Salo A, Lindqvist C et al. The use of bioabsorbable osteofixation devices in craniomaxillofacial surgery. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;94:5-14.
28. Ali SA, Zhong SP, Doherty PJ, Williams DF. Mechanisms of polymer degradation in implantable devices. I. Poly(caprolactone). Biomaterials 1993;14:648-656.
29. Gauthier O, Muller R, von Stechow D, Lamy B, Weiss P, Bouler JM et al. In vivo bone regeneration with injectable calcium phosphate biomaterial: a three-dimensional micro-computed tomographic, biomechanical and SEM study. Biomaterials 2005;26:5444-5453.
30. Muller R, Ruegsegger P. Micro-tomographic imaging for the nondestructive evaluation of trabecular bone architecture. Stud Health Technol Inform 1997;40:61-79.
31. Muller R, Van Campenhout H, Van Damme B, Van Der Perre G, Dequeker J, Hildebrand T et al. Morphometric analysis of human bone biopsies: a quantitative structural comparison of histological sections and micro-computed tomography. Bone 1998;23:59-66.
32. Chu CC. Biodegradable Polymeric Biomaterials: An Update Overview. In: Bronzino J, editor. The Biomedical Engineering Handbook. Boca Raton, FL: CRC Press; 1999. p. cap. 41.
33. Sung HJ, Meredith C, Johnson C, Galis ZS. The effect of scaffold degradation rate on three-dimensional cell growth and angiogenesis. Biomaterials 2004;25:5735-5742.
34. Coimbra MER. Degradation of polylactide acid, bioglass and hydroxyapatite: An in vitro and in vivo study Department of Material Science. Rio de Janeiro: Military Institute of Engineering - IME; 2008: p. 212.
35. Young RA. IU Monographs on Crystallography - 5 The Rietveld Method. New York: Oxford University Press; 1995.

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INTRA-LOCK® SYSTEM

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The evolution of oral implantology over the past 40 years has taken many pathways. The profusion of implant architectures, both in their macro and micro-designs, has made it clear that there is no linear path to a more ideal implant interface or functional design. The ever increasing manufacturing base for implant production has presented an increasing dilemma for clinicians who must select the appropriate implant for a particular case. This conflict between "market versus science-based" implant designs has been played out in clinical practice, often to the detriment of the patients we treat. While the parameters for successful implant design have changed considerably in the past decade, we find that by combining biologic and engineering-driven alternatives, we can more closely meet the new criteria for ideal clinical outcomes.

HISTORICAL PERSPECTIVE

Over ten years ago, an international coalition of dental professionals, biologists, design engineers, and manufacturing craftsmen were assembled to further the evolution of existing dental implant technology. With a fresh slate, they began a design and development project that was global in extent and encompassed many years of research and analysis. By analyzing "proven" designs and the latest and most valid biological principles, they realized that within this multidisciplinary data, there existed the opportunity to provide the field with significant advancements in the design of implant systems. Armed with this new database of knowledge, a prototype implant and delivery system was developed. It was named the Intra-Lock System® and was marketed for almost a decade in Europe and the Americas. Tens of thousands of implants were placed and evaluated. Clinical success, patient acceptance and confidence in this system by the practitioners who used it led to the worldwide debut of Intra-Lock International, Inc.® in 2001.

A CLOSER LOOK AT THE INTRA-LOCK® DENTAL IMPLANT SYSTEM

Any critical analysis of an implant system must take into account all of the features available to the clinician. If we closely examine the Intra-Lock System®, from the prosthetic platform to the apical end, we find a series of advanced designs that address the biologic short-

comings of previous implant systems. Abutment design, macroarchitecture, biologically active surfaces, and the economy of instrumentation needed for clinical use are parameters that give true meaning to the concept of "intelligent engineering". System components should be synergistic in the sense that they simplify surgical placement, have prosthetic variability to address a wide spectrum of applications, and respect the biologic component of healing and maintenance of the soft and hard tissue envelope. The Intra-Lock International® system addresses all of these parameters and provides a total solution for the discerning implantologist.

OSSEAN™ SURFACE TREATMENT

Surgical principles in oral implantology are returning to a paradigm of early or immediate loading of dental implants.[1] Respect for both prosthetic and biologic principles is imperative. When a dental implant is placed, the bone-to-implant interface is weaker at two weeks immediately after implant insertion because of an inflammatory cascade and catabolic events which result in bone breakdown and remodeling.[2] This places the implant at risk if it is placed in immediate function or in an extraction site with a significant defect. Previous implant coatings, such as plasma-sprayed HA, have attempted to address this breakdown phase with mixed success.[3] Earlier amorphous HA coatings were highly osteoinductive because of the bioavailability of free calcium ions.[4] Studies of HA coated implants in the '80s clearly demonstrated earlier osseointegration and a higher bone-to-implant contact.[5] However, the low crystallinity of the HA coating led to fractures of these coatings and severe peri-implant infections after loading.[6] For over a decade, clinicians avoided HA coatings. In an attempt to eliminate these clinical problems, manufacturers subsequently changed the HA formulation to approximately 97% crystallinity. This solved the fracture problem but had the opposite effect on osteoinductivity. Highly dense HA does not resorb to any significant degree. This dramatically reduces the bioavailability of free calcium from the implant surface. Therefore, current HA surfaces have limited biologic interaction when compared to newer acid-etched titanium surfaces and no longer offer any significant clinical advantage.[7]

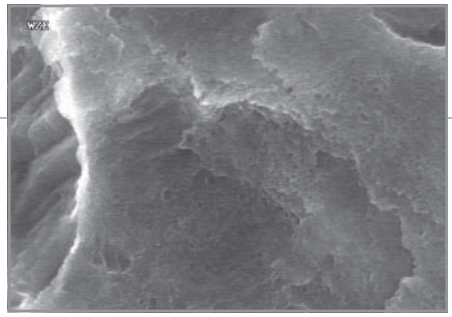


Figure 1: SEM of Ossean surface at high magnification (50,000X).

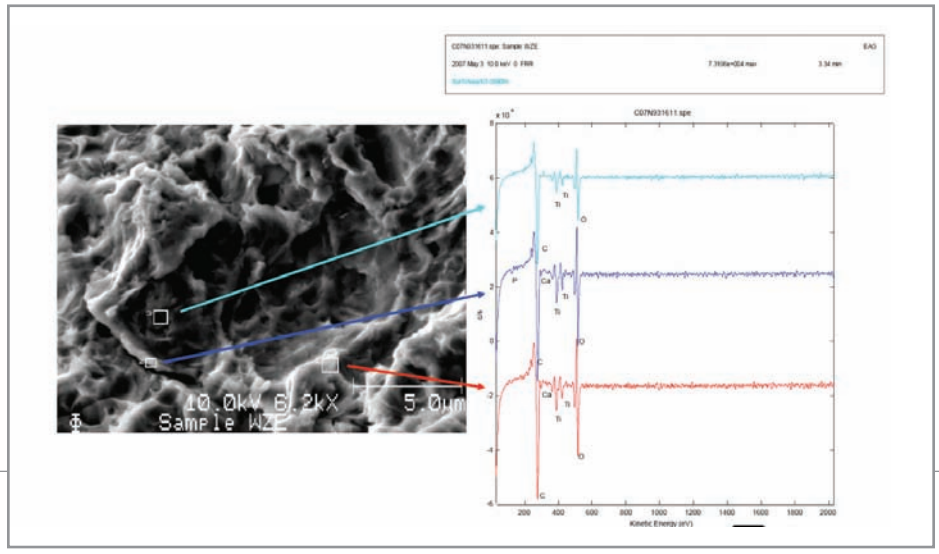


Figure 2:Auger spectroscopy demonstrating even distribution of the calcium phosphate surface.

In 1991, the concept of "bone-bonding" was first described.[8] Different from the type of interface originally described by Branemark and known as osseointegration, bone-bonding is characterized as an interfacial bond between the bone and implant surface that exceeds the cohesive strength of either bone or implant.[9] A chemical interaction occurs between bone and implant that enhances both bone

cystallinity and adhesion, and can be demonstrated when calcium phosphate materials are present in the correct concentrations.[10] The introduction of a nanotextured surface, further enhanced by molecular impregnation with calcium phosphate, has been shown to significantly enhance osteoblastic activity and thereby eliminate the catabolic phase of bone remodeling (Fig. 1).[11]

Figure 3: Drive lock engaging the implant from the sterile delivery system.

In addition, the Ossean™ surface dramatically increases the rate of osteoblastic synthesis of type I collagen, thus promoting osseointegration and reducing the chances of early failure of immediately loaded implants.[12] Even distribution of the calcium phosphate surface is critical to control the physiology of osteoblasts (Fig. 2).

This increase in bone-bonding strength is clearly demonstrated in a study conducted by Coelho, et al, where Intra-Lock™ implants with and without the Ossean™ surface were tested in a reverse torque pullout study.[13] The Ossean™ surface implants at two weeks exhibited a 100% greater bone adhesion than the implants without the surface modification. In a second study, implants from two other competing manufacturers were tested against a similar macro-architecture Intra-Lock® implant. In this study, when compared to a particulate calcium phosphate coating and a TiO blasted + HF etched surface, at one week the Intra-Lock® implants had a 500% greater bone-bonding shear strength as demonstrated in a reverse torque pullout study.[14] The conclusion reached by both studies is that there is a limitation of biologic activity on purely etched surfaces and there is also a qualitative difference in some nanotextured + calcium phosphate impregnated surfaces. The Ossean™ surface is clearly biologically active in the sense that bone goes directly to the anabolic phase without intervening bone breakdown. This is extremely important in immediate load cases and for extraction site defects where the percentage of initial bone-to-implant contact is compromised.[15]

DRIVE-LOCK™

Implant surgeons with experience using multiple implant systems often have a collection of prosthetic instruments that may not be interchangeable. Within a given system, multiple instruments may lead to lost time and confusion in implant placement. Additionally, implant mounts are often discarded as an unnecessary component in implant placement. The Intra-Lock System® replaces multiple instruments with a single driver called "Drive-Lock"™. This driver is not only exceptional in engineering, but is also a perfect example of ergonometric design and economy of movement. In a sterile shipping mount, the implant is suspended on a titanium ring. When the surgeon is ready to deliver the implant to the surgical site, the Drive-Lock™ driver simply engages, with a slight degree of pressure, the implant interface. An O-ring on the driver holds the implant securely in place. The implant can then be removed and carried directly and confidently to the osteotomy site, where the seating of the implant is then initiated, all in one fluid motion.

Another consideration is the strength of the implant system. Every aspect of the implants, the attachments, and the instruments are subjected to clinical, mechanical, and computerized stress analyses. Intra-Lock Systems® are built to withstand forces that exceed applied clinical stresses by a wide margin. Therefore, when the implants are being placed, they can be inserted with confidence, and without fear of deformation of the prosthetic interface connection. Final seating can be accomplished with a motor or, if you prefer, with a compatible hand ratchet driver. The tip of the drive-lock™ attachment is also a 1.3mm hex driver. It carries the cover screw to the implant and threads it into place. There is no need for any other instrumentation and is designed for ease, safety, precision, and speed in implant surgery (Fig. 3).

Drive-Lock™ offers another significant advantage. Most implant systems have a driver that engages the prosthetic platform. As torque values increase during implant placement, there can be a deformation of the platform (internal or external) which compromises both abutment stability and sealing capacity. Significant deformation can result in a cold weld of the mating surface, making removal of the driver quite difficult and potentially micro-fracturing the bone-to-implant interface. Intra-Lock International® created and patented the Drive-Lock™ (US Patent #7,131,840 and International patents pending) to allow easy insertion and removal. It takes over 200 Ncm of torque before any deformation of the prosthetic interface can be detected.

ABUTMENT DESIGN

The first anti-rotational abutment system was the Branemark external hex. Originally designed solely to allow the engagement of a driver to seat the implant, it was ultimately adapted to prevent the rotation of abutments placed on the implant body. The short height of the hex, and the lack of an abutment with correct tolerances, made it a poor choice for stabilization of a single tooth restoration.[16] In addition, the flat to flat abutment connection allowed percolation of fluids and a bacterial component into the implant connection.[17] This pumping action during cyclical loading ultimately places stress on the retaining screw.[18] Screw loosening became endemic in this design with failure of the prosthetic components and soft tissue volume.[19] The move from external to internal attachments began as a means to mitigate the loosening of abutments

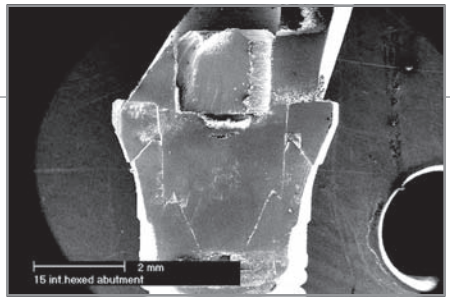
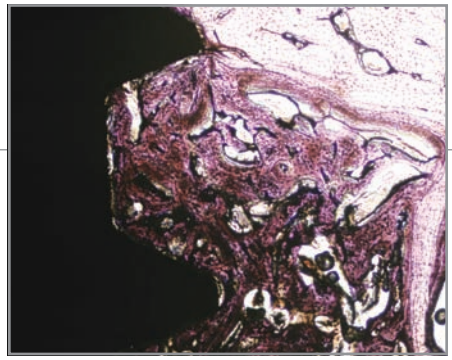
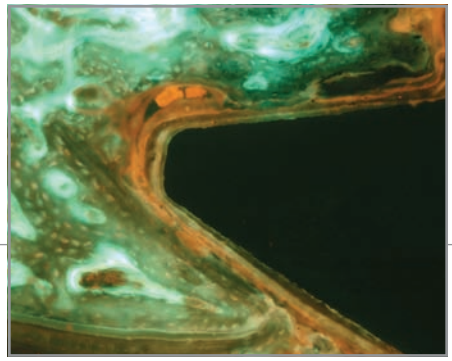


Figure 4: SEM cross section of Intra-Lock® abutment in the implant.



Histological response to MILO® Wide Pitch at two week healing time.



Immunofluorescence optical microscopy showing accelerated bone formation around MILO® Wide Pitch Implants at two week healing time.

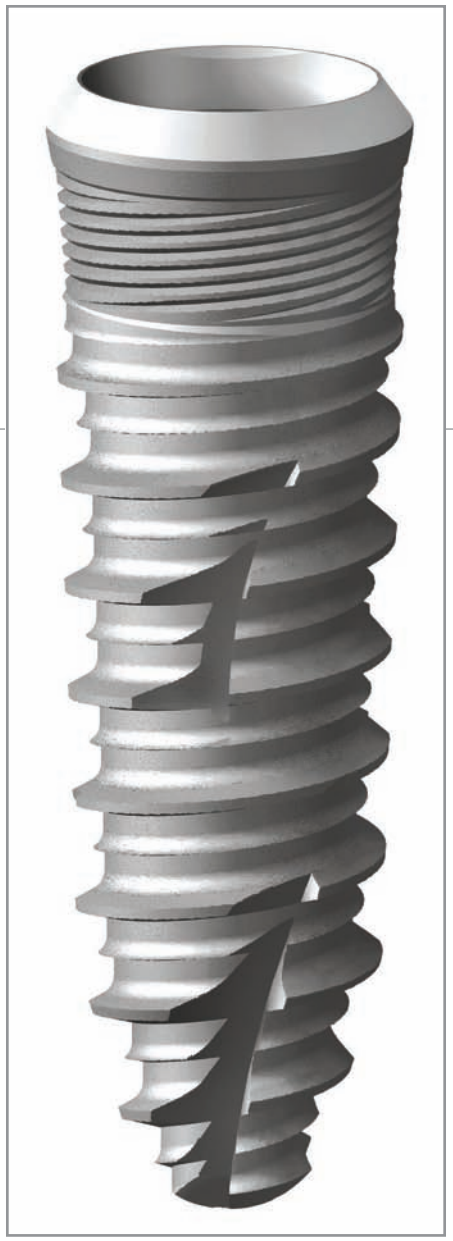
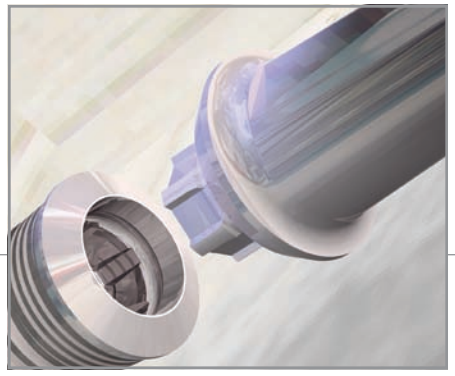


Figure 5: The CT implant design.



Abutment implant interface.

as they were being subjected to clinical functional forces. A clinical byproduct of this change, as noted radiographicaly, was a decrease in crestal bone remodeling.

Percolation of fluids during the normal micro-mobility that occurs at the implant-abutment interface leads to bacterial infiltration.[20] Chronic inflammation results in the production of matrix metalloproteanases (collagenase, gelatinase, elastase) which cause soft tissue breakdown and the potentiation of osteoclastic activity.[21] The challenge, therefore, was to reduce microleakage at the implant/abutment interface.

Intra-Lock™ engineers proceeded to develop a ferrule attachment in combination with both inside and outside stabilization (Fig. 4).

In a recent study conducted by New York University Department of Biomaterials and Biomimetics, some of the leading abutment designs were tested and compared for microleakage. The Intra-Lock® abutment was shown to be superior. It clearly and significantly reduces microleakage when compared to various attachment designs of its competitors.[22] This significant reduction in microleakage will reduce the concentration of inflammatory compounds being produced, which helps to prevent resorption of the interdental bone and thereby preserve papillary support for aesthetics. A more stable joint will also decrease stress to the retaining screw. Retention of the abutment should be borne primarily by the abutment at the joint interface. There should be stability of the abutment after primary torque even if the retaining screw is removed. The ferrule attachment gives us this kind of stability and is responsible for the dramatic reduction in microleakage. In addition, there is a choice of abutment diameters, giving the clinician an option for a platform shifted emergence profile.

When evaluating an abutment system, the clinician should be looking for several features. First, is prosthetic variability. Is there sufficient adaptability for all types of prosthesis planning, from removable overdentures to complex fixed restorations, both in the provisional and definitive stages? Second, is the accuracy of impression and record taking. Third, is the stability of the abutment interface if the final abutment must be transferred from lab model to the mouth several times during the try-in phase. Finally, the abutment should eliminate flexural opening at the margins during function to reduce microleakage at the implant-abutment interface. The Intra-Lock® abutment clearly meets all of these parameters as demonstrated by university studies. This gives clinicians the confidence needed for long-term success in prosthetic rehabilitation.

FULL RANGE OF IMPLANT ARCHITECTURES

Most implant companies manufacture an implant system that consists of a single macroarchitecture with the variables being only length and diameter. This "one-size-fits-all" mentality fails to address the challenges of implant sites where volume, density, and extraction site defects compromise initial stability and healing. Intra-Lock International® offers several different architectures and thread designs, each of which is engineered to be "site-specific". These designs range from relatively parallel-walled to a fully tapered architecture for greater initial stability. Intra-Lock International® researchers and engineers are currently developing an implant architecture based on a patented concept of radial compression threading. This new thread design will offer increased stability and guidance during implant placement in

extraction sites where bone-to-implant contact can be substantially lower than in healed sites. All of these designs employ Drive-Lock™ technology and have the Ossean™ surface treatment for enhanced healing. Collar lengths are also variable so that the restorative dentist can maintain control of the prosthetic emergence profile. In ridges with compromised width, the CT implant line functions as an osteotome to spread the ridge during implant seating (Fig. 5).

As clinicians move towards early and immediate implant loading, having an implant system that allows the surgeon to select an implant architecture with the greatest stability at time of placement provides security and an enhanced healing response, especially in extraction site defects. All two-piece implant deigns from Intra-Lock™ use the same prosthetic parts for both provisionalization and the definitive prosthesis.

From surgical placement to final prosthetic reconstruction, the Intra-Lock System® is designed to give the entire implant team complete control during patient treatment. This makes the Intra-Lock System® the logical choice of discerning implantologists.

SMALL DIAMETER IMPLANTS

For immediate stabilization of dentures, provisional prosthetics, or replacement of mandibular incisors and maxillary lateral incisors, small diameter one-piece implants can be a logical alternative to traditional implants, especially in compromised ridges. Also known in the literature as mini or narrow diameter implants, the MDL® and MILO® implant lines are FDA approved for all intra-boney sites and applications. These implants are available in 2.0 mm and 2.5mm for the MDL® implants, and 3.0 mm diameter for the MILO® implant. Only one 1.2mm externally irrigated drill is necessary for the MDL® line. Light, intermediate introduction of this drill breaches the gingival tissue and periosteum. Drilling depth is approximately one-third the length of the implant body after the cortical plate has been perforated. The MDL® contra-angle attached snaps over the O-ball assembly and engages the square driving feature. The implant is then carried directly to the surgical site and placed in the pilot hole. The MDL® sharp apical guiding point initiates the self-tapping action. The implant starts to rotate at approximately 15 rpm, allowing it to cut through the alveolus. The MDL® implant subsequently threads and expands the bone within the visco-elastic limit. Once in place, the implant is firmly retained by the elastic properties of the bone, and is ready for immediate loading. Retentive o-ring housings can then be picked-up in the patients denture and the patient leaves the office with an implant stabilized overdenture (Fig. 6).

ONE-PIECE IMPLANT. MULTIPLE APPLICATIONS.

Dental Implantology started with one-piece implant designs, however they lacked functionality. With a standard one-piece implant design the practitioner is faced with the limitations of the implant's built in abutment design. Corrections for angulations and margins, have to be accomplished in the mouth. Intra-Lock's small diameter systems address this drawback. They accept a

Figure 6: Immediate stabilization of mandibular denture with small diameter implants.

full selection of abutments that fit over the O-Ball assembly. This patented (US Patent 7,217,130) Cement-Over™ Abutment System permits an impression technique through transfer and analog preserving precious chair time. The abutment is customized at the lab, and the final crown built right at the same stage. In addition to a healing cap, abutment selection includes: wide, straight, angled, castable, and orthodontic.

MILO® small diameter implants also have FDA clearance for replacement of mandibular central/lateral incisors as well as maxillary lateral incisors. Analysis of shear strength of small diameter implants indicates that they are actually stronger than some larger diameter implants with internal attachments. Due to their size and clinical protocol, a factor that must be taken into consideration when deciding which mini implant system to use is its strength in terms of resistance to static and dynamic forces. An accepted method of determining these qualities is set forth by the International Test Standard: ISO 14801:2003(E) – " Dentistry — Fatigue Test for Endosseous Dental Implants." This series of tests were preformed by an independent laboratory. In addition to substantiating the implant's adequate resistance to fatigue forces it also revealed that the static properties of the Intra-Lock Mini Dental Implants had a mean ultimate load that was 37.5% stronger than an mini implant on the market and in widespread use for over a decade.[23]

When used in the appropriate sites, small diameter one-piece implants can be a better choice when the alveolus is compromised or where there is minimal space for implant placement but aesthetics is still critical. A wide variety of abutments are also available, making small diameter MDL and MILO implants an ideal choice in challenging implant cases.

SYNOPSIS

Intra-Lock International® is a full featured implant company that provides the highest quality products to the implant community. This pursuit of excellence is deeply rooted in its corporate philosophy of balancing research, engineering, manufacturing, and marketing. There is an enduring commitment to the clinician to provide implant components that have undergone the most rigorous engineering and biologic analysis. The confidence that this philosophy engenders has made Intra-Lock International® one of the fastest growing implant companies in the world.

REFERENCES

1. Castellon P, Blatz MB, D.M.D., Dr.Med.Dent., Block MS, Finger IM, Rogers B. Immediate loading of dental implants in the edentulous mandible. J Am Dent Assoc 2004 Vol 135, No 11, 1543-1549.
2. Albreksson T, Johansson C. Quantified bone tissue reactions to various metallic materials with reference to the so-called osseointegration concept. In: Davies JE, ed. The Bone-Biomaterial Interface 1991; Toronto: University of Toronto Press:357-363.
3. Block MS. Advantages and disadvantages of hydroxylapatite-coated implants. Oral MaxillofacSurg Clin of North America 1991;3:835-851.
4. Gerner BT, Arth E, Alberktsson T, Ronningen H, Solheim LF, Wie H. Comparison of bone reactions to coated calcium phosphate and pure titanium dental implant in the canine iliac crest. Scan Journ of Denta Res1988; 96:143-148.
5. Engquist B, Bergendal T, Kallus T, Linden U. A retrospective Multicenter evaluation of osseointegrated implants supporting overdentures. Intl J Oral Maxillofac Impl 1988: 3:129-134.
6. MacDonald DE, Betts F, Stranick M, Doty, Boskey AL. Physicochemical study of plasma-sprayed hydroxyapatite-coated implants in humans. Journal of Biomed Mat Res 2000 Vol. 54(4):480-490.
7. Dalton JE,, Cook SD. In vivo mechanical and histological characteristics of HA-coated implants vary with coating vendor. Journal of Biomedical Materials Research 2004 Vol. 29(2): 239 - 245.
8. Lee SC, Song WS. Histomorphometric and Removal Torque Values Comparision of Rough Surface Titanium Implants. J Korean Assoc Maxillofac Plast Reconstr Surg. 2001 Sep;23(5):396-405.
9. Mendes VC, Moineddin R, Davies JE. The effect of discrete calcium phosphate nanocrystals on bone bonding. Biomaterials 28(207)4748-4755.
10. Zhamg Y, Yokogawa Y, Kameyama T. Bimodal porous bi-phasic calcium phosphate ceramics and its dissolution in SBF solution. Key Eng Mat 2007 Vol. 330-332;91-94.
11. Coelho P, Freire J, Coelho A, et al. Nanothickness bioceramic coatings: Improving the host response to surgical implants. In: Leipsch D, ed. World Congress of Biomechanics Conference Proceedings. Munich:Medimont.2006;253-258.
12. Anselme K. Osteoblast adhesion on biomaterials. Biomaterials 200; 21:667-681.
13. Marin C, Granato R, Suzuki M, Gil JN Piattelli A, Coelho PG. Removal torque and histomorphometric evaluation of bioceramic grit-blasted/acid-etched and dual acid-etched implant surfaces. An experimental study in dogs. J Perio 2009 Vol. 79(10):1942-1949.
14. Coelho PG. Personal communication. Manuscript in preparation. 2008.
15. Susarla SM, Chuang SK, Dodson TB. Delayed versus immediate loading of implants: survival analysis and risk factors for dental implant failure. J Oral Maxillofac Surg. 2008;66:251.
16. Binon PP. The effect of implant/abutment hexagonal misfit on screw joint stability. Int J Prosthodont. 1996;9:149.
17. Brogginni N, McManus LM, Hermann JS, Medina R Schenk RK, Buser D et al. Peri-implant inflammation defined by the implant-abutment interface. J Dent Res.2006;85:473.
18. Khraisat A, Hashimoto A, Normura S, Miyakawa O. Effect of lateral cyclic loading on abutment screw loosening of an external hexagon implant system. J Prosthet Dent. 2004;91:326.
19. Brogginni N, McManus LM, Hermann JS, Medina RU, Oates TW, Schenk RK et al. Persistent acute inflammation at the implant-abutment interface. J Rest Dent Res.2003;82:232.
20. Steinebrunner L, Wolfart S, Bossmann K, Kern M. In vitro evaluation of bacterial leakage along the implant-abutment interface of different implant systems. Int J Oral Maxillofac Implants. 2005;20:875.
21. Becker W, Becker BE, Newman MG, Nyman S, Clinical and microbiological findings that cause failure of dental implants. Quintessenz.1991;42:9.
22. Coelho PG, Sudack P, Suzuki M, Kurtz KS, Romanos GE, Silva NRFA. In vitro evaluation of the implant abutment connection sealing capability of different implant systems. J Oral Rehab 2008 35;917-924.
23. Test conducted as per ISO 14801:2003(E) – " Dentistry — Fatigue Test for Endosseous Dental Implants." Test report avalible upon request.

MILO® 3.0

A True 'Convertible' implant

VERSATILITY OF A TWO-PIECE

MILO® Cement-Over™ abutments simply fit over the O-Ball assembly and when cemented in place with resin cement, abutment and implant form one strong and solid unit.

STRENGTH OF A ONE-PIECE

The MILO® one-piece 3.0mm diameter design has greater strength and fatigue resistance than a full-size standard two-piece implant*.

EASE OF DELIVERY

The system's unique Drive-Lock™ feature reduces delivery and placement to one fluid motion.

OSSEAN® SURFACE

Once in place, Intra-Lock's extremely hydrophilic, bio-active OSSEAN® Surface promotes rapid early healing and increased biomechanical fixation*.

With ossean® SURFACE

*Data on File

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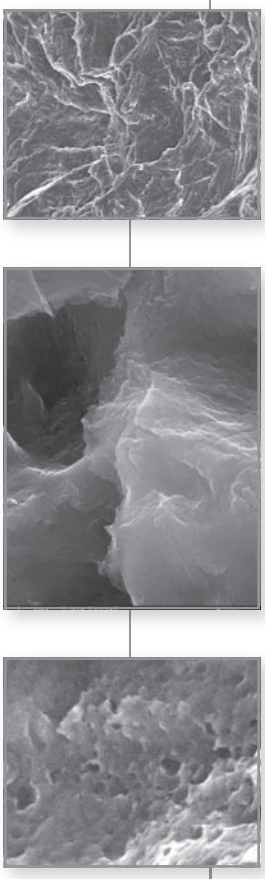

OSSEAN™ Surface: Another Marketing Buzz or a Real Technological Breakthrough?

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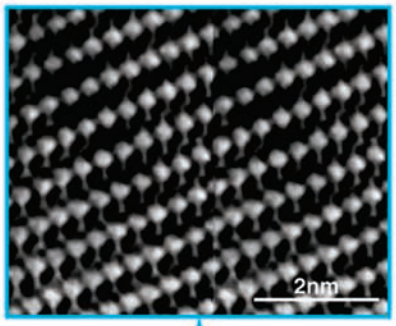
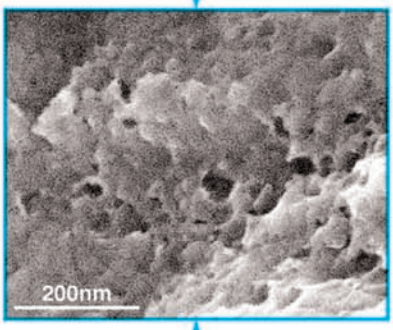
Interaction between an artificial device and living tissue is a fascinating field of exploration and science. In the last 30 years, we have been observing an ever-increasing convergence of diverse and multiple branches of science. Today, a good specialist can no longer rest on what was learned from the elders on the benches of the University. Cross-discipline education and constant perusal of the scientific literature is a must for the contemporary practitioner. Thus, a periodontologist must have a basic understanding of advanced immunology, the immunologist must understand some advanced biochemistry, and the biochemist needs to remain updated in advanced physics and mathematics. In addition, all can benefit from a good dose of philosophy, which is indispensible for gaining a perspective on matters and for the conceptualization of complex theories.

The concept of "gaining a perspective on matters" begs the question, "Are we seeing a new "renaissance" taking revenge on the ultra-specialists from the seventies?" Is this generation, who were sadly lacking in even the most rudimentary skills required for good communication and understanding, isolated in their "ivory towers" and simply "too intelligent" to impart the essence of their arcane knowledge beginning to wane? Is a new generation, a "missing link" between the intellectually gifted and the "generic" practitioner beginning to emerge? If that be true, it would indeed be a boon to all mankind. One can only hope for the best and for the proliferation of this rare "new breed".

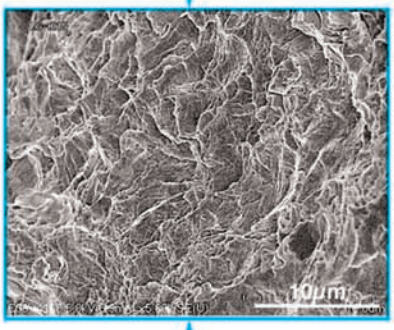
Going back to the interactions between an implant and its surrounding tissues, the works of Professor Per Ingvar Branemark had the merit to clearly establish the basics of osseointegration and show that bone heals uneventfully when properly placed in close contact with Titanium. But that was only a beginning. Too many questions were left with no good answer; why do we witness implant failures, usually at early stage of



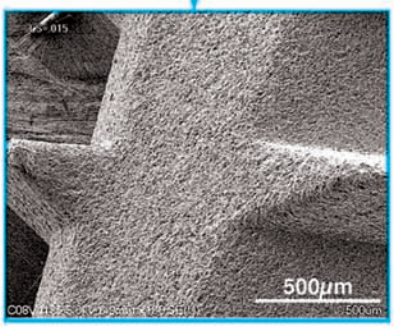
20,000,000X
Molecular Structure
Calcium Phosphate



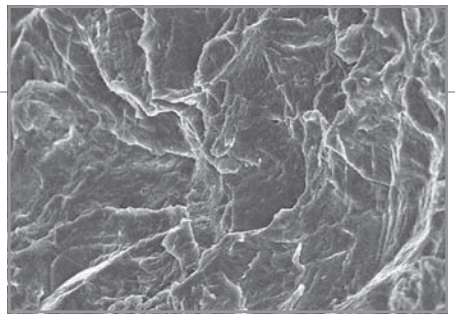
200,000X
Nano Structure



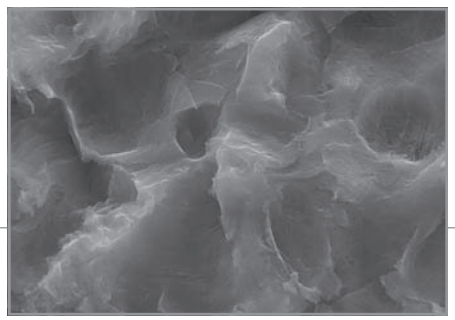
5,000X
Micro Structure



100X
Macro Structure



SEM image of OSSEAN Surface at 10,000X.



SEM image of OSSEAN Surface at 30,000X.

bone repair? Do all the implant designs trigger the same response from the bone? Is bone healing always the same, regardless of thread design, surface characteristics, load or no-load, degree of approximation, etc.?

For example, consider the multitude of screw designs on the market, with so many surface finishes, but no real study to validate the merits of said refinements. Instead, we are bombarded by advertising claims, numerous anecdotal and clinical case reports, a modicum of literature dispensed from Universities (on behalf of those interests providing multi-million dollar grants), but recently we have witnessed some comprehensive research-driven designs.

An example of the latter can be seen in the work of Berglundh et al[1]. This report demonstrated that changing thread design and drilling sequence could significantly alter the kinetics of bone healing. Coelho et al[2] also provided evidenced that a thread design change, combined with a change in drilling sequence would speed up bone formation by a factor as great as ten times. The data are impressive, and even more impressive when you consider the fact that these experiments were still looking at a macro scale; where we can see changes that were made to the implant and could be discerned by the naked eye!

The history of surface modifications has also undergone its share of trial and error, going from machines surfaces to TPS and plasma spray Hydroxyapatite. A consensus has emerged from the scientific community with respect to this characteristic, and the verdict has been established: micro-rough surfaces are the pick of the litter. Despite the fact that the technologies employed to generate these surfaces may vary from one manufacture to another, the same profile and the same surface chemistry will provide the same biological response. An important difference to note in this advance is that whereas the changes in thread design and drilling sequences are discernible by the human eye, the perception of significant surface changes can only be determined via microscopic examination. Kikuchi et al[3] have shown that the surface characteristics that exist at this level are essential for platelet activation. They have concluded that surface profilometry at the microscale level is more important than surface chemistry. At this level of technology, the changes are on the micro scale.

Nowadays, most of the implant manufactures have remained at this level even if many use the "buzz-word" of nanotechnology. Nanotechnology is a relatively new science (under 30 years old) and its development increased exponentially in the nineties with the introduction of the Scanning Tunneling Microscope and the Atomic Force Microscope. The discipline encompasses various areas: nanomaterials and molecular nanotechnology. It is fascinating to discover that a well known periodic element as stable and chemically inert as Gold (Au), functions as a potent catalyst when used at a nanoscale. Molecular nanotechnology consist of building complex structures, using the atoms as elemental bricks.

Nanoprofilometry is often confused and taken for nanotechnology, but they are not synonymous. They are as different as "looking" versus "doing".

Vetrone et al[4] recently showed in 2008 that nanostructured surfaces influence the behavior of various cell types and even alter the potential for the differentiation of stem cells. The Ossean® project was initiated under my

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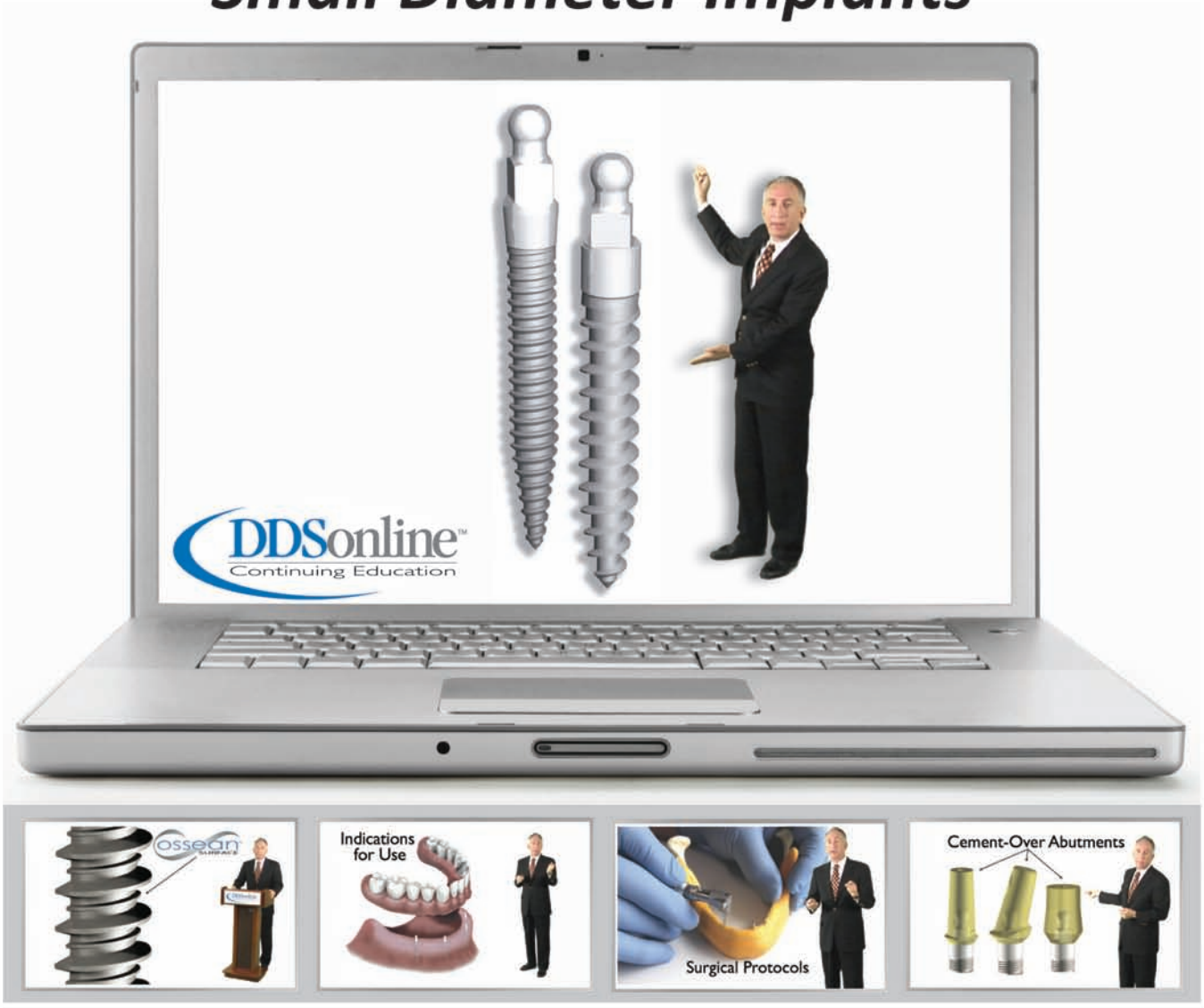

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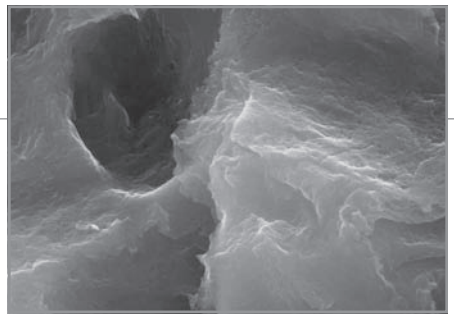
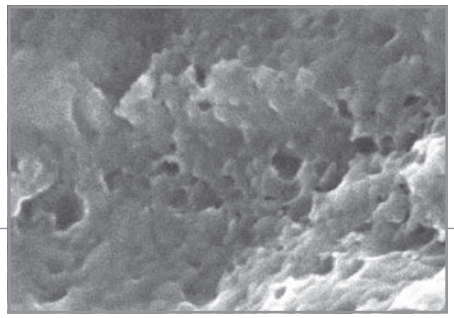


SEM image of OSSEAN Surface at 100,000X.



SEM image of OSSEAN Surface at 200,000X.

responsibility in 2005, and the surface was launched in 2007. It is characterized by a fractal structure with the same pattern repeating itself from the macroscale to the microscale, then to the nanoscale and beyond. There is no addition of any particle of any kind on the surface but rather, an elemental modification of the surface chemistry within the Titanium Oxide layer via the incorporation of Calcium Phosphate. High resolution SEM shows at 200,000X magnification, the nanotexture of the surface. Pristine. Devoid of any particle or contaminant. Calcium Phosphate is evidenced under XPS-ESCA or Auger Spectroscopy. The clinical ramifications are impressive: Marin and Al[5] showed values of torque removal at two weeks post-implantation to be double on Ossean® surface implants when compared to a standard Blasted/Acid etched implant. Another paper from Coelho and Al[6] (to be published), shows a comparative study between Ossean® Intra-Lock implants, and two other leading brand implants, (same size, same shape, same thread pitch.) The first part of the study consists in measuring the torque removal at one week and three weeks after implant placement. The results show a 500% increase of torque value at one week for the Intra-Lock Ossean® surface implant over the other two implants. Those results are mind-boggling when we put in perspective the fact that it was a given that osseointegration has to go through a catabolic phase prior to entering the anabolic phase. It is postulated that the Ossean® surface changes the genetic "fate" or the coding of the surrounding cells.

Similar conclusions can be drawn from another study to be published by Piatelli et al[7]. In this human study, Ossean® surface implants have been compared to the same implant shape with a conventional Blasted/Acid Etched surface. An osteocyte count has been performed adjacent to the implant surface and at distance. The results show a 50% increase of those precious cells compared to the control.

These studies and other data confirm the fact that we are not only working at the nanoscale, but also within the true realm of nanotechnology, at the molecular level.

These recent findings are changing the paradigm of tissue healing around implants, and will enable us to define the process of osseointegration with greater precision and depth of understanding.

REFERENCES

1. Berglundh T, Abrahamsson I, Lang NP, Lindhe J. De novo alveolar bone formation adjacent to endosseous implants. A model study in the dog. Clin Oral Impl Res, 14, 2003, 251–262.
2. Coelho & Al. Early Bone Healing Around Different Implant Bulk Designs and Surgical Techniques. A Study in Dogs. Clinical Implant Dentistry and Related Research. In Press.
3. Lena Kikuchi & Al. Platelet interactions with calcium-phosphate-coated surfaces. Biomaterials, Volume 26, Issue 26, Pages 5267-5426 (September 2005).
4. Vetrone F. & Al. Nanoscale Oxidative Patterning of Metallic Surfaces to Modulate Cell Activity and Fate, NANOLETTERS, Vol. xx, No. x, 2008.
5. Marin C. & Al. Removal Torque and Histomorphometric Evaluation of Bioceramic Grit-Blasted/Acid-Etched and Dual Acid-Etched Implant Surfaces: An Experimental Study in Dogs. J of Perio, Volume 79 • Number 10, Oct 2008. (1942-1949). doi: 10.1902/jop.2008.080106.
6. Coelho PG. Personal communication. Manuscript in preparation. 2008.
7. Piattelli & Al. Histomorphometric Evaluation of Bioceramic Molecular Impregnated and Dual Acid Etched Implant Surfaces in the Human Posterior Maxilla. Clinical Implant Dentistry and Related Research. In Press.

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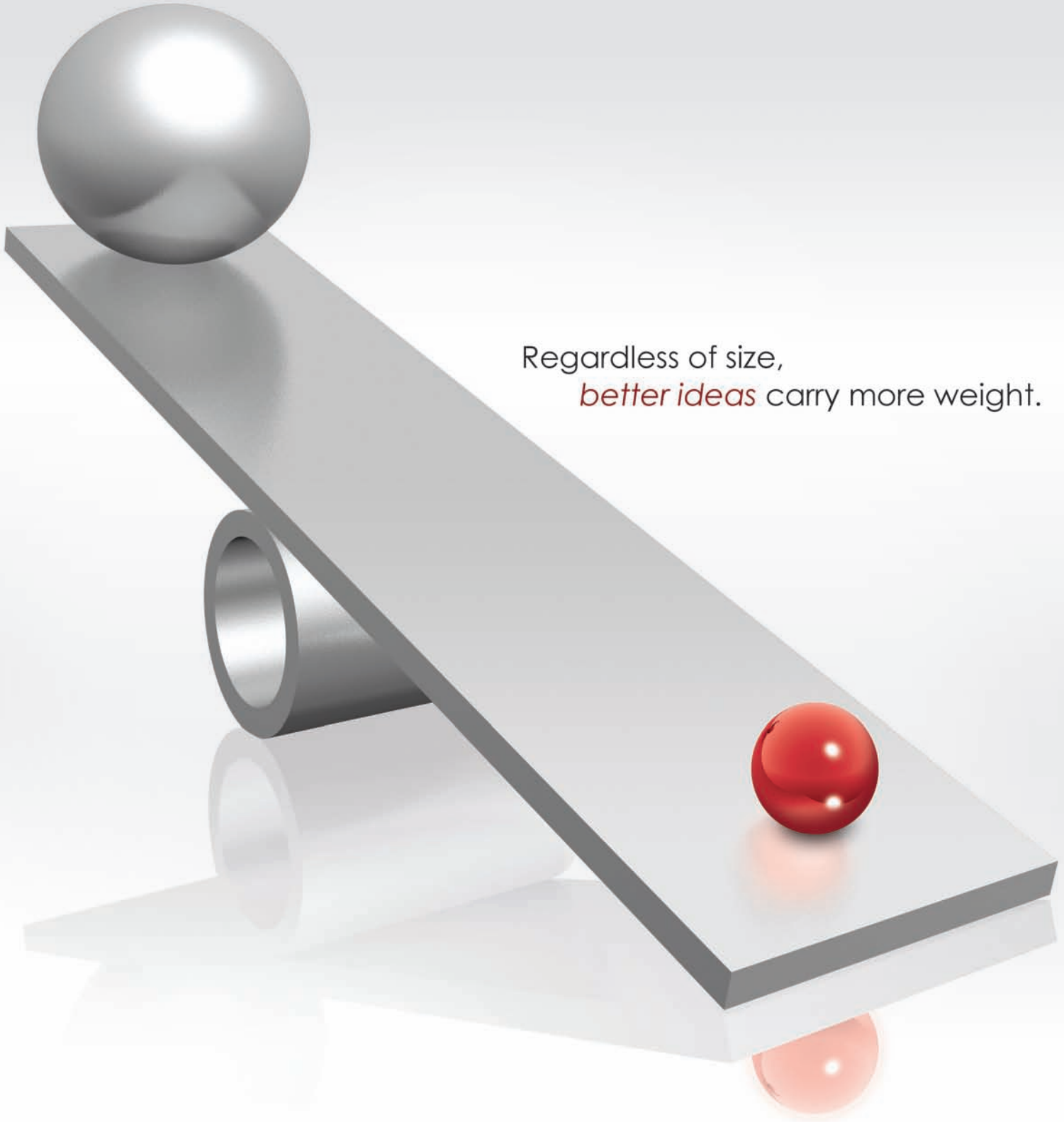

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Buccal Bone Remodeling After Immediate Implantation With a Flap or Flapless Approach: A Pilot Study in Dogs

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Background: *The aim of the present pilot study was to evaluate the buccal bone remodeling after immediate implantation with flap or flapless approach in mongrel dogs.*

Materials and Methods: *The mandibular bilateral premolars of three dogs were extracted and three implants were immediately installed in both hemi-arches of each dog. Randomly, one hemi-arch was treated with the flapless approach (experimental group); while in the contra lateral hemi-arch, tooth extractions and implant placement were done after mucoperiosteal flap elevation (control group). A 1mm gap from the buccal cortical wall to the implant was always left and after the adjustment of the healing caps, a non-submerged healing of 12 weeks was provided for both groups. Histomorphometric analysis was done to compare buccal and lingual bone height loss, bone density, and bone-to-implant contact in the groups.*

Results: *There was a significant association between the surgical flap and the extent of bone resorption around immediate implants. The loss of buccal bone height was significantly lower in the flapless group when compared to the flap group (0.98 mm x 2.14 mm, respectively, $p<0.05$). The coronal and apical buccal bone densities of the flap group were significantly higher when compared to the lingual components, indicating anatomical differences between the bone plates. The marginal gaps between the buccal walls and the implants disappeared without the migration of connective tissue for both groups.*

Conclusion: *The flapless approach for immediate post-extraction implants reduces the buccal bone height loss.*

Key words: *animal model, bone resorption, immediate implants, flapless surgery*

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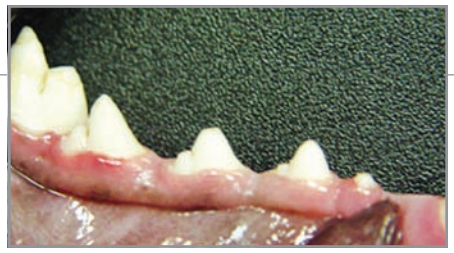

Figure 1: Pre-operative view.

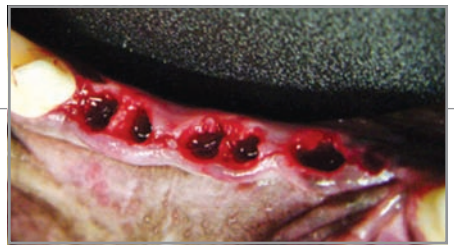

Figure 2: One random hemi-arch was treated with the flapless approach.

Figure 3: The opposite hemi-arch was treated with a mucoperiosteal flap.

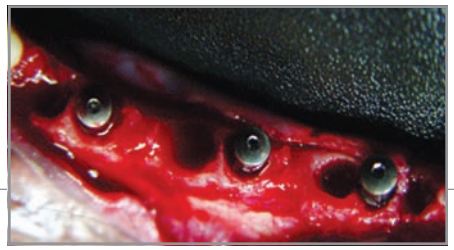

Figure 4: Three implants were immediately inserted in the mesial alveolus of the correspondent three pre-molars in both hemi-arches of each dog. A jumping gap of 1mm from the buccal cortical wall to the implant was always left.

INTRODUCTION

There is a consensus that tooth loss implicates a progressive involution of the alveolar bone in an apico-coronal as well as in a bucco-lingual direction.[1-4]

It has been proven that after the extraction of natural teeth, the greatest reduction of the alveolar bone occurs within the first months.[4-8] As a result, a race towards shortening the treatment period previously required with implant therapy was initiated. The immediate implant placement into fresh extraction sites has been demonstrated as an effective therapy not only because it reduces the number of surgical procedures,[9-15] but also because it facilitates the preservation of the morphological contour of the ridges.[10,13] However, some studies in animals have shown a pronounced resorption of the buccal, and to some extent, the lingual bone plates after implant placement in fresh extraction sockets that resulted in a marked reduction of the thin buccal hard tissue height.[16,17]

The surgical procedure of immediate implant placement must fulfill several important conditions, such as non-traumatic tooth extraction and primary stability. While the primary stability could be obtained allocating the implant 3-5 mm apically in the host bone,[12] a non-traumatic extraction is aimed at the preservation of the integrity of the buccal plate, which could provide a self-contained defect around the implant with higher potential of regeneration.[18]

The buccal bone plates of fresh extraction sockets are significantly thinner when compared to the lingual component as observed in animal and human studies.[5,6,17,19,20] This anatomical characteristic has to be considered to avoid pronounced buccal bone remodeling after immediate implant placement.

The surgical technique for tooth extraction and immediate implant placement frequently involves sulcular incisions and mucoperiosteal flap elevation. However, displacement of the periosteum and exposure of the alveolar bone result in an acute inflammatory response and consequently in bone resorption.[21-23] Araújo and Lindhe[5] observed osteoclasts on surgical exposed alveolar bone areas during the first two weeks of wound healing. Besides, Wood et al.,[23] Yaffe et al.[24] and Araújo et al.[6] also described pronounced loss of the buccal bone wall, but not of the thicker lingual wall, in mucoperiosteal surgeries applied in periodontal treatment of dentate areas. These findings suggest that remodeling of the buccal bone following immediate implantation could be due to the anatomical characteristics of the buccal bone and to the mucoperiosteal flap.

The aim of the present pilot study was to evaluate the buccal bone remodeling after immediate implantation with a flap and flapless approach in mongrel dogs.

MATERIALS & METHODS

Surgical Procedure

The study protocol was approved by the Animal Research Committee of the School of Dentistry of Ribeirão Preto- University of São Paulo and was performed in three young adult male mongrel dogs that weighed approximately 20 kg. The animals presented intact maxillas, no general occlusal trauma, and no oral viral or fungal lesions and were in good general health with no systemic involvement as determined by a veterinarian following clinical examination.

Food was withheld the night preceding surgery. The animals were pre-anaesthetized with acepromazine 0.2% - 0.05 mg/kg IM. An intravenous catheter was then placed in the foreleg for induction with thiopental 2.5% - 5 a 8 mg/Kg IV. Animals were thus moved

to the operating room and maintained on gas anesthesia (1–2% isoflurane/O2 to effect). Conventional dental infiltration anesthesia was used at the surgical sites. The animals received a slow constant rate infusion of lactated Ringer's solution (10–20 ml/kg/h IV) to maintain hydration during surgery. These procedures were undertaken under the supervision of a veterinarian.

The surgical procedures for the mandibular premolar extractions were done in each hemi-arch of each dog (Fig. 1). Randomly, one of the sides was treated with the flapless approach (experimental group Fig. 2), while the contralateral side was treated with mucoperiosteal flaps (Fig. 3). The teeth were sectioned in a bucco-lingual direction at the bifurcation so that the roots could be individually extracted using a periotome, without damaging the bony walls. After alveolar debridement, three Ankylos® implants measuring 3.5 x 9.5 mm (diameter and length, respectively) were immediately inserted in the mesial socket of the correspondent three pre-molars in both hemi-arches of each dog, totaling 18 implants in the experiment. The implants were placed at the level of bone crest and a jumping gap of 1 mm from the buccal cortical wall to the implant was always left (Fig. 4) without invading the lingual bone plate with the drill or the implant. Subsequently, healing caps of 1.5 mm in height were adjusted in order to provide a non-submerged healing in both groups (Fig. 5). The flaps of the control group were repositioned and sutured with absorbable sutures (Vicryl, Ethicon, Inc., Johnson & Johnson Company, São José dos Campos-SP, Brazil.), while the soft tissues were accommodated and then sutured in the experimental group. No grafting materials were used in the gaps between the buccal plates and the implants. The animals received painkillers and anti-inflammatory agents. A broad spectrum antibiotic (penicillin and streptomycin 20,000 IU; 1.0 g/10 kg IM) was administered immediately post-surgery and re-dosed after four days. The animals were maintained on a soft diet for 14 days after the sutures were removed. The healing was evaluated weekly and plaque control was maintained by flushing the oral cavity with chlorhexidine gluconate. The remaining teeth were cleaned monthly with ultrasonic points and all implants remained non-submerged during the experimental period.

Sacrifice and Histological Processing

The animals were sedated and then sacrificed with an overdose of thiopental 12 weeks after implant placement. The hemi-mandibles were removed, dissected and fixed in 4% phosphate-buffered formalin pH 7, for 10 days, and transferred to a solution of 70% ethanol until processing. The specimens were dehydrated in increasing concentrations of alcohol up to 100%, infiltrated and embedded in LR White resin (London Resin Company, Berkshire, England), and hard-sectioned in bucco-lingual direction using the technique described by Donath and Breuner.[25] The most central sections were stained with Stevenel's blue and Alizarin red S for histometric analysis using optic microscopy.

Histomorphometric Analysis

Longitudinal buccal-lingual histological sections from each implant were captured through a video camera Leica DC 300F (Leica Microsystems GmbH, Nussloch, Germany) joined to a stereomicroscope Leica MZFL III (Leica Microsystems GmbH, Nussloch, Germany). The images were analyzed through the Image J program (National Institutes of Health, Bethesda,USA). The buccal bone wall

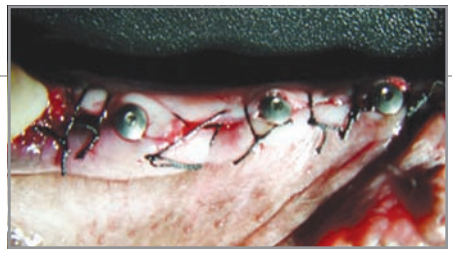

Figure 5: Healing caps of 1.5mm in height were adjusted in order to provide a non-submerged healing.

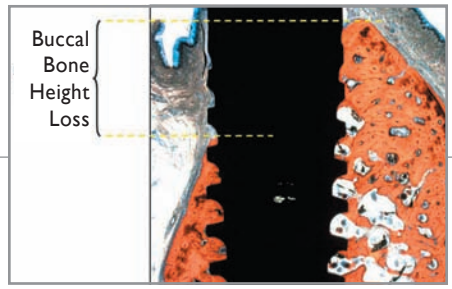

Figure 6: Buccal bone wall resorption represented as a linear measurement.

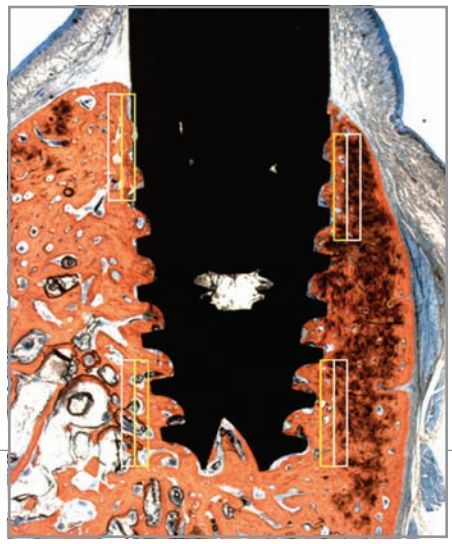

Figure 7: Bone density determined within the rectangles. The yellow rectangles represented the bone density adjacent to the implant surface (BDA), and the white rectangles represented the bone density distant to the implant surface (BDD). This analysis was done coronally and apically.

resorption was determined in relation to the lingual bone wall as a linear measurement (relative measurement, Fig. 6). The buccal and lingual bone plates were also measured from the shoulder of the implant to the first bone-to-implant contact (absolute measurement). The percentages of bone-to-implant contact (BIC) were calculated throughout the implant perimeter, from the first coronal bone-to-implant contact, considering the mineralized bone in direct contact with the implant surface. The bone density was determined within two rectangles, one of them adjacent to the implant surface (BDA), and the other as a mirror image of the first, but distant to the implant surface (BDD, Fig. 7). This analysis was done in two different positions of the implants; one coronal, and the other apical, permitting an intra-group evaluation. The bone density measurements evaluated the percentages of mineralized bone in relation to the percentages of marrow cavities. A single examiner captured the measurements with no knowledge of the experimental groups.

Statistical Analysis

Mean values and standard deviations were calculated. The data were grouped using the dogs as units for analysis. The mean differences between the groups for each histomorphometric parameter were analyzed through the Mann-Whitney nonparametric test with a confidence level of 95%.

RESULTS

Clinical and Histological Observations

Healing was uneventful for all animals and no implant was lost. All implants were osseointegrated after a 12 week postoperative period. The marginal gaps between the buccal walls and the implants disappeared without the migration of connective tissue in both groups.

Histomorphometric Analysis

The loss of buccal bone height, which is a relative measurement that depends on the behavior of the lingual bone plate, was statistically lower in the flapless group when compared to the flap group (0.98 mm x 2.14 mm, Table 1). Additionally, the comparisons of the absolute values of bone loss around the implants for the flapless and flap groups showed statistically significant differences between the buccal bone resorption of the experimental groups, but not between the lingual remaining bone heights (Table 1). The comparisons within the groups showed statistically significant differences between the buccal and lingual bone resorption in the flapless and flap groups (Table 1). The loss of the buccal bone in the flap group was more than 100% greater than the lingual bone.

The buccal bone density was numerically higher in all the parameters evaluated when compared to the lingual bone density. These differences were statistically significant for all of the comparisons tested, except for the flapless coronal buccal bone density (Table 2).

Although the buccal bone density was numerically higher for the flap group compared to the flapless group, these differences were not statistically significant for both coronal and apical parameters (Table 2).

The comparisons between coronal and apical bone density were statistically significant only for the lingual bone for both flapless and flap groups, with the apical bone having a lower density (Table 2).

There were no statistically significant differences between adjacent and distant bone densities for all of the possible comparisons (Table 2).

All of the implants presented considerably good indications of bone-to-implant contact, and the results were remarkably similar between the groups. The buccal BIC in both groups is numerically higher when compared to the lingual BIC results, and statistically significant in the flap group (Table 3).

DISCUSSION

The flapless surgical approach significantly favored the maintenance of the alveolar buccal plate after immediate implant placement, and a reasonable explanation could be the preservation of the periosteal vascular network. In the present pilot study, the only difference between the groups was the flap elevation in the control group and this group exhibited at least twice the buccal bone height loss when compared to the flapless group. Cardaropoli et al.[26] with the same proposition, but using eight beagle dogs, found even better results than the current ones. They recorded a buccal bone loss of 0.6 mm at the test immediate implants treated with the flapless surgery while the bone loss at the control implants was 2.11 mm.

Despite the fact that flapless implant surgery has recently been described as a minimally invasive surgical approach that provides esthetic and comfort,[27,28] the present study focuses on another advantage of this technique in immediate post-extraction implant placement: the non detachment of the periosteum in order to guarantee a source of vascular supply to the buccal and lingual bone plates.

In the '60s, Wilderman et al.[29] has primarily demonstrated that "although the exposure of bone by surgery allows observation, some bone resorption is the penalty for this type of examination," especially when dealing with the buccal bone plate over the tooth root area. In general, the bone surface that is temporarily exposed undergoes a

TABLE 1: LOSS IN BUCCAL AND LINGUAL ALVEOLAR BONE HEIGHT (MM)

	Relative Measurements		Absolute Measurements			
	Flapless	Flap	Flapless		Flap	
	Buccal X Lingual	Buccal X Lingual	Buccal	Lingual	Buccal	Lingual
Mean	0.98 *	2.14 *	2.46 × §	1.48 ×	3.83 ☐ §	1.70 ☐
SD	0.45	0.34	0.42	0.27	0.21	0.31

Legend: * p<0.0001, × p<0.0001, ☐ p<0.0001, § p<0.0001

TABLE 2: BONE DENSITY PERCENTAGES DESCRIBED AS MEAN ± SD

		Buccal		Lingual	
		BDA	BDD	BDA	BDD
Flapless	Coronal	90.37 ± 6.12	91.95 ± 8.84	87.13 ± 7.99 ¨	86.80 ± 7.01 :
	Apical	85.80 ± 13.87 * / ¤	95.52 ± 2.56 * / ×	59.88 ± 13.19 ¤ / ¨	56.82 ± 14.19 × / :
Flap	Coronal	93.42 ± 4.43 #	97.08 ± 2.19 ∞	84.55 ± 4.97 # / »	89.57 ± 5.83 ∞ / o
	Apical	94.39 ± 5.29 &	95.91 ± 2.96 ¥	50.69 ± 9.90 & /»	46.70 ± 9.00 ¥ / o

Legend: * ¨ p=0.0023, : p=0.0006, * p=0.0262, ¤ p=0.0070, × p=0.0006, # p=0.0070, ∞ p= 0.0041, » p=0.0006, o p=0.0006, & p=0.0006, ¥ p=0.0006

necrotic process that finishes with the bone resorption, but a broad bone plate containing marrow spaces could have a reduced bone height loss at the end of the healing period.

According to Nobuto et al.[30] that evaluated the microvascular responses after mucoperiosteal flap surgery in dogs, the elevation of the periosteum may cause circulatory insufficiency and then bone resorption.

It was previously shown that the main function of the periodontal ligament (PDL) blood vessels is to supply nutrients to the PDL as well as to the osteoblasts in the alveolar bone.[31] Thus, after tooth extractions the alveolar bone blood supply coming from the periodontal ligament is eliminated, and consequently, only the vascularization provided by the periosteum remains. However, the elevation of mucoperiosteal flaps also compromises the blood supply from the periosteum. The hypothesis that tooth extraction without the elevation of a mucoperiosteal flap may decrease the post-surgery resorption level was recently evaluated by Fickl et al.[32] The results demonstrated that leaving the periosteum in place decreased the resorption index of the extraction socket. The authors also highlighted a great impact of this finding in thin periodontal biotypes, where the osteoclastic activities of the internal and external sides could merge together and cause a more pronounced buccal bone plate loss. The results of the current study were consistent with these statements, especially for the flap approach group in which the loss of the buccal bone was more than 100% greater than the lingual bone as shown by the absolute measurements of bone loss around the implants. The statistically significant difference between flapless and flap groups, when considering the buccal bone loss, confirmed the importance of periosteum preservation in this kind of implant therapy. Besides, the non-significant differences between the flap and flapless lingual bone plate resorption could indicate that the morphology of the buccal and lingual plates might represent another crucial factor in determining the final bone resorption.

Based on these facts, it could be speculated that the immediate implant therapy was not the only factor influencing the high level of buccal bone height loss of 2.5 mm in relation to the lingual

TABLE 3: BONE TO IMPLANT CONTACT PERCENTAGES DESCRIBED AS MEAN ± SD

	Buccal	Lingual
Flapless	77.39 ± 9.07	70.50 ± 12.17
Flap	77.75 ± 12.58 *	66.00 ± 7.69 *

Legend: p=0.0262 *

bone plate as described by Araujo et al.[16] after flap surgery.

In our histological specimens, the buccal bone crest appeared significantly thinner when compared to the lingual component. This pattern was also observed in different studies.[5,6,17,19,20] Furthermore, the bone densities of buccal and lingual plates were vastly different in both groups. In general, while the buccal plates were constituted by a cortical bone type with sparse and decreased numbers of marrow areas, the lingual bone plates exhibited numerous and large marrow areas. This difference between the buccal and lingual bone densities was statistically significant in the apical portion of test and control groups, and was also statistically significant in the coronal portion of the control group. This last finding could mean that this portion exhibited insufficient bone marrow spaces and source of blood vessels, and consequentially compromised angiogenesis that used to be related to bone loss.[30] There was no statistically significant difference between the bone densities adjacent and distant to the implants in both groups, with the exception of the buccal bone densities of the apical portion of the flapless group. The significantly lower density adjacent to the implant of the buccal bone observed in the intra-group evaluation (85.80% adjacent and 95.52% distant), and also the numerical difference between the groups considering this parameter (85.80% for flapless and 94.39% for flap) could be seen as another advantage of the non-detachment of the periosteum, providing vessels and consequently nutrients to the cortical bone plates.

All of the implants presented good levels of bone-implant contact, and the results were extremely similar between flapless and flap groups. The buccal bone-implant contact was numerically higher in both groups when compared to the lingual BIC which could be related to the higher number of marrow areas found in the lingual bone plate. In summary, the current study supports the existence of a close relationship between angiogenesis and bone resorption/formation,[30] in which the remodeling process is strongly dependent on the interaction between new blood vessels and bone.

Qahash et al.[33] demonstrated a significant association between the width of the buccal alveolar ridge and extent of bone resorption evaluated by incandescent and fluorescent light microscopy. They suggested that the width of the buccal alveolar ridge should be at least 2 mm to maintain the alveolar bone level. These observations have general implications for implant placement with most surgical protocols, and more importantly, for immediate implants. In fact, according to Polimeni et al.[34] and Wikesjö et al.,[35] whom studied the alveolar bone healing potential in peri implant critical-size defects, the thicker lingual bone plate provided a large wound space that was correlated to enhanced bone regeneration, whereas implants placed closer to the buccal plate were associated with increased crestal bone loss.

Blanco et al.[36] whom recently studied the influence of flapless and flap surgeries in bone resorption of immediate post-extraction implants, also found a minor reduction of the buccal bone plate with the flapless approach, emphasizing the importance of the allocation of the implants in the confines of the alveolus, discussing that one reason for the higher buccal bone plate resorption of Araujo et al.[6] study could be due to the use of a 4.1 mm diameter implant in alveoli of 3.5 mm diameter for premolar 3 and of 3.9 mm for premolar 4; which means that the diameter of the implant was greater than the alveoli themselves.

In the present study, the implants were placed 1 mm away from the buccal marginal bone wall without invading the lingual bone plate with the drill or the implant. No residual defect was observed on the histological specimens after 12 weeks of healing and the formation of new bone could be a possible explanation, as well as the bone loss in some extent. This jumping gap distance has already been studied and it was shown that this defect may heal with new bone and a high degree of osseointegration without the use of barrier membranes.[18] It was described that this kind of defect "allowed the formation of a coagulum that, even in the absence of a barrier membrane, was properly protected by the periosteum of the soft tissue flap. In other words, during the healing of a 'self-contained' bone defect and in the presence of a proper periosteum, the use of a barrier membrane may not be required," but this is dependent on the implant surface and time of healing allowed after implant installation.

CONCLUSION

Within the limitations of this pilot study, it can be concluded that the flapless approach for immediate post-extraction implants reduces the buccal bone plate resorption.

REFERENCES

1. Lekovic V, Camargo PM, Klokkevold PR, Weinlaender M, Kenney EB, Dimitrijevic B et al. Preservation of alveolar bone in extraction sockets using bioabsorbable membranes. J Periodontol 1998;69:1044-1049.
2. Lekovic V, Kenney EB, Weinlaender M, Han T, Klokkevold P, Nedic M et al. A bone regenerative approach to alveolar ridge maintenance following tooth extraction. Report of 10 cases. J Periodontol 1997;68:563-570.
3. Pietrokovski J, Massler M. Alveolar ridge resorption following tooth extraction. J Prosthet Dent 1967;17:21-27.
4. Schropp L, Wenzel A, Kostopoulos L, Karring T. Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. Int J Periodontics Restorative Dent 2003;23:313-323.
5. Araujo MG, Lindhe J. Dimensional ridge alterations following tooth extraction. An experimental study in the dog. J Clin Periodontol 2005;32:212-218.
6. Araujo MG, Sukekava F, Wennstrom JL, Lindhe J. Ridge alterations following implant placement in fresh extraction sockets: an experimental study in the dog. J Clin Periodontol 2005;32:645-652.
7. Johnson K. A study of the dimensional changes occurring in the maxilla following tooth extraction. Australian Dental Journal 1969;14:241-244.
8. Carlsson GE, Bergman B, Hedegard B. Changes in contour of the maxillary alveolar process under immediate dentures. A longitudinal clinical and x-ray cephalometric study covering 5 years. Acta Odontol Scand 1967;25:45-75.
9. Knox R, Caudill R, Meffert R. Histologic evaluation of dental endosseous implants placed in surgically created extraction defects. Int J Periodontics Restorative Dent 1991;11:364-375.
10. Lazarra RJ. Immediate implant placement into extraction sites: surgical and restorante advantages. . Int J Periodontics Restorative Dent 1989;9:333-343.
11. Lundgren D, Rylander H, Andersson M, Johansson C, Albrektsson T. Healing-in of root analogue titanium implants placed in extraction sockets. An experimental study in the beagle dog. Clin Oral Implants Res 1992;3:136-143.
12. Nemcovsky CE, Artzi Z, Moses O, Gelernter I. Healing of marginal defects at implants placed in fresh extraction sockets or after 4-6 weeks of healing. A comparative study. Clin Oral Implants Res 2002;13:410-419.
13. Paolantonio M, Dolci M, Scarano A, d'Archivio D, di Placido G, Tumini V et al. Immediate implantation in fresh extraction sockets. A controlled clinical and histological study in man. J Periodontol 2001;72:1560-1571.
14. Rosenquist B, Ahmed M. The immediate replacement of teeth by dental implants using homologous bone membranes to seal the sockets: clinical and radiographic findings. Clin Oral Implants Res 2000;11:572-582.
15. Wilson TG, Jr., Schenk R, Buser D, Cochran D. Implants placed in immediate extraction sites: a report of histologic and histometric analyses of human biopsies. Int J Oral Maxillofac Implants 1998;13:333-341.
16. Araujo MG, Sukekava F, Wennstrom JL, Lindhe J. Tissue modeling following implant placement in fresh extraction sockets. Clin Oral Implants Res 2006;17:615-624.
17. Araujo MG, Wennstrom JL, Lindhe J. Modeling of the buccal and lingual bone walls of fresh extraction sites following implant installation. Clin Oral Implants Res 2006;17:606-614.
18. Botticelli D, Berglundh T, Buser D, Lindhe J. The jumping distance revisited: An experimental study in the dog. Clin Oral Implants Res 2003;14:35-42.
19. Spray JR, Black CG, Morris HF, Ochi S. The influence of bone thickness on facial marginal bone response: stage 1 placement through stage 2 uncovering. Ann Periodontol 2000;5:119-128.
20. Adell R, Lekholm U, Rockler B, Branemark PI, Lindhe J, Eriksson B et al. Marginal tissue reactions at osseointegrated titanium fixtures (I). A 3-year longitudinal prospective study. Int J Oral Maxillofac Surg 1986;15:39-52.
21. Bragger U, Pasquali L, Kornman KS. Remodelling of interdental alveolar bone after periodontal flap procedures assessed by means of computer-assisted densitometric image analysis (CADIA). J Clin Periodontol 1988;15:558-564.
22. Staffileno H, Levy S, Gargiulo A. Histologic study of cellular mobilization and repair following a periosteal retention operation via split thickness mucogingival flap surgery. J Periodontol 1966;37:117-131.
23. Wood DL, Hoag PM, Donnenfeld OW, Rosenberg DL. Alveolar crest reduction following full and partial thickness flaps. J of Periodontology 1972;43:141-144.
24. Yaffe A, Fine N, Binderman I. Regional accelerated phenomenon in the mandible following mucoperiosteal flap surgery. J Periodontol 1994;65:79-83.
25. Donath K, Breuner G. A method for the study of undecalcified bones and teeth with attached soft tissues. The Sage-Schliff (sawing and grinding) technique. J Oral Pathol 1982;11:318-326.
26. Cardaropoli G MF, Osorio R, Toledano M, Pisani Proenca T, Thomsen P, Tarnow D. Healing following tooth extraction and immediate implant installation with flapless surgery. Clin Oral Impl Res 2007;18; 5: XXX.
27. Rocci A, Martignoni M, Gottlow J. Immediate loading in the maxilla using flapless surgery, implants placed in predetermined positions, and prefabricated provisional restorations: a retrospective 3-year clinical study. Clin Implant Dent Relat Res 2003;5 Suppl 1:29-36.
28. Zeren KJ. Minimally invasive extraction and immediate implant placement: the preservation of esthetics. Int J Periodontics Restorative Dent 2006;26:171-181.
29. Wilderman MN, Wentz F, Orban BJ. Histogenesis of repair after mucogingival surgery. J Periodontol 1960;31 283-299.
30. Nobuto T, Suwa F, Kono T, Taguchi Y, Takahashi T, Kanemura N et al. Microvascular response in the periosteum following mucoperiosteal flap surgery in dogs: angiogenesis and bone resorption and formation. J Periodontol 2005;76:1346-1353.
31. Matsuo M, Su C, Saito M, Kishi Y, Takahashi K. Vascularization an unsuccessful case following guided bone regeneration. Jpn J Oral Biol 2000;42:573-579.
32. Fickl S, Zuhr O, Wachtel H, Bolz W, Huerzeler M. Tissue alterations after tooth extraction with and without surgical trauma: a volumetric study in the beagle dog. J Clin Periodontol 2008;35:356-363.
33. Qahash M, Susin C, Polimeni G, Hall J, Wikesjo UM. Bone healing dynamics at buccal peri-implant sites. Clin Oral Implants Res 2008;19:166-172.
34. Polimeni G, Koo KT, Qahash M, Xiropaidis AV, Albandar JM, Wikesjo UM. Prognostic factors for alveolar regeneration: bone formation at teeth and titanium implants. J Clin Periodontol 2004;31:927-932.
35. Wikesjo UM, Susin C, Qahash M, Polimeni G, Leknes KN, Shanaman RH et al. The critical-size supraalveolar peri-implant defect model: characteristics and use. J Clin Periodontol 2006;33:846-854.
36. Blanco J NV, Aracil L, Munoz F, Ramos I. Ridge alterations following immediate implant placement in the dog: flap versus flapless surgery. J Clin Periodontol 2008;35:640-648.

Implant Considerations in the Anticoagulated Patient: A Review

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Abstract: *Oral anticoagulation therapy is one of the most prevalent forms of treatment used in contemporary medicine. It is estimated that more than 50 million Americans adhere to some type of anticoagulation regimen. With an increase in implants and implant related surgical procedures done in the dental office, dentists should be well versed in the management and potential complications that can arise from patients undergoing anticoagulation therapy. The purpose of this article is to review contemporary oral anticoagulation therapy and offer literature-based recommendations on the perioperative management of these patients in the practice of implant dentistry.*

Key words: *dental implants, pharmacologic protocol, oral medicine, implant complications*

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BACKGROUND

Oral anticoagulation therapy is one of the most prevalent forms of treatment used in contemporary medicine. It is estimated that more than 50 million Americans adhere to a low dose daily aspirin protocol and other anticoagulants such as warfarin sodium (Coumadin®, Bristol-Myers Squibb, 345 Park Ave., New York, NY 10154) and Clopidogrel bisulfate (Plavix®, Bristol-Myers Squibb), which routinely rank among the top 50 medications prescribed in the United States.[1,2] As the make up of the American population ages with the majority of "baby boomers" now reaching retirement age, trends of increased oral anticoagulant use are expected to continue. Perioperative management of these patients for dental procedures has been a controversial issue for quite some time with debates regarding the risk of uncontrolled bleeding, if medication is continued, versus the possibility of thromboembolic complications if the medication is discontinued. Since the late '50s, a menagerie of different recommendations has been issued with protocols that often contradict one

another. With implant surgery becoming the standard of care to replace the missing tooth, dental practices are increasing their exposure to surgical ramification. With the increasing age of the population, it is inevitable that the dentist will be faced with treating the anticoagulated patient within the implant setting. Dentists should be well versed in the management of the anticoagulated patient and the potential complications. The purpose of this article is to review contemporary oral anticoagulation therapy and offer literature-based recommendations on the perioperative management of these patients in the practice of implant dentistry.

ANTICOAGULATION RATIONALE

Improved understanding of cardiovascular physiology and advances in the management and treatment of cardiovascular disease have rendered oral anticoagulation therapy a mainstay of modern medicine. Reduction in the occurrence of thromboembolism is often the goal of oral anticoagulation therapy for patients with a history of various conditions including, but not limited to; angina, atherosclerosis, atrial fibrillation, cerebrovascular occlusion, coronary stents, deep vein thrombosis, ischemic heart disease, myocardial infarction, prosthetic heart valves, and pulmonary embolism.[3-6]

In order to understand the manner in which oral anticoagulants treat these conditions, a basic understanding of hemostasis is necessary (Fig. 1). Briefly, hemostasis is a three part mechanism consisting of vascular spasm, platelet plug formation, and coagulation.[7] Traumatic blood vessel injury induces protective vasoconstriction via neural reflexes and myogenic spasm.[8] As the vessels contract, resulting in a narrowed lumen diameter, newly exposed collagen fibers activate nearby platelets causing them to morph their shape, express multiple pseudopodia, and release stored granules. Platelet secretion of adenosine disphosphate and prostaglandins leads to further platelet recruitment and eventual formation of a platelet plug that occludes the narrowed vessel lumen. The final coagulation cascade is initiated by exposed subendothelial collagen and extravasated thromboplastin which respectively activate the intrinsic and extrinsic coagulation pathways. The ensuing interaction of multiple coagulation factors ultimately triggers activation of the common coagulation cascade. Subsequent interactions of Factor X and Factor V form a prothrombin activator complex that promotes cleavage of prothrombin to thrombin. Thrombin interacts with fibrinogen to form fibrin monomers that ultimately crosslink and occlude the narrowed vessel lumen with entrapped vascular components such as platelets, blood cells, and plasma.

Lab Evaluation of the Anticoagulated Patient

Bleeding problems can be screened by various lab tests which include the platelet count, bleeding time, prothrombin time, partial thromboplastin time, and International Normalized Ratio (INR).

The platelet count provides a quantitative evaluation of platelet function. A normal platelet count should be 100,000-400,000 cells/mm3. A platelet count of less than 100,000 cells/mm3 is called thrombocytopenia and is often associated with major postoperative bleeding. The average lifespan of a platelet ranges from 7 to 12 days.

The bleeding time provides an assessment of adequacy of platelet count and function. The test measures how long it takes a standardized skin incision to stop bleeding by the formation of a temporary hemostatic plug. The normal range of bleeding time depends on the way the test is performed, but is usually between 1-6 minutes. The bleeding time is prolonged in patients with platelet abnormalities, or taking medications which affect platelet function. This test assesses platelet function.

The prothrombin time (PT) measures the effectiveness of the extrinsic pathway to mediate fibrin clot formation. It is performed by measuring the time it takes to form a clot when calcium and tissue factor are added to plasma. A normal prothrombin time indicates normal levels of Factor VII and those factors common to both the intrinsic and extrinsic pathways (V, X, prothrombin, and fibrinogen). A normal prothrombin time is usually between 10-15 seconds. Prothrombin time is most often used by physicians to monitor oral anticoagulant therapy such as warfarin.

The partial thromboplastin time (PTT) measures the effectiveness of the intrinsic pathway to mediate fibrin clot formation. It tests for all factors with the exception of Factor VII. The test is performed by measuring the time it takes to form a clot after the addition of kaolin, a surface activating factor, and cephalin, a substitute for platelet factor, to the patient's plasma. A normal partial thromboplastin time is usually 25-35 seconds. Partial thromboplastin time is most often used by physicians to monitor heparin therapy.

The INR was designed for patients on chronic anticoagulant therapy. It allows comparisons from one hospital to another. A patient with normal coagulation parameters has an INR of 1.0. The therapeutic range for a patient on anticoagulant therapy is between 2.0-3.5.

ANTICOAGULATION MEDICATIONS

In the United States, contemporary anticoagulation therapy (Table 1) commonly utilizes one, or a combination of the following medications:

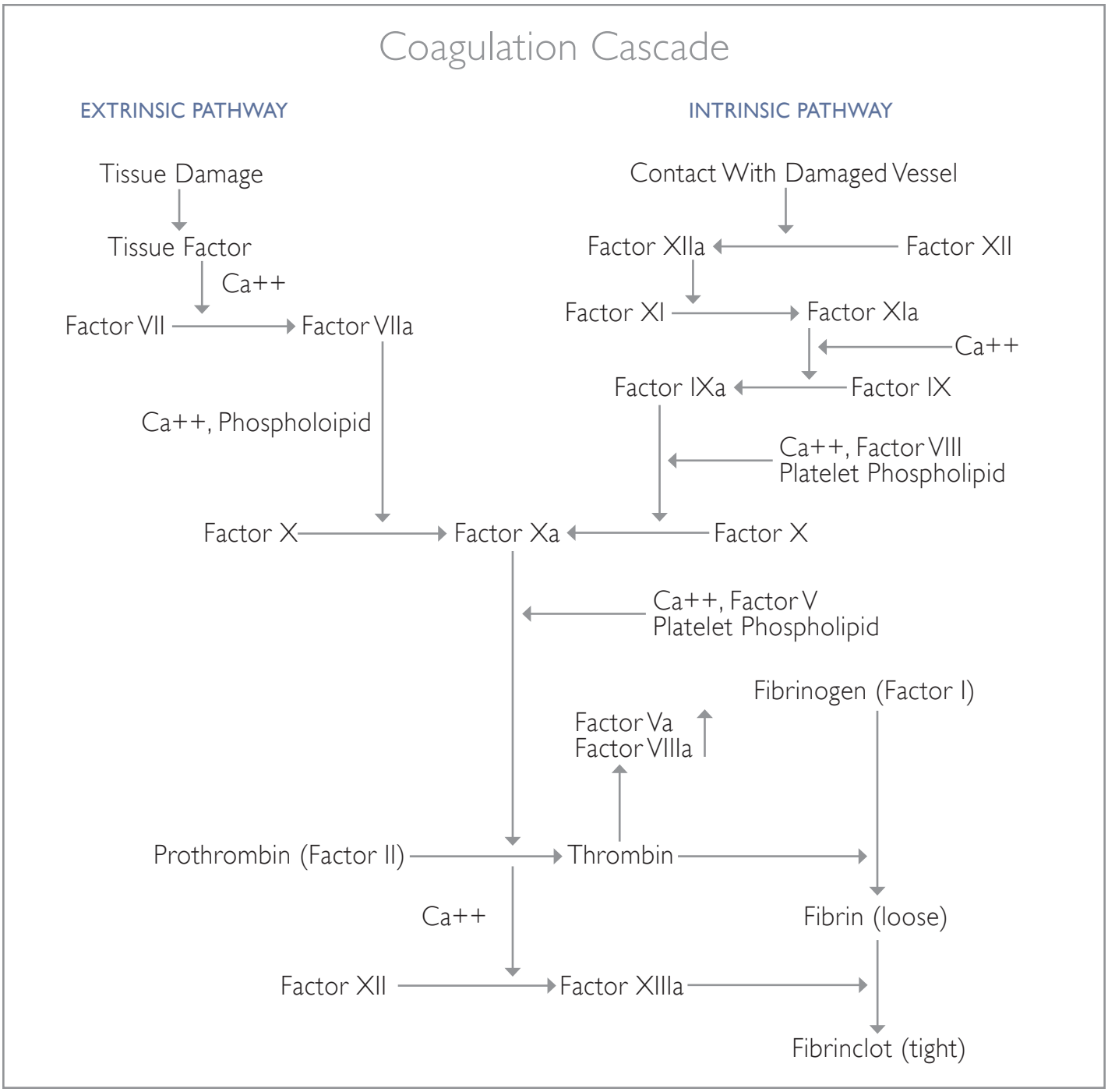


Figure 1: Intrinsic and extrinsic pathways of the coagulation cascade.

Acetylsalicylic Acid (Aspirin)

Aspirin is the most widely utilized oral anticoagulant with use by more than one third of the United States population. Among patients with known cardiovascular disease, the prevalence of aspirin-based oral anticoagulant therapy exceeds 80%.[1] When used as an oral anticoagulant, aspirin is typically prescribed in an 81mg or 325mg once-daily dosing. Aspirin affects platelets through the inhibition of cyclooxygenase 1 (COX-1). With inhibition of COX-1, platelet production of thromboxane A2 (TXA2), a potent vasoconstrictor, platelet activator and platelet aggregator, is impaired. Aspirin's effects on platelet function are irreversible and span the 8-10 day lifecycle of the platelet. Due to platelet turnover, approximately 10% of platelets with normal COX-1 activity are recovered daily following cessation of low dose aspirin

therapy.[9] As such, it may take up to 10 days to fully recover COX-1 activity, although full COX-1 activity may not be required for adequate hemostasis.

Clopidogrel Sulfate

Clopidogrel sulfate (Plavix®, Bristol-Myers Squibb/Sanofi Aventis, 345 Park Ave., New York, NY 10154) is an oral anticoagulant used in the prevention of atherosclerotic events for patients with medical histories similar to those treated with low dose daily aspirin therapy.[10] In fact, clopidogrel sulfate is often prescribed as a dual therapy with aspirin as the combination has proven more effective than aspirin alone in the treatment of certain cardiovascular conditions.[11] Clopidogrel sulfate prevents platelet aggregation by selectively inhibiting the binding of adenosine diphosphate to platelet receptors.[12] Like aspirin, the effects of clopidogrel sulfate on platelet function are irreversible and last for the life span of the platelet. Platelet aggregation and bleeding time typically return to baseline levels five days after the clopidogrel sulfate cessation.

Warfarin Sodium (Coumadin®, Bristol-Myers Squibb, 345 Park Ave., New York, NY 10154)

Warfarin sodium acts by inhibiting vitamin-K dependent clotting factors II, VII, IX, X and the anticoagulant proteins C and S.8 warfarin sodium half-life is approximately 36 hours and the duration of action for a single dose may last anywhere from 2 to 5 days.[13] As such, this medication is often utilized for long term anticoagulation therapy on patients with a history of the following: atrial fibrillation, cardiac valve replacement, cerebrovascular accident, coronary stent, deep venous thrombosis, myocardial infarction, and pulmonary embolism.[14] Patients treated with warfarin sodium are generally considered higher risk, both in terms of overall health and risk for bleeding, than patients taking oral antiplatelet therapy.[15]

Heparin

Heparin is a short acting, highly sulfated glycosaminoglycan that is naturally produced by basophils and mast cells.[16] Heparin complexes with antithrombin III to facilitate the removal of circulating thrombin and ultimately leads to reduced fibrin formation. Unlike other anticoagulants, heparin is traditionally administered by continuous intravenous infusion for short-term inpatient use due to a narrow therapeutic window.[7] Newly developed low molecular weight heparin, however, is reported to have improved pharmacokinetics that may allow the drug to be utilized on an outpatient basis.[17]

PERIOPERATIVE MANAGEMENT

The decision to interrupt oral anticoagulation therapy prior to implant procedures is multifactorial. In the perioperative management of these patients, factors to consider include the overall health of the patient, the type of oral anticoagulant therapy utilized, anticipated blood loss associated with the planned procedure, and a contingency plan for excessive or uncontrolled bleeding.

Patients receiving oral anticoagulation therapy can have widely disparate medical histories. Consider the following pair of patients: a 40-year-old male with mild hypertension and early atherosclerosis versus a 60-year-old male with a history of myocardial infarction and coronary stent placement. Certainly, the ASA classifications

TABLE 1: COMMON ANTICOAGULATION MEDICATIONS

Medication	Type	Action	Comment
Acetylsalicylic Acid (Aspirin)	Oral antiplatelet anticoagulant	Inhibition of cyclooxygenase-1 (COX-1)	Irreversibly affects platelets
Clopidogrel sulfate (Plavix®)	Oral antiplatelet anticoagulant	Inhibits binding of adenosine diphosphate to platelet receptors	Irreversibly affects platelets
Warfarin sodium (Coumadin®)	Oral coagulation cascade anticoagulant	Inhibition of vitamin-K dependent clotting factors II, VII, IX, X and anticoagulant proteins C and S	Half-life ~36 hours. Single dose duration of action may last 2-5 days depending on the patient
Heparin	Intravenous coagulation cascade anticoagulant	Complexes with antithrombin III to reduce circulating thrombin and reduce fibrin formation	Half-life ~1 hour. Typically given intravenously in an inpatient treatment setting

of these patients differ markedly as should the decisions on how to treat and manage them. When treating these patients, it is important to keep an eye on the "big picture" by considering the patient's overall health, and not solely focusing on their dental needs.[18] As such, it is important to consult with the patient's medical treatment provider regarding any questions about his or her medical status.

While the underlying goal of oral anticoagulation therapy is universal, the mechanisms of action by which medications achieve this goal vary. As such, it is important for practitioners to distinguish between antiplatelet anticoagulants and anticoagulants that interfere with the coagulation cascade.[12] Studies examining the hemorrhagic effects of antiplatelet anticoagulants on dental procedures have found negligible increases in intraoperative and postoperative bleeding.[19-21] Likewise, similar studies evaluating coagulation cascade anticoagulants have generally found no increased risk of intraoperative or postoperative bleeding that could not be controlled with local measures when International Normal Ratio (INR) values were within therapeutic levels.[22-25]

INR measures the extrinsic pathway of coagulation and commonly ranges between 0.8–1.2 in healthy adults. Therapeutic INR values differ for various cardiovascular conditions, but typically range between 2.0-3.0.[26] For mechanical cardiac valves, higher INR values up to 4.0 are recommended.[27] INR values are not typically verified preoperatively for patients treated with antiplatelet anticoagulants. For patients treated with coagulation cascade anticoagulants, however, numerous authors obtain preoperative INR values on the day of surgery.[28-30] Depending on the extent and complexity of the planned dental procedure, INR values of 3.0 or less are typically recommended for patients treated with coagulation cascade anticoagulants.[31] If a tranexamic acid rinse protocol is utilized, patients with INR values up to 4.5 have been safely treated without complication.[31-34]

When making decisions on the management of anticoagulated patients, anticipated blood loss from the planned procedure must be considered. Expectant blood loss from a restorative procedure such as a dental amalgam will be considerably different from that of a surgical procedure such as a connective tissue graft or impacted third molar extraction.

TABLE 2: LOCAL HEMOSTATIC AIDS

Product or Action	Composition	Action
Positive Pressure	N/A	Manual occlusive aid to clot formation
Vasoconstrictor	1:100,000 Epinephrine	Activation of adrenergic receptors
Gelfoam®	Porcine derived gelatin sponge	Occlusive matrix; activation of intrinsic pathway
Surgicel®	Plant derived -cellulose	Occlusive matrix; activation of intrinsic pathway; antibacterial properties
CollaCote®, CollaPlug® CollaTape®, UltraFoam™ UltraWrap™	Bovine derived collagen	Occlusive matrix; activation of intrinsic pathway
HemCon®	Crustacean derived chitosan	Positively charged chitosan attracts negatively charged red blood cells; antibacterial properties
4.8% Tranexamic Acid Mouth Rinse	Tranexamic acid	Binds to lysine receptor sites on plasmin and plasminogen inhibiting fibrin binding and fibrinolysis
Topical Thrombin	Bovine derived thrombin	Enhances conversion of fibrinogen to fibrin
Electrocautery	N/A	High frequency electric current cauterizes tissue and induces blood coagulation

Studies evaluating blood loss from restorative procedures have reported minimal hemorrhagic complications, while those evaluating surgical operations such as flap-osseous procedures have found up to 592 ml of blood loss from a single surgical site.[35,36] Blood loss from surgical procedures is also influenced by the experience level of the provider. Surgeries performed by less experienced providers have been shown to take up to three times longer and may result in nearly twice as much blood loss as those performed by more experienced practitioners.[36] In general, however, most studies have found that blood loss from dental procedures is under 200 ml and even less when the duration of the procedure does not exceed two hours.[36-39] When you consider that a pint of blood, the amount generally taken during blood donation, is 473 ml, the amount of blood lost during dental procedures is well within the limits of safety.

HEMORRHAGE MANAGEMENT

This risk of moderate to severe bleeding induced by dental procedures is less than 1% for the average patient.[40] While this risk increases with the anticoagulated patient, nearly all scenarios of excessive bleeding can be adequately managed with relatively simple local measures (Table 2) such as:

Positive Pressure

Positive pressure to intraoral wounds is typically accomplished by compressing moistened gauze on the site of hemorrhaging (i.e. bone or socket) or compression of the flap itself. Suturing wound margins is another method in which compressive force may be applied to bleeding areas.[7] Positive pressure aids hemostasis by promoting occlusion of the site of injury and providing mechanical aid to clot formation.[41] Minor hemorrhaging is often controlled with positive pressure alone and may not require further intervention.

Oxidized Regenerated Cellulose

Oxidized regenerated cellulose based products such as Surgicel® (Ethicon, Johnson & Johnson, One Johnson & Johnson Plaza, New Brunswick, NJ 08933) are derived from plant based alpha-cellulose and function hemostatically in a manner similar to absorbable gelatin sponges.[44] A unique property this product has is relatively low pH. The low pH has an antibacterial effect. A broad range of gram negative, gram positive, and antibiotic-resistant bacteria have proven to be locally susceptible to oxidized regenerated cellulose.[45] When used for oral applications, this product typically resorbs within 7-14 days.

Vasoconstrictor

Dental anesthetics contain vasoconstrictor primarily to increase their duration of action and minimize the risk of local anesthetic toxicity.[42] Epinephrine, the most commonly utilized vasoconstrictor in dental local anesthetics, is a catecholamine that facilitates vasoconstriction, which when injected into the site in question will help slow the bleeding and aid in coagulation.

Absorbable Collagen Products

Absorbable collagen products such as CollaPlug®, CollaTape®, and CollaCote® (Integra Life Sciences Corp, 311 Enterprise Dr., Plainsboro, NJ 08536) are derived from bovine deep flexor tendons and typically resorb completely within 14 days. Additional bovine-derived products such as Avitene® (Traatek, Inc., 3848 SW 30th Ave., Fort Lauderdale, FL 33312) have similar properties. These products aid in coagulation by either acting as a simple occlusive matrix, or promote hemostasis by their collagen content which activates the intrinsic coagulation cascade.

Absorbable Gelatin Sponge

Gelfoam® (Pfizer Inc., 235 E. 42nd St., New York, NY 10017) is a resorbable gelatin sponge of porcine origin that is capable of absorbing up to 45 times its weight in whole blood.[43] Absorbable collagen sponges aid in hemostasis by providing a simple occlusive matrix and additionally through contact activation of the intrinsic pathway.[44] The gelatin sponge is usually resorbed within 2-5 days.

Chitosan Derived Products

Chitosan-derived products such as HemCon® (HemCon Medical Technologies, Inc., 10575 SW Cascade Ave., Ste. 130, Portland, OR 97223) are extremely effective at promoting hemostasis and have recently been used by United States military medical personnel for treatment of battlefield injuries. Chitosan is a naturally occurring polysaccharide that is commercially produced via the deacetylation of crustacean chitin. Positively charged chitosan molecules readily attract negatively charged red blood cells and the two form an extremely strong seal that acts as a primary occlusive barrier for hemorrhagic sites. With hemorrhaging limited and/or stopped by this initial seal, the natural coagulation cascade ensues. Like oxidized regenerated cellulose, chitosan-derived products have locally active antibacterial properties.[46] Chitosan-derived products also have antibacterial properties which it achieves via active cell wall disruption.[47]

Tranexamic Acid

Tranexamic acid is an anticoagulant oral rinse that binds to lysine receptor sites on plasmin and plasminogen, and which results in inhibiting fibrin binding and fibrinolysis.[48] Rinsing with tranexamic acid solution results in therapeutic levels (>100mg/ml) within the saliva for 2-3 hours. Wounds healing in the presence

of tranexamic acid have demonstrated increased tensile strength, thus making the clot more resistant to mechanical disruption.[50] Tranexamic acid is supplied as a 4.8% solution and patients may be instructed to rinse with 10 ml, four times daily for seven days following surgery.[49]

Topical Thrombin

Topical thrombin facilitates clot stabilization by enhancing the conversion of fibrinogen to fibrin for the initial platelet plug. Medical grade topical thrombin is often bovine derived and is typically supplied as a freeze dried sterile powder that must be reconstituted with sterile saline. For general use in dental applications, a topical thrombin solution of 100 IU/ml is delivered via pump/syringe spray or combined with a carrier such as a hemostatic gelatin sponge.

Electrocautery

Electrocautery involves the application of a high-frequency electric current to cauterize tissue and induce blood coagulation, and is useful in severe hemorrhaging scenarios.

CONCLUSION

Although most patients experience no problems when their oral anticoagulation therapy is interrupted for dental procedures, complications ranging from nonfatal cerebral emboli to death can occur. Typically, these complications are associated with prolonged discontinuation of anticoagulants that interfere with the coagulation cascade such as warfarin sodium. While there are currently no reports of fatal complications associated with the discontinuation of oral antiplatelet medications prior to dental treatment, such action places these patients at risk of developing thromboembolic complications.[12,15]

If patients are instructed to continue their oral anticoagulation therapy prior to dental treatment in an effort to avoid thromboembolic complications, do they pose a risk for unmanageable hemorrhaging? According to dental literature, the answer is a resounding "no." Multiple studies have demonstrated that most dental procedures can be safely performed without interrupting oral antiplatelet therapy.[12,19-21] Likewise, a number of studies have demonstrated that patients taking coagulation cascade anticoagulants can be safely treated as long as INR values are within therapeutic ranges.[29-34] In nearly all patients involved with these studies, hemorrhaging was easily controlled with local measures.

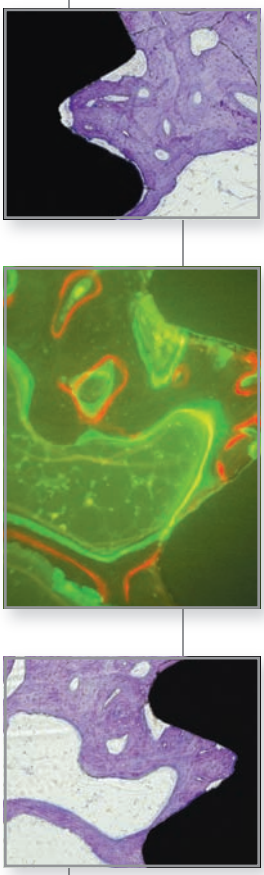
DISCLAIMER

Although specific, commercially-available products are mentioned in this article, this does not constitute an endorsement of the United States Government, Department of Defense, or Navy Medicine. The opinions and assertions contained in this article are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of the Navy, Department of Defense, or the United States Government.

REFERENCES

1. Ajani U, Ford E, Greenland K, Giles W, Mokdad A. Aspirin use among U.S. adults: Behavioral Risk Factor Surveillance System. Am J Prev Med 2006; 30(1):74-7.
2. Top 50 Drugs Prescribed 2007. Humana Inc. Publication 2007: 1-2.
3. Fuster V, Pumphrey C, McGoon M, Chesebro J, Pluth J, McGoon D. Systemic thromboembolism in mitral and aortic Starr-Edwards prostheses: a 10-19 year follow-up. Circulation 1982; 66:157-61.
4. Hirsch J, Dalen J. Introduction (with recommendations of the American College of Chest Physicians). Chest 1992; 102: 303s-4s.
5. Campbell C, Smyth S, Montalescot G, Steinhubl S. Aspirin dose for the prevention of cardiovascular disease: a systematic review. J Am Med Assoc 2007; 297(18): 2018-24.
6. Smith S, Feldman T, Hirshfeld J, Jacobs A, Kern M, King S, et al. ACC/AHA/SCAI 2005 guideline update for percutaneous coronary intervention: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. (ACC/AHA/SCAI Writing Committee to Update 2001 Guidelines for Percutaneous Coronary Intervention). Circulation 2006; 113(7):166-286.
7. Purcell C. Dental management of the anticoagulated patient. N Z Dent J 1997; 93(413): 87-92.
8. Ball J. Management of the anticoagulated dental patient. Compend Contin Educ Dent 1996; 17(11): 1100-2.
9. Burch J, Stanford N, Majerus P. Inhibition of platelet prostaglandin synthetase by oral aspirin. J Clin Invest 1978; 61(2): 314-9.
10. Gerschutz G, Bhatt D. The CURE trial: using clopidogrel in acute coronary syndromes without ST-segment elevation. Cleve Clin J Med 2002; 69(5): 377-8.
11. Depta J, Bhatt D. Aspirin and platelet adenosine diphosphate receptor antagonists in acute coronary syndromes and percutaneous coronary intervention: role in therapy and strategies to overcome resistance. Am J Cardiovasc Drugs 2008; 8(2): 91-112.
12. Grines C, Bonow R, Casey D, Gardner T, Lockhart P, Moliterno D; American Heart Association; American College of Cardiology; Society for Cardiovascular Angiography and Interventions; American College of Surgeons; American Dental Association; American College of Physicians. Prevention of premature discontinuation of dual antiplatelet therapy in patients with coronary artery stents: a science advisory from the American Heart Association, American College of Cardiology, Society for Cardiovascular Angiography and Interventions, American College of Surgeons, and American Dental Association, with representation from the American College of Physicians. J Am Dent Assoc 2007; 138(5): 652-5.
13. Anticoagulant: Coumadin® Tablets (Warfarin Sodium Tablets, USP) Crystalline. Prescription Insert, Bristol-Myers Squibb Company Publication 2007: 1-6.
14. Scully C, Wolff A. Oral surgery in patients on anticoagulant therapy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002; 94(1): 57-64.
15. Jafri S, Zarowitz B, Goldstein S, Lesch M. The role of antiplatelet therapy in acute coronary syndromes and for secondary prevention following a myocardial infarction.Prog Cardiovasc Dis 1993; 36(1): 75-83.
16. Cox M, Nelson D. Lehninger Principles of Biochemistry 4th Edition. Freeman 2004: 1100.
17. Hull R. Treatment of pulmonary embolism: The use of low-molecular-weight heparin in the inpatient and outpatient settings. Thromb Haemost 2008; 99(3): 502-10.
18. Bridbord J. Another view on the anticoagulated patient. J Oral Maxillofac Surg 2002; 60(3): 342.
19. Ardekian L, Gaspar R, Peled M, Brener B, Laufer D. Does low-dose aspirin therapy complicate

oral surgical procedures? J Am Dent Assoc 2000; 131(3): 331-5.
20. Madan G, Madan S, Madan G, Madan A. Minor oral surgery without stopping daily low-dose aspirin therapy: a study of 51 patients. J Oral Maxillofac Surg 2005; 63(9): 1262-5.
21. Partridge C, Campbell J, Alvarado F. The effect of platelet-altering medications on bleeding from minor oral surgery procedures. J Oral Maxillofac Surg 2008; 66(1): 93-7.
22. Ward B, Smith M. Dentoalveolar procedures for the anticoagulated patient: literature recommendations versus current practice. J Oral Maxillofac Surg 2007; 65(8): 1454-60.
23. Alexander R, Ferretti A, Sorensen JR. Stop the nonsense not the anticoagulants: a matter of life and death. NY State Dent J 2002; 68(9): 24-6.
24. Cannon P, Dharmar V. Minor oral surgical procedures in patients on oral anticoagulants--a controlled study. Aust Dent J 2003; 48(2): 115-8.
25. Evans I, Sayers M, Gibbons A, Price G, Snooks H, Sugar A. Can warfarin be continued during dental extraction? Results of a randomized controlled trial. Br J Oral Maxillofac Surg 2002; 40(3): 248-52.
26. Steinberg M, Moores J. Use of INR to assess degree of anticoagulation in patients who have dental procedures. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995; 80(2): 175-7.
27. Hirsh J, Fuster V, Ansell J, Halperin J. American Heart Association/American College of Cardiology Foundation. American Heart Association/American College of Cardiology Foundation guide to warfarin therapy. J Am Coll Cardiol 2003; 41(9): 1633-52.
28. Blinder D, Manor Y, Martinowitz U, Taicher S. Dental extractions in patients maintained on oral anticoagulant therapy: comparison of INR value with occurrence of postoperative bleeding. Int J Oral Maxillofac Surg 2001; 30(6): 518-21.
29. Devani P, Lavery K, Howell C. Dental extractions in patients on warfarin: is alteration of anticoagulant regime necessary? Br J Oral Maxillofac Surg 1998; 36(2): 107-11.
30. Dodson T. Strategies for managing anticoagulated patients requiring dental extractions: an exercise in evidence-based clinical practice. J Mass Dent Soc 2002; 50(4): 44-50.
31. Borea G, Montebugnoli L, Capuzzi P, Magelli C. Tranexamic acid as a mouthwash in anticoagulant-treated patients undergoing oral surgery. An alternative method to discontinuing anticoagulant therapy. Oral Surg Oral Med Oral Pathol 1993; 75(1): 29-31.
32. Ramström G, Sindet-Pedersen S, Hall G, Blombäck M, Alander U. Prevention of postsurgical bleeding in oral surgery using tranexamic acid without dose modification of oral anticoagulants. J Oral Maxillofac Surg 1993; 51(11): 1211-6.
33. Carter G, Goss A, Lloyd J, Tocchetti R. Tranexamic acid mouthwash versus autologous fibrin glue in patients taking warfarin undergoing dental extractions: a randomized prospective clinical study. J Oral Maxillofac Surg 2003; 61(12): 1432-5.
34. Sindet-Pedersen S, Ramström G, Bernvil S, Blombäck M. Hemostatic effect of tranexamic acid mouthwash in anticoagulant-treated patients undergoing oral surgery. N Engl J Med 1989; 20(13): 840-3.
35. Rooney T. General dentistry during continuous anticoagulation therapy. Oral Surg Oral Med Oral Pathol 1983; 56(3): 252-5.
36. Baab D, Ammons W, Selipsky H. Blood loss during periodontal flap surgery. J Periodontol 1977; 48(11): 693-8.
37. McIvor J, Wengraf A. Blood-loss in periodontal surgery. Dent Pract Dent Rec 1966; 16(12): 448-51.
38. Hecht A, App A. Blood volume lost during gingivectomy using two different anesthetic techniques. J Periodontol 1974; 45(1): 9-12.
39. Berdon J. Blood loss during gingival surgery. J Periodontol 1965; 36: 102-7.
40. Curtis J, McLain J, Hutchinson R. The incidence and severity of complications and pain following periodontal surgery. J Periodontol 1985; 56(10): 597-601.
41. Meehan S, Schmidt M, Mitchell P. The international normalized ratio as a measure of anticoagulation: Significance for the management of the dental outpatient. Spec Care Dentist 1997; 17(3): 94-6.
42. Malamed S. Handbook of Local Anesthesia 5th Edition. Mosby 2004: 416.
43. Council on Pharmacy and Chemistry: Absorbable gelatin sponge – new and nonofficial remedies. JAMA 1947; 135: 921.
44. Ongkasuwan J. Hemostatic agents. Baylor College of Medicine Grand Rounds Archive 2005; 10: 1-9.
45. Spangler D, Rothenburger S, Nguyen K, Jampani H, Weiss S, Bhende S. In vitro antimicrobial activity of oxidized regenerated cellulose against antibiotic-resistant microorganisms. Surg Infect 2003; 4(3): 255-62.
46. Muzzarelli R, Tarsi R, Filippini O, Giovanetti E, Biagini G, Varaldo P. Antimicrobial properties of N-carboxybutyl chitosan. Antimicrob Agents Chemother. 1990; 34(10): 2019-23.
47. Andres Y, Giraud L, Gerente C, Le Cloirec P. Antibacterial effects of chitosan powder: mechanisms of action. Environ Technol 2007; 28(12): 1357-63.
48. Gaspar R, Brenner B, Ardekian L, Peled M, Laufer D. Use of tranexamic acid mouthwash to prevent postoperative bleeding in oral surgery patients on oral anticoagulant medication. Quintessence Int 1997; 28(6): 375-9.
49. Bandrowsky T, Vorono A, Borris T, Marcantoni H. Amoxicillin-related postextraction bleeding in an anticoagulated patient with tranexamic acid rinses. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996; 82(6): 610-2.
50. Björlin G, Nilsson I. The effect of antifibrinolytic agents on wound healing. Int J Oral Maxillofac Surg 1988; 17(4): 275-6.



Comparing Light and Fluorescence Microscopic Data: A Pilot Study of Titanium and Magnesium Oxide Implant Integration in Rabbit Bone

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Background: *Implant integration in pre-clinical experimental studies often involves 3D in vivo biomechanical techniques. The advantage with such tests is that the results are obtained quickly. However, the interface is ruptured due to implant loosening, and bone-to-implant integration cannot be quantified. Histomorphometrical 2D techniques are time consuming but necessary. In order to gain more insight into tissue reactions to implants, a combination of various techniques is warranted.*

The aim of this study was to quantify bone tissue on nondecalcified (i) routinely stained and (ii) fluorochrome labelled sections to determine if there is a resemblance between various methods. The aim was also (iii) to compare the bone integration to titanium implants with control native and test magnesium oxidized surfaces, and whether or not our 2D histomorphometrical data would support our earlier 3D biomechanical data.

Materials and Methods: *Ten implants each were inserted in rabbit tibiae. Oxytetracycline, alizarin complexone and calcein green, respectively, were administered. The follow-up was six weeks. Quantifications were performed semi-computerized in the light microscope, and manually on images of the fluorescent sections.*

Results: *The Mg implants revealed a significantly greater bony contact percentage compared to the controls. No significant differences in bone area or newly formed bone were obtained between the implants. Tetracycline could not be observed on any sections, but alizarin and calcein were visualized. The bone activity was greater around the Mg implants compared to controls, as deduced from quantifications of fluorescence occurrence. Combining and comparing the amount of fluorochrome occurrence to the amount of newly formed bone in the threads demonstrated a subjective correlation between the methods.*

Conclusion: *The present pilot study demonstrated better bone integration of implants with Mg incorporation in the oxide compared to native titanium oxides. These results support our earlier biomechanical data with greater integration of similar Mg implants. Moreover, by adding in vivo florescence labelling, the amount of newly formed bone can be quantified and this provides additional information when compared to newly formed bone measured on histologically stained sections.*

Key words: *commercially pure titanium implants, bone, fluorescent labelling, nondecalcified, histomorphometry*

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INTRODUCTION

Pre-clinical in vivo animal tests of tissue formation around biomaterials intended for medical devices in bone tissue often involve quantitative data concerning, for example, the amount of bone in close vicinity to the implant surface. This is considered a measure of the tissue acceptance of the implant.

A large number of such studies have been carried out at our laboratories over the years, resulting in 15 PhD theses between 1991-2007.[1-6]

The techniques for quantifying bone tissue reactions to biomaterials vary among different laboratories, and there is no consensus concerning how and what to quantify. Each laboratory seems to have its own set of standard methods which, naturally, may be relevant for the purpose of the study. However, since several of the methods that are used are both time consuming and expensive, it would be of interest to apply and perform additional/complementary analysis on the very same samples. Fluorochromes are intra-vital dye bone markers, i.e. they contain calcium-seeking molecules that bind to the mineralization front in bone tissue formation sites and show specific fluorescence when illuminated. Such substances have been available for a long time and have recently attained new popularity, possibly in conjunction with new technologies such as laser scanners.[7, 8, 9] One of the most common fluorochromes is Tetracycline, which is used clinically as an antibioticum. Fluorochromes present specific fluorescence colours, and by using various sequential markers, visualization of different colours in the bone tissue is possible using appropriate excitation and emission filters in the microscope. Several published studies involving fluorescence markers are qualitative and demonstrate colourful images revealing "newly formed bone".[10] In a quantitative confocal laser scanning study, distances between the dyes were reported to reflect the formation and remodelling rate of bone.[11] In the present study, we have analyzed in vivo fluorescent labelling with oxytetracycline, alizarin complexone, and calcein green[12] for bone tissue quantification around turned (control) and oxidized (test) commercially pure titanium implants, the former with a native oxide film and the latter with magnesium ions incorporated in the oxide.

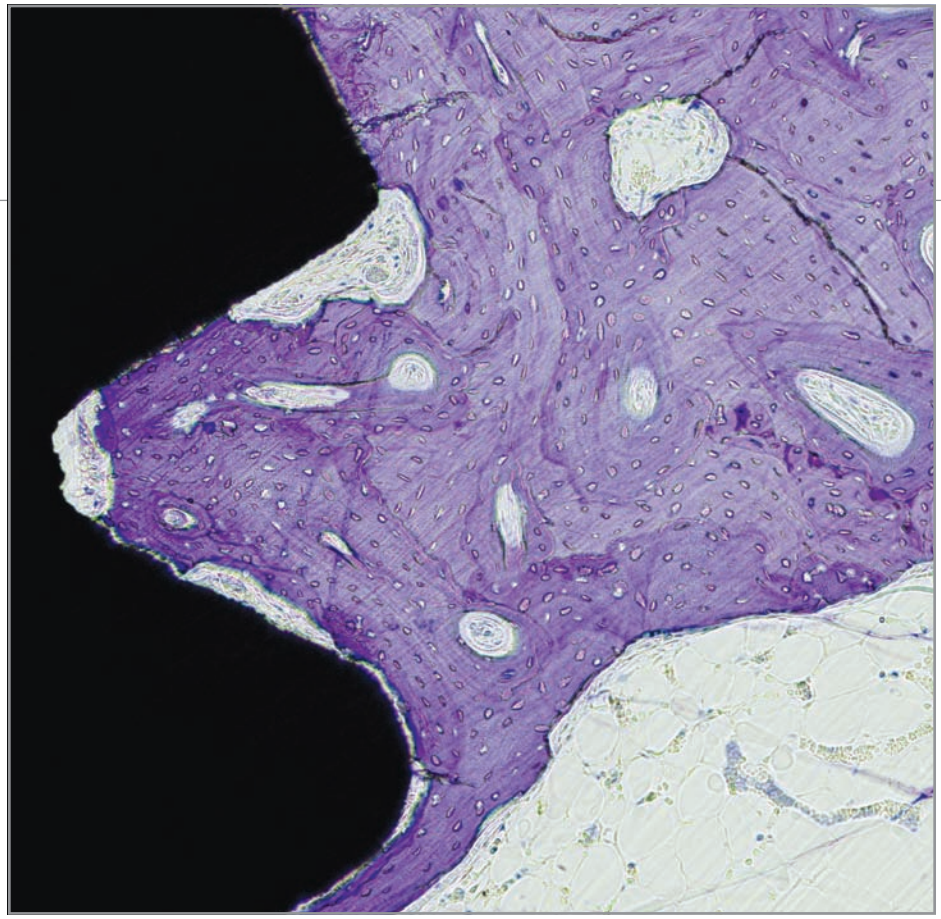


Figure 1: Toluidine blue stained cut and ground control implant section. The distance between the thread peaks is 600 um.

The aim of this study was to quantify bone tissue on nondecalcified (i) routinely stained and (ii) fluorochrome labelled sections in an effort to discover a resemblance between various methods. The aim was also to (iii) compare the

bone integration to titanium implants with control native and test magnesium oxidized surfaces. It was considered of interest to find out if the 2D histomorphometrical data would support our earlier 3D biomechanical data.[13]

MATERIALS AND METHODS

Animals and surgical technique

Ten adult male New Zealand white rabbits were used in this study, which was approved by the National Ethics Committee for Animal Experimentation, Stockholm, Sweden. The rabbits were kept in separate cages and fed ad libitum with standard laboratory diet.

Anaesthetics included intramuscular injections of fentanyl and fluanizone (Hypnorm Vet, Janssen, Saunderton, England) at a dose of 0.5 ml per kg body weight and intraperitoneal injections of diazepam (Stesolid, Kabi Pharmacia, Helsingborg, Sweden) at a dose of 2.5 mg per animal. Local anaesthesia comprised of 1.0 ml 5% Xylocaine (AstraZeneca, Södertälje, Sweden) was injected into the surgical area. The skin was shaved and washed with a mixture of 70% ethanol and 2% iodine solution prior to surgery. Analgesia was given post surgically at a dose of 0.5 ml Temgesic (0.3 mg/ml, Reckitt and Colemann, Hull, England) subcutaneously.

The skin and the fascia layers were opened and closed in separate layers. The periosteum was gently pulled away and not resutured. Each animal had a total of six different types of implants randomly inserted, with three in each tuburositas tibia region, resulting in one proximal, one mid, and one distal insertion site. Of these six various implants, two types, i.e. 10 control and 10 test implants, were selected for this study, while the remaining four implant types will be reported elsewhere. Both groups included n=3 proximal-, n=4 mid- and n=3 distal samples. However, in this study we will not divide the various insertion sites but rather treat them as 10 versus 10 observations.

The animals were sacrificed with an intravenous overdose of Pentobarbital 100 mg/ml (Apoteksbolaget, Malmö, Sweden).

Implants

Both the control and the test implants were made of commercially pure titanium (c.p. Ti). The macro-design of the implants was thread-shaped (0.6 mm between the thread peaks) with an outer diameter of 3.75 mm and a total length of 7 mm. The test implants were further anodized, resulting in a magnesium-incorporated titanium oxide thickness of 3.40 µm. The control turned implants had an oxide thickness of 0.02 µm. For further information regarding the surface properties of the test and control implants see Sul et al. 2005.[13]

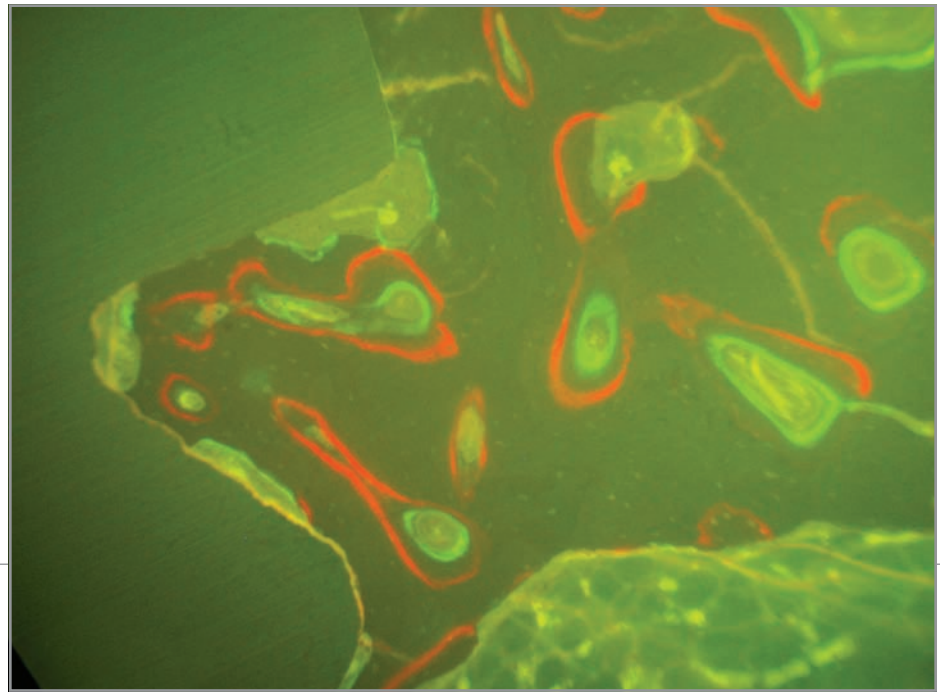


Figure 2: The corresponding fluorochrome labelled bone surrounding a control implant (red = alizarin and green = calcein). The distance between the thread peaks is 600 um.

Fluorochrome Labelling

During the follow-up time, three different fluorochromes were injected

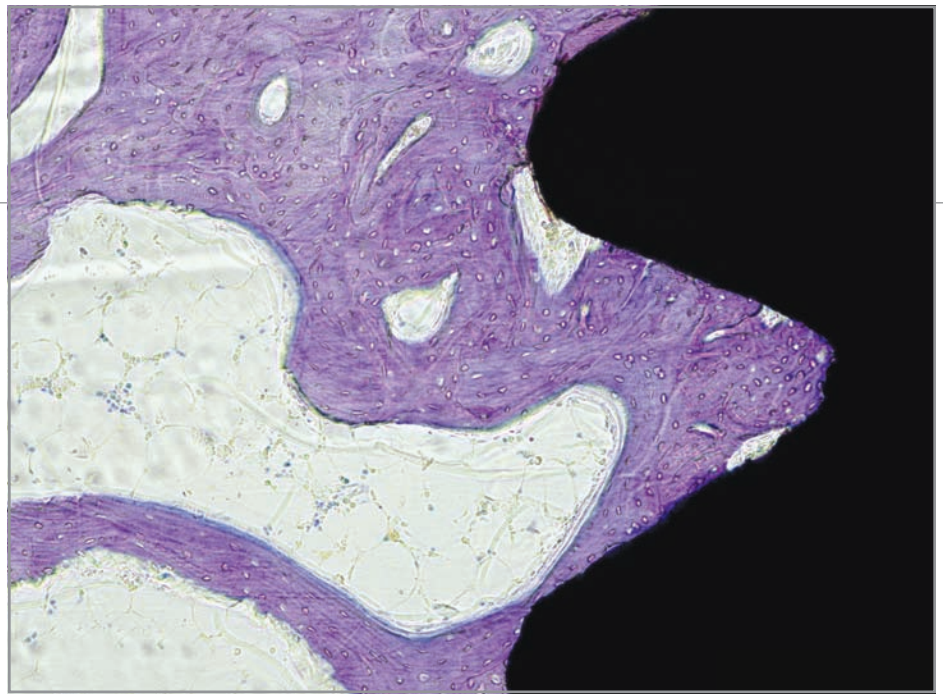


Figure 3: A Toluidine blue stained cut and ground test control section. The distance between the thread peaks is 600 um.

subcutaneously. The labelling regime was according to the recommendations of Rahn[14] and was as follows: (i) the first label of 25 mg oxytetracycline/kg body weight (Sigma-Aldrich, St. Louis, Missouri, USA) was administered on day 14 after implant insertion, (ii) the second label of 30 mg alizarin complexone/kg body weight (Sigma-Aldrich, St. Louis, Missouri, USA) was administered on day 24, and (iii) the third label of 15 mg calcein green/kg body weight (Sigma-Aldrich, St. Louis, Missouri, USA) was administered on day 38, i.e. four days prior to termination of the study. The animals were sacrificed six weeks after insertion. Remnants of these solutions were kept in a freezer for about six months for later analysis of the fluorescent colour appearance using various filters. After thawing, each solution was diluted 10, 100 and 1,000 times with PBS (phosphate buffered saline, pH 7.3) and administered separately on lens-cleaning tissue followed by observations in the fluorescence microscope (below) with an objective of x40.

Sample Preparation

Each implant with surrounding tissue was retrieved en bloc and immediately immersed in 4 % neutral buffered formaldehyde (pH 7.1) fixative. Nondecalcified cut and ground sections were prepared using the so-called Exakt technique (Exakt Apparatebau, Norderstedt, Germany) initially described by Donath and Breuner in 1982, and Donath in 1988.[15,16] In brief, this technique involves one week of fixation, dehydration in ethanol (from 70% to 100%), pre-infiltration in diluted resins, infiltration in pure resin and finally embedding in light-curing resin (Technovit 7200 VLC, Kulzer & Co., Germany). After polymerisation, the samples were divided in the long axis of the implant. The surface was ground parallel and plexi glass was glued onto the sample surface, followed by sectioning and grinding using the Exakt cutting and grinding equipment. The initial sections of 200 µm were ground to about 25 µm. [17] Two ground sections were prepared from each sample. One section was stained with toluidine blue mixed with pyronin G and the other section remained unstained. In this study, the stained sections were used for both fluorescence and routine histomorphometry. Toluidine blue staining differentiates between young and old bone; the former is stained dark purple and the latter is stained pale purple. The soft tissue including the cell nucleus is stained blue.

Analysis

Histomorphometrical analyses were performed in all threads on both sides of the implant and involved measurements of bone-to-implant contact, total bone area, new bone area, and fluorochrome occurrence. The histologically stained sections were investigated both qualitatively and

quantitatively in a Metallux 3 light microscope coupled to a Leitz Microvid computer based unit (Leitz, Wetzlar, Germany) with x10 (NA 0.30), x16 (NA 0.45), and x40 (NA 0.70) objectives and a x10 eyepiece. Quantifications were performed by the same person using a x10 objective and a zoom, enabling the entire area of interest to be visualized in the eyepiece of the microscope.[1]

For detection of fluorescent dyes, the same sections were observed in a fluorescence microscope, Axioskop (Zeiss, Oberkochen, Germany), with x16 (NA 0.35) and x40 (NA 0.75) objectives and a x10 eyepiece. Fluorescence microscopy images were captured with a digital camera (Canon Powershot G3, Canon Inc., Tokyo, Japan) coupled to the microscope and visualized with the software program ZoomBrowser EX (ver. 4.0.2.120). A grid, composed of 18x18 squares, was superimposed on the images. In every square, the presence of the different fluorochromes was counted manually. The results were presented in percentages.

The percentages of new bone obtained by measurement on routinely stained sections were compared to the percentages of fluorochrome occurrence, which are related to newly formed bone on the very same sections.

Statistical analysis

The SPSS (11.5, SPSS Inc., Chicago, Illinois, USA) statistical package was used for the statistical analysis. The Mann-Whitney U-test was used for statistical comparisons of the 10 versus 10 implants. Differences were considered statistically significant at $p < 0.05$. The quantitative results in the various figures and graphs are given as mean values with the standard error of the mean (SEM). No statistical comparisons were conducted between the implants located in the three various regions due to the small number of samples.

RESULTS

Qualitative light microscopy analysis

Irrespective of test or control implant, the survey inspections of the histologically-stained nondecalcified cut and ground sections revealed variations in the bone tissue structures around the implants, depending on the insertion sites, i.e. proximal, mid, or distal regions of the tuburositas tibia. The proximal samples, inserted closest to the knee joint, demonstrated a more spongeous type of bone. This was especially noted on the anterior side of the implants. Moreover, it appeared that the proximal samples were surrounded by bone tissue around almost the entire implant. Further down in the tuburositas tibia region, i.e. around the mid- and distally-located implants, less spongeous bone tissue could be observed. The

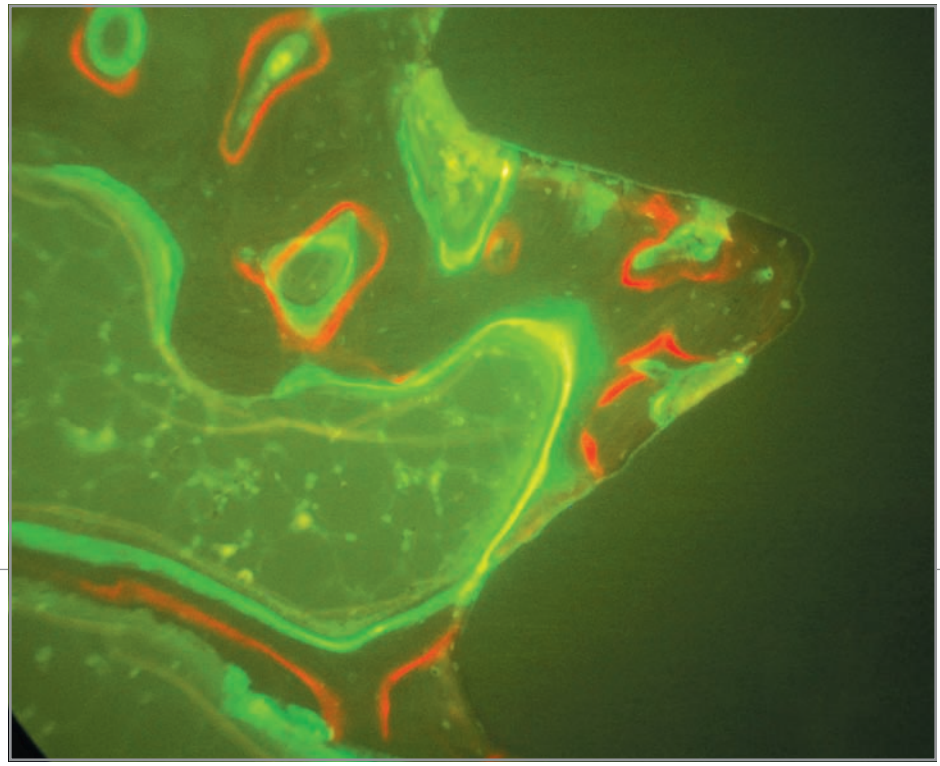


Figure 4: The corresponding fluorochrome labelled bone surrounding a test implant (red = alizarin and green = calcein). The distance between the thread peaks is 600 um.

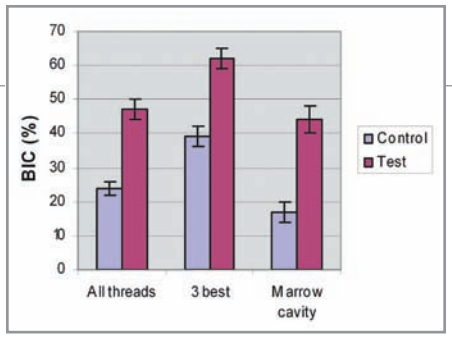


Figure 5: Bone-to-implant contact: Mean percentage of the bone-to-implant contact (BIC), within all threads, the three best consecutive threads in the cortical region and the threads in the marrow cavity. Error bars represent Standard Error of the Mean (SEM). Control = c.p. Ti (n=10). Test = Mg implants (n=10).
A p-value of $p < 0.001$ could be revealed for each comparison.

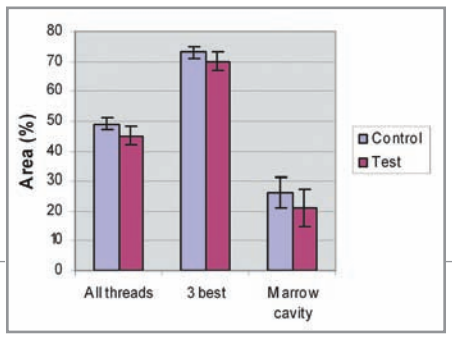


Figure 6: Bone Area: Mean percentage of total bone area, within all threads, the three best consecutive threads in the cortical region and the threads in the marrow cavity in the control c.p. Ti group and in the test Mg group.
Error bars represent Standard Error of the Mean (SEM). There was no statistically significant difference between the two groups.

sections from the most distally-inserted screws revealed the thickest cortical bone and, in general, the corticalization was greater on the posterior side of the mid- and distally-located implants.

The light-microscopic investigations demonstrated new bone tissue formation above and below the cortical region, i.e. in the periosteal and endosteal regions. The periosteal up-growth appeared as a spongeous type of bone tissue with the presence of an immature woven bone quality, easily recognized in higher magnification with the aid of polarising filters. The trabeculaes were often darker stained than the old cortical bone and contained large vacuoles. The endosteal bone tissue appeared more mature than that observed in the periosteal region. Higher magnification also revealed new bone tissue formation in close vicinity to the implants, i.e. in the interface region, as well as at some distance away from the implant surface. The histological staining used in this study results in bone tissue stained in various shades of purple, and soft tissue stained blue. Moreover, a clear differentiation between dark stained younger bone tissue compared to pale stained elderly bone tissue could be noted. This was clearly observed in the thread area. Cement lines could be revealed separating newly formed bone tissue from old cortical bone. Osteons were observed both close to the implants and at some distance away from the implants. Ongoing bone tissue remodelling was also noted, with cavities containing bone formation and bone resorption sites in the same regions. The bone formation cavities revealed the presence of osteoid seams covered with ostoblasts. The resorption cavities often showed osteoclasts, both darker and lighter stained. Irrespective of sample surface, both macrophages and some multinucleated giant cells could be seen. The latter were both light stained and elongated, as well as dark stained and less elongated (figure not shown).

Some regions around both implant types demonstrated an intimate bone contact, i.e. osseointegration could be seen, and as judged by the naked eye, there seemed to be more bone tissue in "direct" contact with the test Mg-treated implants compared to the untreated control screws. In the test Mg group the intimate bone contact was particularly observed in the marrow cavity region, with a very thin rim of bone tissue in close proximity to the test implant surfaces.

Qualitative Fluorescence Microscopy Analysis

Lens Cleaning Tissue

Analysis of the fluorochromes on lens cleaning tissue with fluorescence microscopy demonstrated calcein green in extremely low concentrations as bright green when exposed to blue light. Oxytetracycline and alizarin complexone required higher concentrations than calcein green to be detected. Oxytetracycline fluoresced yellow light when it was illuminated with blue light, and alizarin complexone fluoresced red light when it was illuminated with green light. The alizarin complexone sample demonstrated very weak yellow fluorescence when exposed to blue light.

Cut and Ground Sections

Irrespective of whether or not the sections were stained, oxytetracycline could not be detected in any sections. Both alizarin and calcein could be clearly observed. Figures 1–4 illustrate toluidine blue stained and fluorochrome labelled control and test samples.

The qualitative investigations of

the sections in the fluorescence microscope revealed that there was continuous bone activity in the vicinity of the implants during the healing period, since large amounts of alizarin and calcein were detected in this region. An intense labelling of the fluorochromes revealed irregular patterns in the proximity of the implants and in the periosteal and endosteal regions. Bone formation, bone resorption, and bone remodelling cavities were observed around both the control and the test implants. In the control group, the alizarin staining (2nd label) was seen most extensively, while the opposite, i.e. more calcein (3rd label) than alizarin, could be observed around the test Mg-implants. The occurrence of fluorochromes could be observed close to both implants, although this phenomenon seemed to be more commonly noted in close vicinity to the test implants. The latter observation revealed intensely-stained calcein rims in the test implant interfaces, especially in the threads located in the marrow cavity region.

Quantitative Analysis

Bone-to-Implant Contact

The bone-to-implant contact (BIC) measurements revealed a significant difference between the control implants with a native oxide film compared to the test Mg-implants (Fig. 5). The mean value of BIC in all threads was 24% ± 6 (19-39) for the control implants and 47% ±9 (36-67) for the test implants ($p < 0.001$). In the three best consecutive threads in the cortical region the mean value of BIC was 39% ±8 (24-52) for the control implants and 62% ±10 (40-75) for the test implants ($p < 0.001$). Corresponding measurements from the threads in the marrow cavity were 17% ± 9 (5-37) and 44% ±13 (20-62) for the control and the test implants, respectively ($p < 0.001$).

Bone Area

There were no significant differences between the two groups with respect to the bone area, although the numbers were a bit higher for the control group (Fig. 6). The mean values for the total bone area in the control group and the test group were 49% ± 6 (38-56) and 45% ±10 (31-68) in all threads, 73% ± 6 (61-80) and 70% ±10 (53-81) in the three best consecutive inner threads in the cortical region, 71% ±8 (55-83) and 73% ±13 (50-88) in their corresponding mirror images (not shown in Fig. 6), and 26% ±17 (9-51) and 21% ±20 (4-68) in the threads in the marrow cavity, respectively.

New Bone Area

The comparisons of newly formed bone area measurements performed on toluidine blue sections revealed no significant differences between the two groups. The respective mean values for the newly formed bone area in the control group and the test group were 40% ±7 (25-47) and 36% ± 9 (28-57) in all threads, 58% ±8 (40-68) and 53% ± 6 (45-64) in the three best consecutive inner threads in the cortical region and 44% ±9 (32-56) and 41% ±8 (26-55) in their outfolded mirror images (not shown in Fig. 7). The mean values for the newly formed bone area in the threads in the marrow cavity were 24% ±14 (9-42) and 20% ±18 (4-59) for the control and test implants, respectively (Fig. 7).

Fluorochrome Occurrence

The fluorescent labelled bone sites surrounding the control implants revealed a tendency toward more alizarin

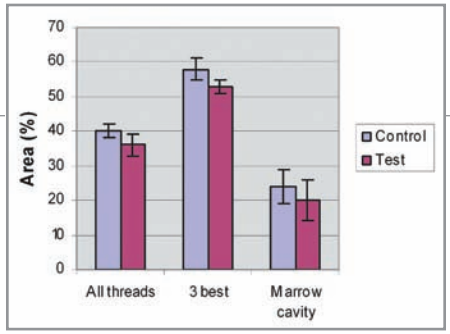
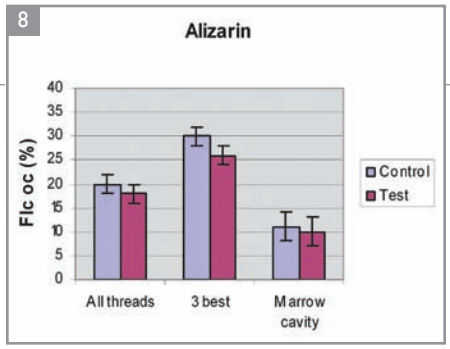
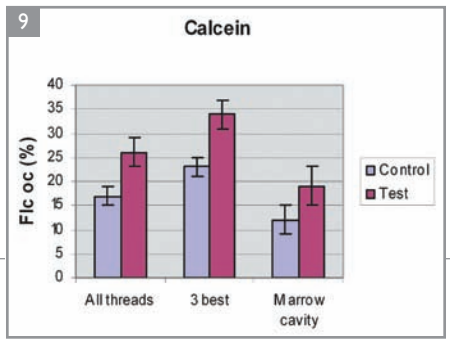
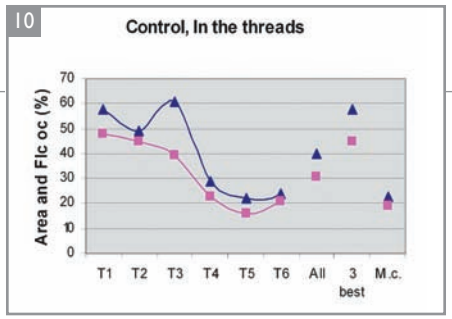


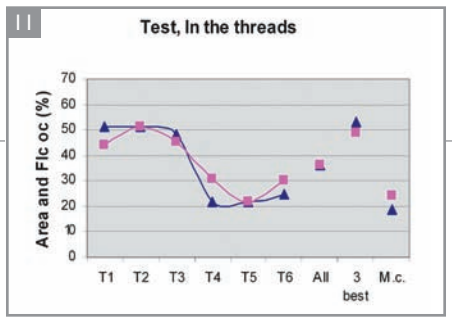
Figure 7: Newly formed bone area (toluidine blue staining). Mean percentage of newly formed bone area within all threads, the three best consecutive threads in the cortical region and the threads in the marrow cavity in the control c.p. Ti group and in test Mg group. Error bars represent Standard Error of the Mean (SEM).

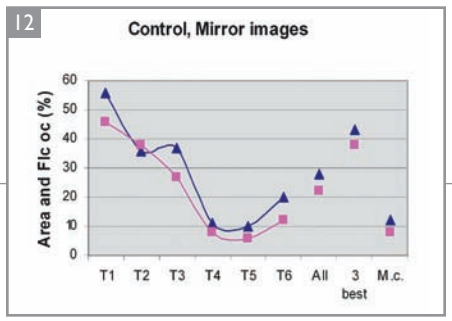


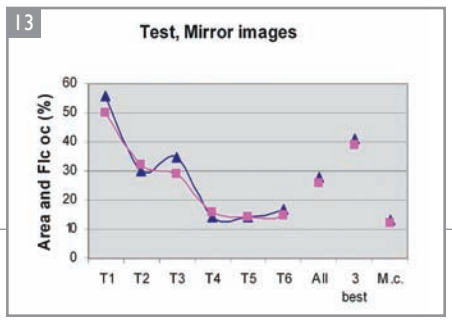


Figures 8 and 9: Fluorochrome occurrence (Flc oc). Alizarin (Fig. 8) and Calcein (Fig 9.) within all threads, the three best consecutive threads in the cortical region and the threads in the marrow cavity in the control c.p. Ti group and in test Mg group. Error bars represent Standard Error of the Mean (SEM).









Figures 10, 11, 12 and 13: Area and Flc oc: Comparison of the two methods, i.e. newly formed bone area (triangle) measured on toluidine blue stained section and fluorochrome occurrences (square) (Area and Flc oc). Control (Fig. 10) and Test (Fig. 11): mean values of measurements performed within each thread (T1- T6), all threads, three best consecutive threads in the cortical region and the threads in the marrow cavity (M.c.). T1 is the most upper thread and T6 the most apical thread located in the marrow cavity. Control (Fig. 12) and Test (Fig. 13): mean values of measurements performed in the mirror images. Note the different scales on the axis.

compared to the tissue surrounding the test implants. However, the bone labelling around test Mg-implants showed significantly more calcein compared to the control implant sites when all threads and the three best consecutive threads in the cortical region were measured ($p < 0.05$). The mean values for the occurrence of alizarin in the control group and the test group were 20% ±7 (8-31) and 18% ±6 (12-33) in all threads, 30% ±9 (15-45) and 26% ±6 (19-37) in the three best consecutive threads, and 11% ±9 (1-25) and 10% ±10 (1-33) in the threads in the marrow cavity, respectively (Fig. 8). The mean values for the occurrence of calcein in the control group and the test group were 17% ±6 (10-25) and 26% ±8 (10-37) in all threads, 23% ±7 (16-38) and 34% ±8 (19-44) in the three best consecutive threads, and 12% ±8 (2-25) and 19% ±12 (1-38) in the threads in the marrow cavity, respectively (Fig. 9). All implants revealed a similar trend, i.e. the occurrence of fluorochrome was greater within the threads than in the mirror images. In the non-threaded upper regions of the implants there was greater calcein activity than alizarin activity in both groups (data not shown), and most often there were more fluorochromes on the posterior side of the implants.

Comparison Between New Bone Area (Toluidine Blue Staining) and Fluorochrome Occurrence

A "subjective correlation", i.e. a relation, could be observed between the new bone area measurements and the fluorochrome occurrence measurements (Figs. 10-13). The distribution of new bone area and of fluorochrome occurrence followed the same trend within the threads and in the mirror images.

DISCUSSION

The present study demonstrated differences in bone-to-implant contact between a native titanium oxide implant surface and magnesium incorporated oxide surfaces where the latter were significantly better integrated. These 2D histomorphometrical data are in agreement with earlier 3D biomechanical tests performed with similar types of implants as in the present study.[13] There may be several reasons for the obtained results, and differences in surface morphology are of great importance.[18, 19]

The three fluorochromes used in the present study, i.e. oxytetracycline, alizarin complexone, and calcein green, were injected on days 14, 24, and 38. This order of fluorochrome administration followed the recommendation of Rahn and is currently routinely used by several laboratories.[12] It is highly important to use a well-proven labelling regime as different labelling sequences can produce different histomorphometrical results. For example, when tetracycline is given as the final label it is difficult to recognize and separate tetracycline from the background, as compared to when tetracycline is given as the first label, followed by other labels.[20]

Fluorochrome occurrences in connection with bone formation have often been reported qualitatively, and in several quantitative studies the analyzed bone tissue response is not related to implants.[21-24]

Our subjective qualitative observations of more bone and a greater fluorescence occurrence/activity were confirmed in our quantitative data. The region of interest for observation of bone tissue formation in the present study focused on the inner threads and on the area in close vicinity to the implant surface. These areas were

involved in the initial surgical trauma. However, the bone tissue in a rabbit is not fully remodelled after six weeks, and thus straight lines of fluorescence bands cannot be observed as is the case, for example, in a fluorescent-labelled cortical plate. This was one reason for not measuring bone tissue growth over time, and instead focusing on comparisons of new bone and the occurrence of fluorocromes (newly formed bone) in similar regions. However, when comparing our figures to the ones in the paper by Wheinlaender et al.,[25] our bone labelling was more differentiated and revealed sharper and more colourful lines. There may naturally be several reasons for such qualitative differences, and some may be related to the different animals (dogs versus rabbits), follow-up times (12 versus 6 weeks), and section quality/thickness (up to 100 versus 25 um).

In this study, oxytetracycline could not be observed in the sections when either UV or blue light was used to excite the fluorochrome. This was also confirmed by using another microscope (Donath K, personal communication), revealing that no oxytetracycline was visible in the sections. One explanation for this may be the phenomenon called label escape error. This type of error occurs when the interval between two fluorochrome injections, the marker interval (MI), is too short in relation to the formation period (FP). The formation period is known as the length of the bone formation phase of the remodelling cycle.[26] The result is double labelling of the bone. The smaller the MI/FP ratio, the greater the amount of double-labelled bone. If the marker interval is longer than the formation period, no double labels will be found.[27] The FP in the bone is different in different regions of the bone, and therefore some bone would be single-marked with oxytetracycline. However, in this study no oxytetracycline was observed at all. Another theory is that the remodelling rate was so rapid that the bone marked with oxytetracycline was resorbed and then remodelled when the other fluorochromes were injected. The remodelling rate at the beginning of the healing period may be rapid close to the implant, and that would support this theory. At some distance from the implant the remodelling rate is slower and oxytetracycline should therefore be observed in the outer regions. However, this was not the case in the present study. Another reason could be the slight bleaching of the sections before the histological staining. This could have weakened the labelling. However, observations on unbleached sections did not reveal any oxytetracycline. All quantifications were done on sections with similar treatment. Yet another theory is that something was wrong with the chemical used in this study or that something went wrong during the administration of the fluorochrome. However, the chemical was pure and fresh, and samples from another study with a new batch of oxytetracycline and a similar fluorochrome labelling regime did not show oxytetracycline (CB Johansson unpublished data). The subcutaneous administration was performed in a similar way as with the other fluorochrome used, indicating a successful injection technique. Unfortunately, the exact reason for not observing tetracycline is therefore unknown to us. In the study by Weinlaender et al. thicker sections (up to 100 um) were used for subjective quantifications of the amount of newly formed bone, labelled with oxytetracycline, alizarin-complexone and calcein, around screws and cylinder type implants inserted in dogs.[25] They reported, "diffusely yellow (oxytetracycline) labeled" tissue both in the periosteal and endosteal regions. The reason for such diffuse labelling was not speculated on, but it may have to do with bone turnover. There was no significant difference reported between amounts of alizarin and calcein outside machined c.p. ti implants, however, the amount of calcein was significantly higher than the amount of alizarin outside the plasma sprayed rougher implants. The amount of alizarin was similar outside of both implants, while the amount of calcein was greater around the rougher implants. These results are in good agreement with our data in the present study. The reason for these observations can either be that there was more activity at the end of the healing period than in the middle of the period or that there was high bone activity during the whole healing period. A constantly high bone activity would result in the alizarin-marked bone already being remodelled when calcein was injected, and therefore more calcein than alizarin would be observed on the specimens.

The new bone area (toluidine blue stained) and the manual fluorochrome occurrence measurements followed the same trend. Both of these measurements depict newly formed bone, but in various ways. For the new bone area measurements, the colour of the stained bone revealed whether the bone was old or new, whereas in the fluorochrome occurrence measurements, the amount of bound fluorochromes revealed the amount of newly formed bone. The fluorochrome method may be preferable since it was seldom difficult to distinguish whether or not the fluorochromes were present. This method also gives information about approximately when new bone really is formed (albeit

not quantified in the present report). In the new bone area measurements it was sometimes difficult to distinguish between new and old bone since the bone was not only stained very pale (= old bone) or very dark purple (= new bone), but all grades of purple were observed.

CONCLUSION

The present pilot study demonstrated significantly better bone integration of implants with Mg incorporation in the oxide as compared to a turned native titanium oxide surface. These 2D results support our earlier biomechanical 3D data with greater integration of similar Mg implants. Additional information about existing new bone can be achieved by adding in vivo fluorochrome dyes that reveal the location of newly formed bone in the sample. In order to gain more knowledge about bone quality, formation, and activity around implants inserted in compromised bone beds, for example such comparisons performed with various techniques will be of great interest.

ACKNOWLEDGEMENTS

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REFERENCES

1. Johansson CB. On Tissue Reaction to Metal Implants. PhD Thesis.University of Göteborg. Department of Biomaterials Science/Handicap Research, Göteborg, Sweden 1991.
2. Sennerby L. On the Bone Tissue Response to Titanium Implants. PhD Thesis.University of Göteborg. Department of Biomaterials Science/Handicap Research, Göteborg, Sweden 1991.
3. Wennerberg A. On Surface Roughness and Implant Incorporation. PhD Thesis.University of Göteborg. Department of Biomaterials Science/Handicap Research, Göteborg, Sweden 1996.
4. Sul Y-T. On the Bone Response to Oxidised Titanium Implants: The role of microporous structure and chemical composition of the surface oxide in enhanced osseointegration. PhD Thesis.University of Göteborg. Department of Biomaterials Science/Handicap Research, Göteborg, Sweden 2002.
5. Franke Stenport V. On Growth Factors and Titanium Implant Integration in Bone. PhD Thesis.University of Göteborg. Department of Biomaterials Science/Handicap Research, Göteborg, Sweden 2002.
6. Meirelles L. On Nano Size Structures For Enhanced Early Bone Formation. PhD Thesis. University of Göteborg. Department of Biomaterials Science/Handicap Research, Göteborg, Sweden 2007.
7. Piattelli A, Trisi P, Passi P, Piattelli M, Cordioli G.P. Histochemical and confocal laser scanning microscopy study of the bone-titanium interface: an experimental study in rabbits. Biomaterials 1994; 15;3: 194-200.
8. Suzuki K, Aoki A, Ohya K. Effects of surface roughness of titanium implants on bone remodeling activity of femur in rabbits. Bone 1997; 21;6: 507-14.
9. Papalexiou V, Noaves Jr A.B, Grisi MFM, Souza SSLS, Taba Jr M, Kajiwara JK. Influence of microstructure on the dynamics of bone healing around immediate implants placed into periodontally infected sites. A confocal laser scanning microscopic study. Clin. Oral Impl. Res. 2004; 15:44-53.
10. Nkenke E, Kloss F, Wiltfang J, Schultze-Mosgau S, Radespiel-Tröger M, L Kerstin, et al. Histomorphometric and fluorescence microscopic analysis of bone remodeling after installation of implants using an osteotome technique. Clin.Oral Impl.Res. 2002; 13: 591-602.
11. Kajiwara H, Yamaza T, Yoshinari M, Goto T, Iyama S, Atsuta I, et al. The bisphosphonate pamidronate on the surface of titanium stimulates bone formation around tibial implants in rats. Biomaterials. 2005; 26: 581-87. doi:10.1016/j.biomaterials.2004.02.072
12. Rahn B.A. Fluorochrome Labelling of Bone Dynamics. European Cells and Materials. 2003; 5: 41.
13. Sul YT, Johansson C, Byon E and Albrektsson T. The bone response of oxidized bioactive and non-bioactive titanium implants. Biomaterials. 2005; 26: 6720-6730.
14. Rahn BA. Polychrome fluorescence labelling of bone formation. Instrumental aspects and experimental use. Zeiss Inf, 1977; 22: 36.
15. Donath K. and Breuner G. A method for the study of undecalcified bone and teeth with attached soft tissues. The Säge-Schliff (sawing and grinding) technique. Journal of Oral Pathology. 1982; 11: 318-326.
16. Donath K. Die Trenn-Dunnschliff-Technik sur Herstellung Histologischer Präparate von Nicht Schnneidbaren Geweben und Materialien. Der Preäparator, 1988; 34: 197-296.
17. Johansson CB and Morberg P. Importance of ground section thickness for reliable histomorphometrical results. Biomaterials. 1995: 16,13: 91-95.
18. Sul YT, Jeong Y, Johansson CB and Albrektsson T. Oxidized, bioactive implants are rapidly and strongly integrated in bone: Part 1-experimental implants. Clin Oral Impl Res. 2006; 17: 521-526.
19. Sul YT, Johansson CB and Albrektsson T. Which surface properties enhance bone response to implants? Comparison of oxidized Magnesium, TiUnite and Osseotite implant surfaces. Int J Prosthodont. 2006; 19: 319-329.
20. Sun T. C., Mori S., Roper J., Brown C., Hooser T. and Burr D. B., 1992, Do Different Fluorochrome Labels Give Equivalent Histomorphometric Information, Bone. 1992;13: 443-446.
21. Pinholt EM and Kwon P H.J. Triple bone labeling of canine mandibles. Oral Surgery, Oral Medicine, and Oral Pathology. 1990; 70: 401-405.
22. Erben RG. Trabecular and Endocortical Bone Surfaces in the Rat: Modeling or Remodeling? The Anatomical Record. 1996; 249: 39-46.
23. Erben R.G, Scutt AM, Miao D, Kollenkirchen U and Haberey M. Short-Term Treatment of Rats with High Dose 1,25-Dihydroxyvitamin D3 Stimulates Bone Formation and Increases the Number of Osteoblast Precursor Cells in Bone Marrow. Endocrinology. 1997; 138: 4629-35.
24. Ma B, Sampson, Wilson D, Wiebkin O and Fazzalari N. A histomorphometric study of adaptive responses of cancellous bone in different regions in the sheep mandibular condyle following experimental forward mandibular displacement. Archives of Oral Biology. 2002; 47: 519-527.
25. Weinlaender M, Beumer III J, Kennedy E.B, Lekovic V, Holmes R, Moy P, et al. Histomorphometric and fluorescence microscopic evaluation of interfacial bone healing around 3 different dental implants before and after radiation therapy. The Int J of Oral & Maxillofac Impl. 2005; 21; 2: 212-24.
26. Parfitt AM, Drezner MK, Glorieux FH, Kanis J, Malluche H, Meunier P, et al. Bone Histomorphometry: Standardization of Nomenclature, Symbols, and Units. Report of the ASBMR Histomorphometry Nomenclature Committee. J Bone and Miner Res. 1987; 2: 595-610.
27. Erben RG. Bone-Labeling Techniques. In Y.H An and K.L. Martin, eds. Handbook of the Histology Methods for Bone and Cartilage. Humana Press, 2003; 99-117.

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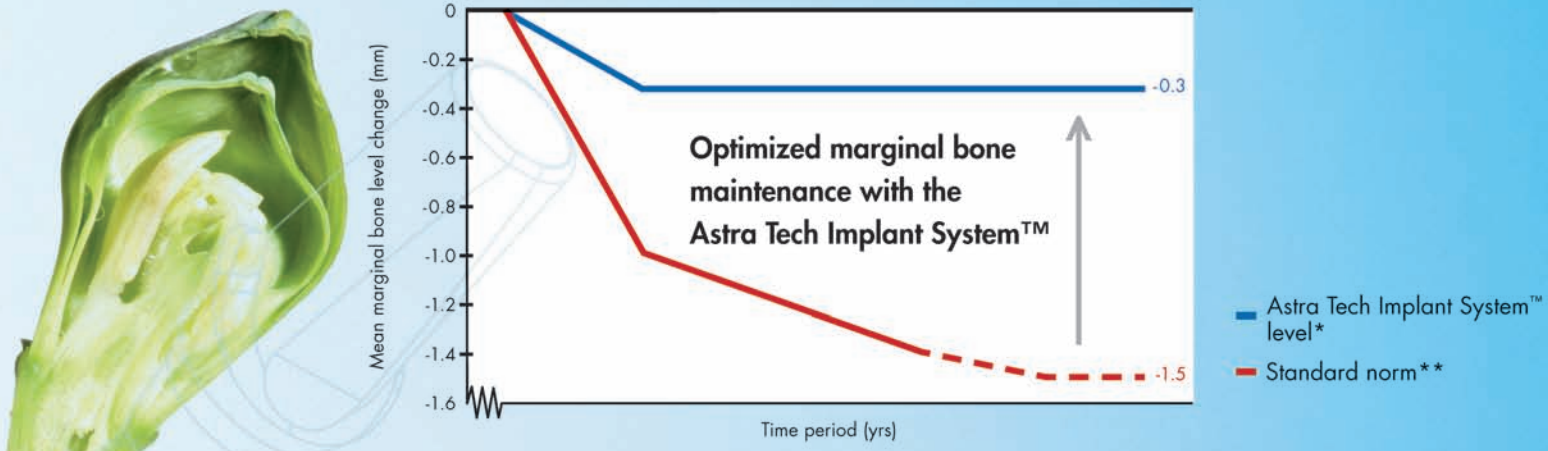

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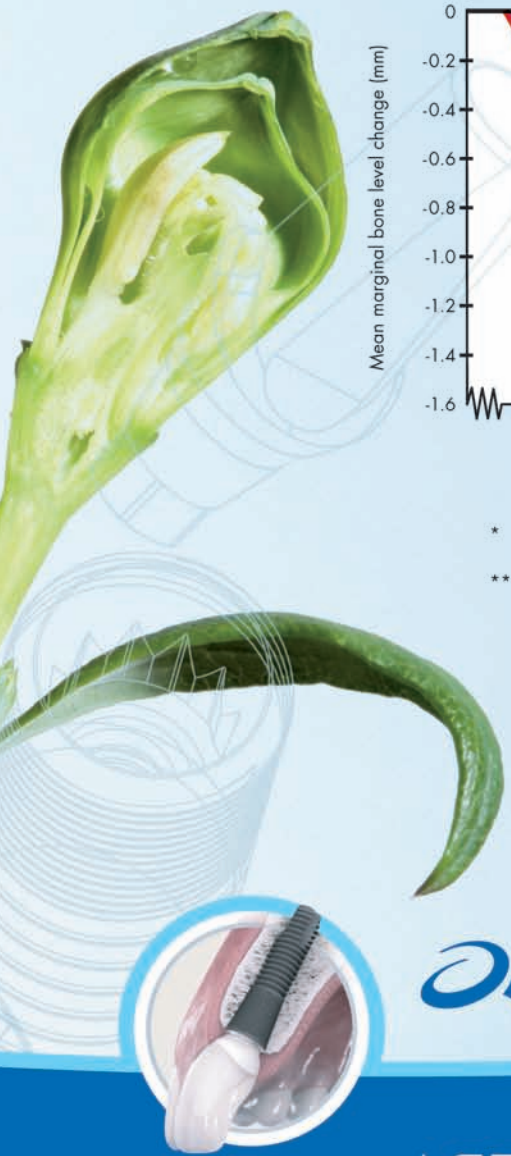

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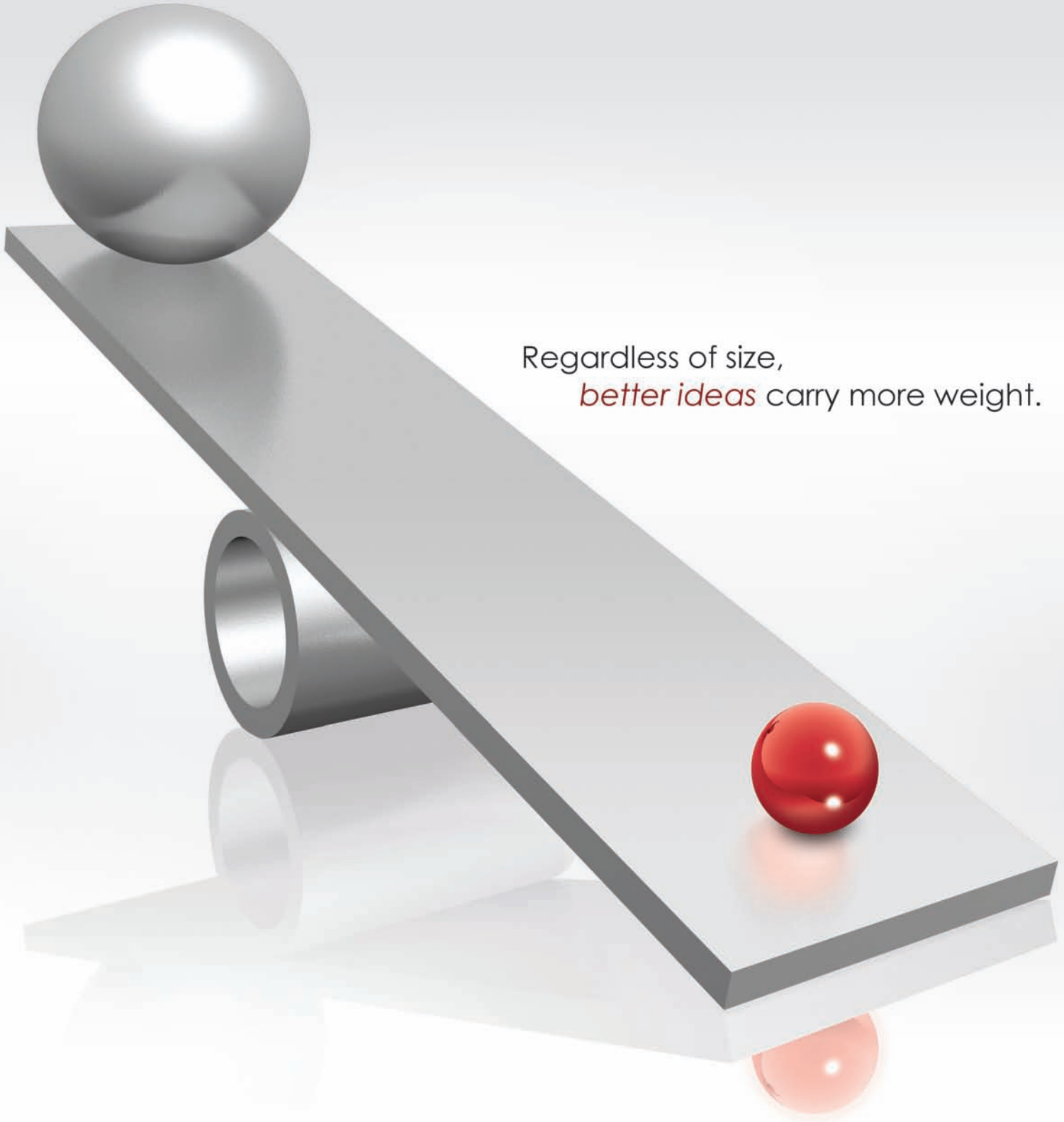

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