Healing of Fresh Extraction Sockets Filled with Bioactive Glass Particles: Histological Findings in Humans

Emanuele Clozza, DDS;* Maurizio Pea, MD;† Fabio Cavalli, MD;‡ Loredana Moimas, MEng, PhD;§ Roberto Di Lenarda, DDS;¶ Matteo Biasotto, DDS, PhD, MSc**

ABSTRACT

Purpose: The aim of the present study was to histologically evaluate fresh human sockets filled with bioactive glass after 6 months of healing.

Materials and Methods: In 13 patients, 32 single extraction sites in the anterior area underwent socket ridge preservation procedure (RPP) with a bioactive glass (BioRestore™, Inion Oy, Tampere, Finland). At implant installation, 22 bone cores were trephined out and processed for histomorphometric and immunohistochemical analysis.

Results: Newly formed immature bone around residual particles of bioactive glass was found in all 22 biopsies. The histomorphometry of the amount of bone, provisional matrix, and residual graft returned a mean ± SD value of 54 ± 31%, 37.9 ± 25.6%, and 8.1 ± 7.8, respectively, 6 months after RPP.

Conclusion: The use of this grafting material in fresh extraction sockets appears to delay the healing processes of the alveolar bone; therefore, its indication as a material for RPP when implant placement is considered within 6 months after extraction should be revised.

KEY WORDS: alveolar sites, bioactive glass, bone graft, bone regeneration, histology

INTRODUCTION

Biomaterials science has developed rapidly in recent decades. The ability to create scaffolds with highly reproducible architecture and compositional variation across the entire scaffold is offered by recent tissue engineering concepts. Focusing on oral application, substitute materials have become popular because of their low postoperative morbidity and the unlimited amount available. Healing of extraction sockets filled with bioactive glass has been investigated in different animal model studies.[1–3] Histological analysis carried out in these studies has clearly showed the osteoconductive properties of the bioactive glass particles and their osteoinductive effects on bone cell functions.[1–3]

Bioactive glass was used also in human studies, that is, in the treatment of periodontal osseous defects[4] and to preserve ridge dimensions in postextraction sockets.[5,6] Although no signs of periodontal regeneration on a previously diseased root surface were observed,[4] the bioactive glass particulates seemed to be able to preserve the ridge contour and allow osseointegration of titanium implants.[5,7]

*Resident, Ashman Department of Periodontology and Implant Dentistry, New York University College of Dentistry, New York, NY, USA; †head, Department of Pathology, Ospedale Orlandi, Bussolengo, Verona, Italy; ‡director, Research Unit of Paleoradiology and Allied Sciences, Ospedali Riuniti di Trieste, Trieste, Italy; §R&D manager, Inion Oy, Tampere, Finland; ¶full professor, Division of Dental Sciences and Biomaterials, Department of Biomedicine, University of Trieste, Trieste, Italy; **assistant professor, Division of Dental Sciences and Biomaterials, Department of Biomedicine, University of Trieste, Trieste, Italy

ClinicalTrials.gov identifier: NCT01105026.

Name of trial registry: A Clinical Investigation to Evaluate the Healing of Tooth Extraction Sites Filled With BioRestore™.

Conflict of interest and sources of funding statement: The authors declare that they have no conflict of interests. The study was self-supported, but company Inion Oy delivered free of charge the Inion BioRestore™ and implants.

Reprint requests: Dr. Emanuele Clozza, Ashman Department of Periodontology and Implant Dentistry, New York University College of Dentistry, 345 East 24th Street, New York, NY 10010, USA; e-mail: leleclo@libero.it

DOI 10.1111/j.1708-8208.2012.00463.x

Limited human studies have described the behavior of the bioactive glass by means of histology,[5–7] while the majority of these clinical studies have only used clinical parameters such as probing depth and radiographic appearance.[8–11]

Despite the encouraging results obtained in animal models, some unsatisfactory outcomes affecting human studies were seen, such as long-term resorption[12] and connective tissue encapsulation of the bioglass particulate.[3]

A three-dimensional glass fiber scaffold composed of Na_2O-K_2O-MgOCaO-B_2O_3-P_2O_5-SiO_2 was recently used in a rabbit pilot study for the regeneration of surgically created bone defects.[13] Preliminary results indicated that in 6 months the tibial defects were healed, new bone formation was found in the medullary cavities, and glass fiber scaffolds were completely resorbed.

These interesting results led our research group to investigate whether the grafting material could promote bone repair in human fresh sockets.

The aim of this paper was restricted to present the histology of specimens retrieved 6 months after ridge preservation procedures (RPP) using a bioactive glass material. The RPP outcome (with horizontal and vertical radiographic measurements over a 3-month period) has been described in a previous manuscript written by the same research group.[14]

MATERIALS AND METHODS

Study Design

Thirteen patients (3 males and 10 females, mean age 55 ± 10 and between 41 and 76 years of age) who required implant therapy were enrolled for the extraction of 32 anterior teeth (18 maxillary teeth; 14 mandibular teeth). The recruitment and active treatment period was December 2008 to October 2009 at the Dental School, University of Trieste, Trieste, Italy.

Each patient signed a consent form to participate in the study approved by the Independent Ethical Committee of "The United Hospitals of Trieste," Trieste, Italy on October 13, 2008 (Approval Number: 30).

All the chosen patients were systemically healthy. They did not smoke and did not take any medications. Any patients with uncontrolled periodontal disease, less than 18 years of age, with current alcohol or drug abuse, with systemic/local conditions that would interfere with wound healing or osseointegration, and with a history of chemotherapy and radiotherapy in the head and neck region also were excluded from this study. The alveolar sockets presented the following characteristics: absence of peri-apical pathology, a four-wall architecture, and a depth greater than 5 mm. The architecture of the defect had to be confirmed by direct observation during the surgical treatment. If the architecture was not confirmed, the defect was excluded from the study.

The patients were informed that implant procedure after the regenerative surgery included a bone biopsy in order to evaluate the results obtained.

Surgical Procedures

Once the patient was enrolled in the study, then the surgery was scheduled. Peri-apical and panoramic radiographs were utilized for the preoperative examination of the patients. The RPPs have been already presented in an earlier publication.[14] Briefly, patients underwent surgery under local anesthesia (2% mepivacaine with 1:100,000 epinephrine, Optocain, Molteni Dental, Florence, Italy) and minimally invasive periotomy was carried out with a surgical blade. Atraumatic extraction with forceps was performed. The extraction socket was carefully rinsed with a physiologic solution. To evaluate the eligibility of the surgical site, the alveolar wall integrity was checked clinically and by means of extemporaneous peri-apical radiography.

No attempt was carried out to obtain a first intention closure of the site in order to avoid a coronal displacement of the mucogingival junction. Single interrupted (5-0) sutures (Vicryl®; Johnsons & Johnson, Woluwe, Belgium) were tightened to promote the stability of grafted particles without using any membranes. Peri-apical radiography was used to control the grafting procedure. Sutures were removed 1 week after RPP.

Postoperative Period

Postoperative pain was treated with paracetamol (Tachipirina®; Angelini, Ancona, Italy). Patients were instructed to take 500 mg two times per day if necessary. A protocol for the control of bacterial contamination consisting of 0.2% chlorhexidine (Corsodyl®; Glaxo SmithKline, Verona, Italy) mouth rinsing three times daily per 2 weeks was prescribed. Patients were requested to avoid brushing and chewing in the treated area for a period of 2 weeks. Then, patients resumed full oral hygiene.

TABLE 1 Specificities, Isotypes, and Dilutions of the Monoclonal Antibodies Used for Immunohistochemical Stainings

Antibodies	Clone	Specificity	Isotype (MOUSE)	Source	Dilutions	Incubation	Processing
CD31	JC70A	Endothelial cell	IgG1, k	Dakocytomation, Glostrup, Denmark	1/30	15′	Heat
CD68	514H12	Osteoblast Histiocyte	IgG2a, k	A. Menarini Diagnostics, Florence, Italy	1/80	15′	Heat
α-SMA	1A4	Smooth muscle cell Pericyte	IgG2a, k	Dakocytomation, Glostrup, Denmark	1/80	15′	Heat
Cathepsin-k	3F9	Osteoclast	IgG2b	D.B.A. Italia Srl, Milan, Italy	1/500	15′	Heat
BMP-7	45912	BMP-7	IgG2b	Santa Cruz Biotechnology, Inc., CA, USA	1/20	15′	None

α-SMA = α-smooth-muscle actin; BMP-7 = bone morphogenetic protein 7; CD31 = cluster of differentiation 31; CD68 = cluster of differentiation 68.

Follow-Up

Oral wound examinations of the study treatment sites were performed at the first, second, and fourth postoperative week to monitor the occurrence of commonly seen postoperative complications (pain, edema, and abscess).

Collection and Storage of the Specimens

Six months after RPP, patients were recalled for implant surgery. At the time of biopsy, harvest local infiltration of anesthetic was administered. A crestal incision was performed and a mucoperiosteal flap was elevated on both the buccal and oral sides. Of 32 extraction sites filled with bioactive glass, 22 biopsies of bone were harvested with a trephine bur (external diameter of 3 mm, internal diameter of 2 mm; 1 trephine bur, Hu-Friedy®; Rotterdam, the Netherlands), where titanium implants (EHC Implant, Hexcel by Plan1Health®; Amaro, Udine, Italy) would be inserted. The depth of trephine cut was set at 10 mm. A total of 22 osseointegrated implants were placed.

Histological Processing

The specimens were immediately stored in 10% formalin and embedded in paraffin according to standard methods. All tissue samples were cut in the mesio-distal plane and parallel to the long axis of the extraction socket with a microtome set at 5 μm. Six sections representing the central part of the grafted socket were obtained from each biopsy. One section, stained in hematoxilyn and eosine, was used to study various aspects of tissue modeling. The other five were deparaffinized and immunostained with the following antibodies: cluster of differentiation 31 (CD31), cluster of differentiation 68 (CD68), α-smooth-muscle actin (α-SMA), cathepsin-k, and bone morphogenetic protein 7 (BMP-7). Monoclonal antibody specificities, their isotypes, and dilutions are presented in Table 1.

All immunostained samples were processed using a sensitive "Bond Polymer Refine" detection system in an automated Bond immunostainer (Vision-Biosystem™; A. Menarini Diagnostics, Florence, Italy).

Histomorphometric and Histological Analysis

Histologic and histomorphometric analyses were conducted using a light microscope (Leica, Leica Microsystem, Milan, Italy).

Various aspects of tissue modeling were studied by means of morphometric measurements to determine the percentage of residual grafting material (IB), provisional matrix (PM), and bone (BN). A single trained and calibrated examiner (M.P.) carried out all the histomorphometric measurements. Immunohistochemical staining was utilized for histological description.

RESULTS

Clinical Findings

None of the subjects enrolled in this study reported any unusual pain or discomfort, abscess, swelling, and no sign of acute inflammation, and wound dehiscence was detected.

Histomorphometric Measurements

Patients contributed more than one socket to the study. A total of 22 bone cores were analyzed. The

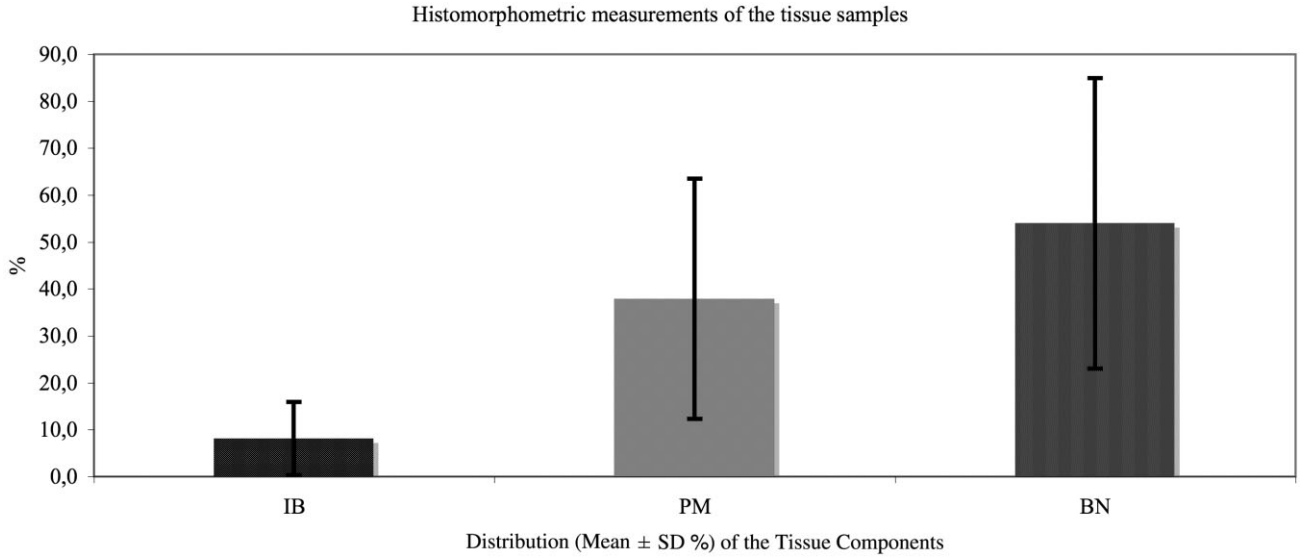

Figure 1 Histomorphometric measurements of the tissue samples. Distribution (mean ± SD [%]) of the tissue components. BN = bone; IB = residual graft; PM = provisional matrix.

histomorphometric evaluation of the amount of BN, PM, and IB returned a mean ± SD value of 54 ± 31%, 37.9 ± 25.6%, and 8.1 ± 7.8, respectively, 6 months after RPP (Figure 1).

Bone

New formed bone was detected in all the sections comprised primary spongiosa, whereas appearance of lamellar bone and bone marrow was never seen (Figure 2). This acidophilic structure occurred as fingerlike projections of mineralized tissue in a connective tissue matrix. Ridges of trabeculae were lined with basophilic osteoblasts and contained several osteocytes (Figure 3A). The mineralized bone was invaded by vascular structures. Vessels were found in the intratrabecular mesenchyma, containing erythrocytes and surrounded by mild infiltration of plasma cells.

Provisional Matrix

A tissue contained densely packed mesenchymal cells present in a collagen-rich connective tissue matrix was found in all of the 22 samples. Provisional matrix harbored a large number of histiocytes but few neutrophilic leukocytes.

Residual Graft

Only few residual grafting particles were found in all the samples. The remaining granules had a diameter of about 20 to 50 μm, with different shapes and degrees of dissolution. These glass particles were intimately associated with newly formed trabeculae.

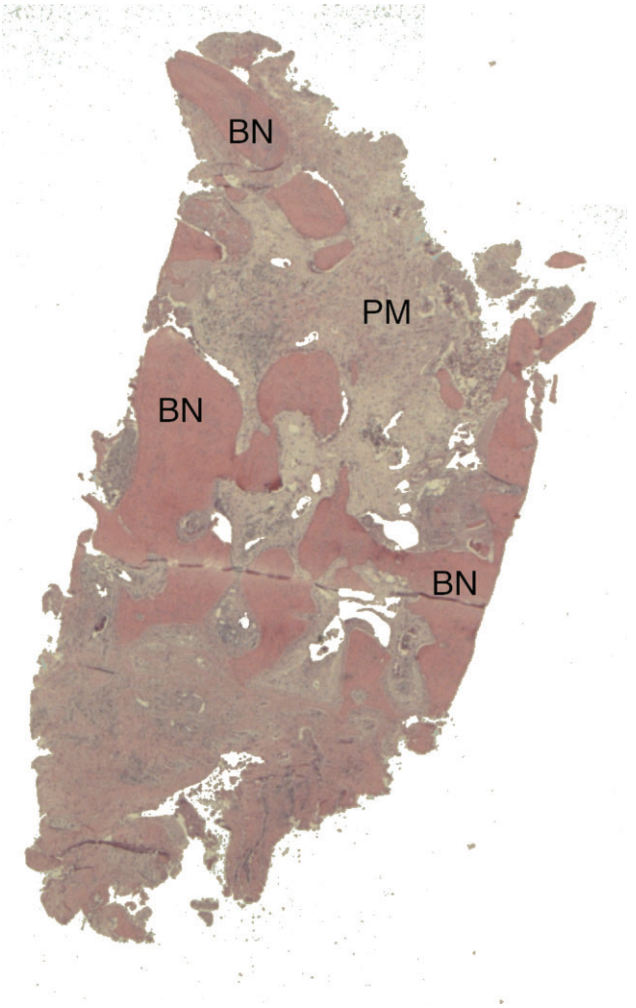

Figure 2 Overview of the bone (BN) core in a apicocoronal section. The sample is comprised of woven bone in the middle area, while provisional matrix (PM) surrounded newly formed BN. Hematoxylin-eosin stain. Original magnification ×12.

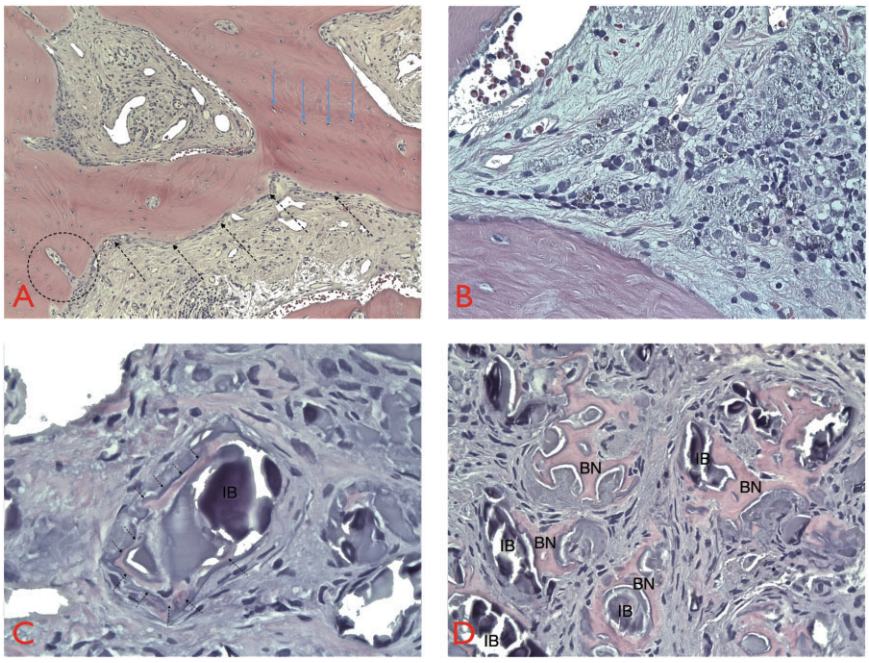

Figure 3 Hematoxylin-eosin stain. (A) Newly formed bone (BN). Note the large number of osteoblasts (*black arrows*) that are present on the BN surface. The trabeculae surrounding the vascular structure (*black circle*). BN contained several osteocytes (*blue arrows*). Original magnification ×100. (B) Cell and fiber-rich provisional matrix. Note the degradation of bioactive glass particles promoted by histiocytes. Original magnification ×400. (C) BN growth (*arrows*) surrounded the residual particle (IB). Note the calcium-rich layer on the outer surface of the particles. Original magnification ×630. (D) Osteoid formation (BN) on the surface of residual graft (IB). Original magnification ×400. IB = residual graft.

The specimens stained in hematoxilyn and eosin revealed the formation of calcium-rich layer on the outer surface of the particles, which was associated with the surrounding bone. Intratrabecular histiocytes contained dusts of residual bioactive glass (see Figure 3B). A large number of residual particles exhibited a central disintegration. Osteoid tissue and active osteblasts were observed in many of these excavations.

Bone tissue formed within the glass particle was not necessarily connected to the bone that proliferated from the cavity walls (see Figure 3C). In addition, the degree of maturation of newly mineralized tissue found within the cracked particles was more pronounced compared with the tissue surrounding them (see Figure 3D).

Immunohistochemical Findings

CD31. A considerable number of blood vessels were detected within the provisional matrix, indicating that these areas were very well supplied with blood (Figure 4).

CD68. The high amount of histiocytic infiltrate was never present massively in the vicinity of bioactive glass residuals (Figure 5).

α-SMA. Smooth muscle cells and pericytes were completely positive *for* α-SMA, thus clearly highlighting the occurrence of vascular structures within the provisional matrix (Figure 6).

Cathepsin-k. Osteoclasts were detected in low numbers (three to four cells) on each specimen, which were present on the surface of newly formed bone. This indicated that the woven bone in such locations was in the process of remodeling (Figure 7).

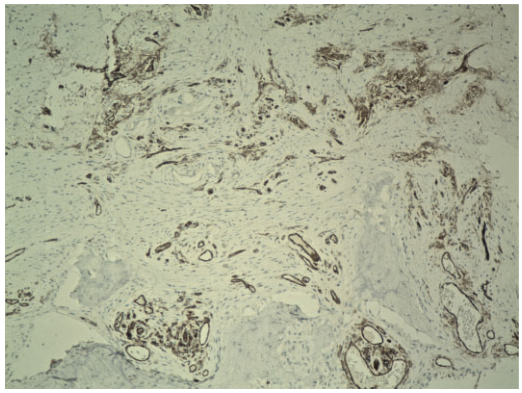

Figure 4 Expression of CD31 illustrating the vascular structure. CD31 stain. Original magnification ×100. CD31 = cluster of differentiation 31.

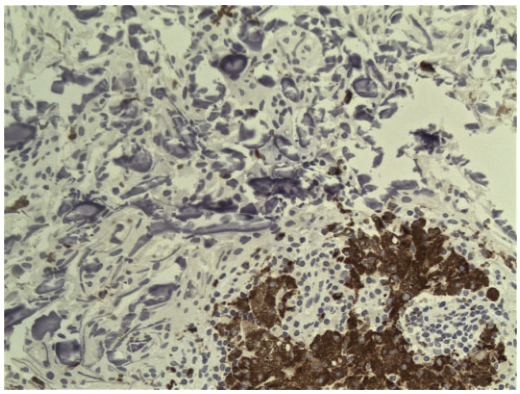

Figure 5 Expression of CD68 (brown) illustrating histiocytes distant from residual bioactive glass. CD68 stain. Original magnification ×200. CD68 = cluster of differentiation 68.

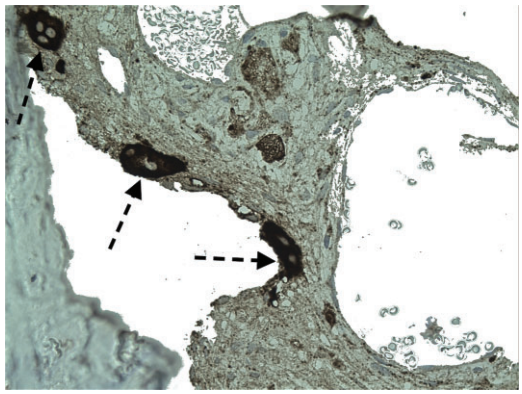

Figure 7 Note the presence of osteoclasts (*arrows*) on the surface of newly formed bone. Cathepsin-k stain. Original magnification ×630.

BMP-7. A mild immunoreactivity was demonstrated using BMP-7 specific antibodies, mainly confined to trabeculae of mineralized bone (Figure 8A). It is important to note that BMP-7 expression was observed also closed to calcium-rich residual particles (see Figure 8B).

DISCUSSION

Although the clinical use of bioactive glass has been most widely adopted in oral applications (i.e., RPP, for the repair of periodontal defects and maxillary sinus floor augmentation), histology has been available for only a few clinical studies. Furthermore, the existing literature provides conflicting data on the outcome of placing bioactive glass materials in the regeneration of bone defects.

In this study, we placed bioactive glass as grafting material in fresh extraction sockets and performed a histological examination of the grafted sites 6 month later.

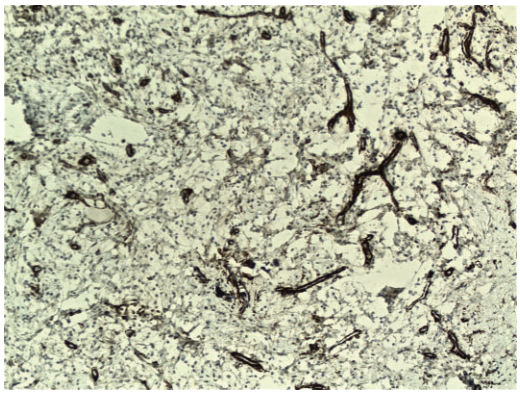

Figure 6 Expression of α-SMA illustrating the occurrence of large vascular structures within the provisional matrix. α-SMA stain. Original magnification ×200. α-SMA = α-smooth-muscle actin.

Different from repairing periodontal defects, the protocol of grafting first and placing implants provided a unique opportunity to gain reentry and harvest bone cores.

A finding of remarkable interest was that 6 months after healing the alveolar sites treated with bioactive glass exhibited on average more than half of the total trephine area to be occupied by newly formed bone, which is not far from data presented by Froum and colleauges[15] about extraction sockets filled with bioactive glass.

In all the specimens representing the implant installation, woven bone was present and occupied 54 ± 31% of the tissue examined, while lamellar bone and bone marrow were not found. These present findings demonstrated that 6 months after RPP, the implant sites were not characterized by mature bone.

Low percentage of residual graft in histomorphometric data revealed that the scaffold was almost completely resorbed, which was a predictable response substantiating the histological results presented in the previous rabbit pilot study.[13]

The residual particles exhibited a central excavation. Schepers and colleagues[1] previously reported this phenomenon in an animal study and noticed that most glass particles are eroded internally via small cracks. In the above-mentioned study, it was hypothesized also that the excavated area presented a protected environment with minimal fluid flow, allowing mesenchymal stem cells (MSCs) to adhere to the internally formed calcium-phosphate layer. At this time, when primitive cells were immobilized on a bone-like surface, differentiation of the MSCs into osteoblasts would have occurred.

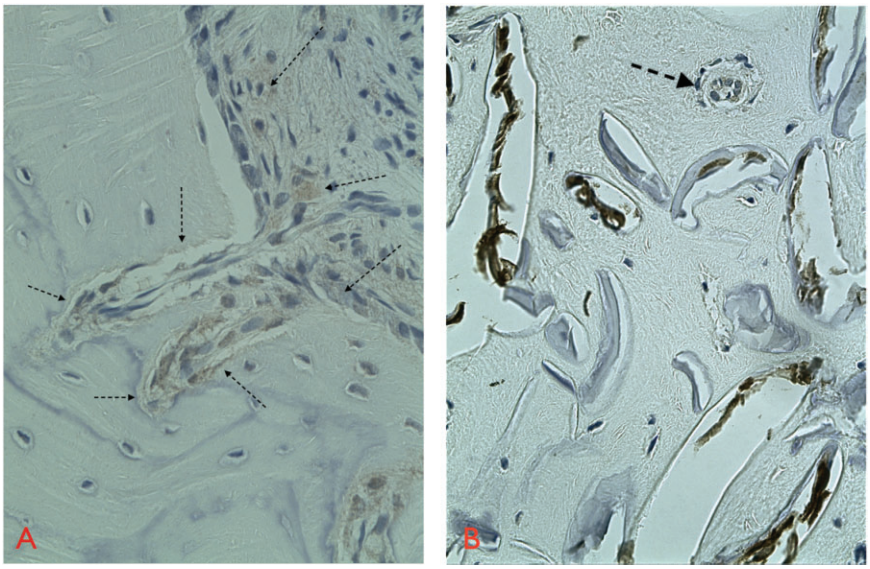

Figure 8 BMP-7 stain. (A) Expression of BMP-7 in osteoblasts (*arrows*) proliferating on the vascular structure. Original magnification ×630. (B) Expression of BMP-7 (*brown*) on the surface of needle-shaped residuals. *Arrow*: vascular structure. BMP-7 stain. Original magnification ×400. BMP-7 = bone morphogenetic protein 7.

Bioactive Glass and Bioactive Bonding

The bone-bonding behavior is referred to as bioactivity and has been summarized in a previous study.[16] Briefly, bioactive materials are said to exhibit class A or class B bioactivity. The former is related to the reaction of the material at cellular level in the body to enhance bone proliferation; the latter is related to the material's osteoconduction properties, which is the process of bone growth along the implant surface. It is universally recognized that BMP-7 plays a key role in the transformation of mesenchymal cells into bone and cartilage.[17]

In the present study, we demonstrated that mesenchymal cells exhibited an expression of BMP-7 not only close to newly formed bone but also on the surface of calcium-rich residual particles of bioactive glass residual particles.

This observation enlarges the knowledge regarding the stimulatory effects of bioactive glass materials in osteoblast differentiation and suggests a useful new marker for investigating reactions between the glass surface and surrounding tissues. This behavior was originally hypothesized by Ohgushi and coworkers,[18] who had investigated the osteoblastic phenotype expression of marrow stromal stem cells on the surface of bioactive materials. They speculated that binding of biologically active molecules to the surface activated the cell membrane receptors of stromal stem cells resulting in osteoblastic differentiation. Many other research groups have made further studies of this phenomenon and most of them have demonstrated the presence of bioactive activity as having a stimulatory effect on osteoblasts.[19–26] Hematoxylin and eosin staining revealed a basophilic layer surrounding the residual particle. This "gelation" represented the interfacial ion exchange between the glass particles and the surrounding tissue fluids results in the formation of a silicate-rich gel, which extended throughout the particle. At the same time, phagocytosing cells could penetrate this silicate-rich gel layer via small cracks in the calcium-phosphate layer and start the resorption of the gel. Then, MSCs penetrated via the small ducts between the excavated center and the surrounding tissue. Hench and colleagues produced a series of publications,[27–29] in which the layer formed between the biomaterial and tissue had been widely investigated. An amorphous layer with thickness of 800 to 1,000 Å developing between bioactive glass and healing bone was identified in their in vitro studies. It seemed reasonable to assume that this amorphous layer comprised of SiO_2, CaO, and P_2O_5 could be equivalent to the basophilic substance found in this study.

Bioactive Glass and Histiocyte Reactions

The histological examination revealed that none of the residual particles were separated from bone tissue by connective tissue capsule. It meant that the biomaterial did not lead to "foreign" body reaction. Variegated data

exist regarding the tissue response when relying on bioactive glass as a grafting material. In the Norton and Wilson clinical study,[5] bone cores were trephined out at the time of implantation and examined to evaluate the tissue response. Authors concluded that connective tissue was present without an inflammatory reaction for up to 6 months. Increasing evidence of bone formation was seen to exist in apposition to the ceramic material beyond 6 months. The histological study carried out by Stvrtecky and colleagues[4] revealed mature host bone, bioactive glass particles attached to host bone, and bioactive glass particles surrounded by connective tissue with no inflammatory response. In contrast, Knapp and colleauges[30] observed in their histological examination of the grafted sites a connective tissue encapsulation of most residual graft particles.

CONCLUSION

In this study, it was demonstrated that bioactive glass particles were harbored by osteoid tissue, exhibiting de novo bone formation by means of their both osteoconductive and osteoinductive properties. It seems that the use of this synthetic bone graft material may, in fact, have retarded bone formation in human alveolar sites as the histologic analysis failed to detect lamellar bone and bone marrow in all the specimens.

The reason for this delayed healing is not presently understood and further investigations should be addressed in this direction. From a clinical point of view, bioactive glass as a material for RPP when implant placement is considered within 6 months after extraction should be revised.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Lorenzo Bevilacqua (Division of Dental Sciences and Biomaterials, Department of Biomedicine, University of Trieste) for his assistance in collecting bone samples and to Dr. Flaviu Dunca (Ashman Department of Periodontology and Implant Dentistry, New York University College of Dentistry) for reviewing the manuscript.

REFERENCES

1. Schepers E, de Clercq M, Ducheyne P, Kempeneers R. Bioactive glass particulate material as a filler for bone lesions. J Oral Rehabil 1991; 18:439–452.
2. Schepers E, Barbier L, Ducheyne P. Implant placement enhanced by bioactive glass particles of narrow size range. Int J Oral Maxillofac Implants 1998; 13:655–665.
3. Araújo MG, Liljenberg B, Lindhe J. Dynamics of Bio-Oss Collagen Incorporation in fresh extraction wounds: an experimental study in the dog. Clin Oral Implants Res 2010; 2:55–64.
4. Nevins ML, Camelo M, Nevins M, et al. Human histologic evaluation of bioactive ceramic in the treatment of periodontal osseous defects. Int J Periodontics Restorative Dent 2000; 20:458–467.
5. Norton MR, Wilson J. Dental implants placed in extraction sites implanted with bioactive glass: human histology and clinical outcome. Int J Oral Maxillofac Implants 2002; 17:249–257.
6. Furusawa T, Mizunuma K. Osteoconductive properties and efficacy of resorbable bioactive glass as a bone-grafting material. Implant Dent 1997; 6:93–101.
7. Stvrtecky R, Gorustovich A, Perio C, Guglielmotti MB. A histologic study of bone response to bioactive glass particles used before implant placement: a clinical report. J Prosthet Dent 2003; 90:424–428.
8. Zamet JS, Darbar UR, Griffiths GS, et al. Particulate bioglass as a grafting material in the treatment of periodontal intrabony defects. J Clin Periodontol 1997; 24:410–418.
9. Low SB, King CJ, Krieger J. An evaluation of bioactive ceramic in the treatment of periodontal osseous defects. Int J Periodontics Restorative Dent 1997; 17:358–367.
10. Froum S, Weinberg MA, Tarnow D. Comparison of bioactive glass synthetic bone graft particles and open debridement in the treatment of human periodontal defects. A clinical study. J Periodontol 1998; 69:698–709.
11. Ong MM, Eber RM, Korsnes MI, et al. Evaluation of a bioactive glass alloplast in treating periodontal intrabony defects. J Periodontol 1998; 69:1346–1354.
12. Garg AK. Bone biology, harvesting, and grafting for dental implants: rationale and clinical applications. In: Garg AK, ed. Review of bone-grafting materials. Chicago, IL: Quintessence Publishing Co, Inc., 2004:52.
13. Moimas L, Biasotto M, Di Lenarda R, Olivo A, Schmid C. Rabbit pilot study on the resorbability of three-dimensional bioactive glass fibre scaffolds. Acta Biomater 2006; 2:191–199.
14. Clozza E, Biasotto M, Cavalli F, Moimas L, Di Lenarda R. Three-dimensional evaluation of bone changes following ridge preservation procedures. Int J Oral Maxillofac Implants 2012:27. (In press).
15. Froum S, Cho SC, Rosenberg E, Rohrer M, Tarnow D. Histological comparison of healing extraction sockets implanted with bioactive glass or demineralized freeze-dried bone allograft: a pilot study. J Periodontol 2002; 73:94–102.
16. Rezwan K, Chen QZ, Blaker JJ, Boccaccini AR. Biodegradable and bioactive porous polymer/inorganic composite

scaffolds for bone tissue engineering. Biomaterials 2006; 27:3413–3431.

17. Lavery K, Hawley S, Swain P, et al. New insights into BMP-7 mediated osteoblastic differentiation of primary human mesenchymal stem cells. Bone 2009; 45:27–41.
18. Ohgushi H, Okumura M, Yoshikawa T, Tamai S, Tabata S, Dohi Y. Bone bonding biomaterials. In: Ducheyne P, Kokubo T, van Blitterswijk CA, eds. Regulation of bone development and the relationship to bioactivity: osteoblastic phenotype expression of marrow stromal stem cells on the surface of bioactive materials. The Netherlands: Reed Healthcare Comm. Publ., 1992:47–56.
19. Bielby RC, Christodoulou IS, Pryce RS, Radford WJ, Hench LL, Polak JM. Time and concentration-dependent effects of dissolution products of 58S sol-gel bioactive glass on proliferation and differentiation of murine and human osteoblasts. Tissue Eng 2004; 10:1018–1026.
20. Bosetti M, Cannas M. The effect of bioactive glasses on bone marrow stromal cells differentiation. Biomaterials 2005; 26:3873–3879.
21. Foppiano S, Marshall SJ, Marshall GW, Saiz E, Tomsia AP. The influence of novel bioactive glasses on in vitro osteoblast behavior. J Biomed Mater Res A 2004; 71:242–249.
22. Gough JE, Notingher I, Hench LL. Osteoblast attachment and mineralized nodule formation on rough and smooth 45S5 bioactive glass monoliths. J Biomed Mater Res A 2004; 68:640–650.
23. Lossdörfer S, Schwartz Z, Lohmann CH, Greenspan DC, Ranly DM, Boyan BD. Osteoblast response to bioactive glasses in vitro correlates with inorganic phosphate content. Biomaterials 2004; 25:2547–2555.
24. Radin S, Reilly G, Bhargave G, Leboy PS, Ducheyne P. Osteogenic effects of bioactive glass on bone marrow stromal cells. J Biomed Mater Res A 2005; 73:21–29.
25. Valerio P, Pereira MM, Goes AM, Leite MF. The effect of ionic products from bioactive glass dissolution on osteoblast proliferation and collagen production. Biomaterials 2004; 25:2941–2948.
26. Välimäki VV, Yrjans JJ, Vuorio EI, Aro HT. Molecular biological evaluation of bioactive glass microspheres and adjunct bone morphogenetic protein 2 gene transfer in the enhancement of new bone formation. Tissue Eng 2005; 11:387–394.
27. Hench LL, Paschall HA. Direct chemical bond of bioactive glass-ceramic materials to bone and muscle. J Biomed Mater Res 1973; 7:25–42.
28. Hench LL, Paschall HA. Histochemical responses at a biomaterial's interface. J Biomed Mater Res 1974; 8:49–64.
29. Hench LL, Andersson O. An introduction to bioceramics. In: Hench LL, Wilson J, eds. Bioactive glasses. Boca Raton, FL: World Scientific, 1993:1–24.
30. Knapp CI, Feuille F, Cochran DL, Mellonig JT. Clinical and histologic evaluation of bone replacement grafts in the treatment of localized alveolar ridge defects. Part 2: bioactive glass particulate. Int J Periodontics Restorative Dent 2003; 23:129–137.