Influence of Bioactive Glass and/or Acellular Dermal Matrix on Bone Healing of Surgically Created Defects in Rat Tibiae: A Histological and Histometric Study

Luciana Liarte Gasparini Ribeiro, DDS, MS[1]/Alvaro Francisco Bosco, DDS, PhD[2]/
Maria José Hitomi Nagata, DDS, PhD[2]/Luiz Gustavo Nascimento de Melo, DDS, MS[1]

Purpose: *The purpose of this study was to histologically analyze the influence of bioactive glass and/or acellular dermal matrix on bone healing in surgically created defects in the tibiae of 64 rats (*Rattus norvegicus, albinus, *Wistar).* ***Materials and Methods:*** *A 4-mm × 3-mm unicortical defect was created on the anterolateral surface of the tibia. Animals were divided into 4 groups: C, control; BG, the defect was filled with bioactive glass; ADM, the defect was covered with acellular dermal matrix; and BG/ADM, the defect was filled with bioactive glass and covered with acellular dermal matrix. Animals were sacrificed at 10 or 30 days postoperatively, and the specimens were removed for histologic processing. The formation of new bone in the cortical area of the defect was evaluated histomorphometrically.* ***Results:*** *At 10 and 30 days postoperatively, groups C (39.65% ± 5.63%/63.34% ± 5.22%) and ADM (38.12% ± 5.53/58.96% ± 7.05%) presented a larger amount of bone formation compared to the other groups (*P < .05*). In the same periods, groups BG (13.10% ± 6.29%/29.5% ± 5.56%) and BG/ADM (20.72% ± 8.31%/24.19% ± 6.69%) exhibited statistically similar new bone formation. However, unlike the other groups, group BG/ADM did not present a significant increase in bone formation between the 2 time points.* ***Conclusion:*** *Based on these results, it can be concluded that all of the materials used in this study delayed bone healing in non–critical-size defects.* Int J Oral Maxillofac Implants 2008;23:811–817

Key words: *acellular dermis, bioactive, bioglass, bone regeneration, bone substitutes*

Several investigations have been conducted on the reconstruction of anatomic structures lost because of trauma, infection, or resection of tumors to provide functional and esthetic rehabilitation of the patient. The utilization of biomaterials in combination with tissue engineering techniques has shown encouraging outcomes with respect to new bone formation.[1–3]

Bioactive glass, especially in its granular form, is an alloplastic material that has been used to fill bone defects.[4,5] The healing of these defects is achieved because of the osteoconductive and osteostimulatory properties of this material. The osteostimulatory property is related to the composition of the bioactive glass, which promotes the formation of an apatite-rich gel layer on the particle surface. This layer attracts osteoprogenitor cells and osteoblasts, thus stimulating the formation of bone tissue.[4]

The acellular dermal matrix is an allograft obtained from fresh cadaveric skin; all cellular components of the dermis and epidermis are eliminated during matrix fabrication, which reduces the risk of rejection and transmission of viral diseases.[6] In dentistry, this matrix has been used to increase the width of keratinized gingiva,[7,8] in root coverage techniques,[9,10] and as a membrane in guided bone regeneration, for which its main advantage is the possibility of being left exposed to the oral cavity with no damage to the regenerative potential of the treated area.[11,12]

[1]Postgraduate, Division of Periodontics, Department of Surgery and Integrated Clinic, Dental School of Araçatuba, University of State of São Paulo "Júlio de Mesquita Filho"–UNESP, Araçatuba, São Paulo, Brazil.
[2]Adjunct Professor, Division of Periodontics, Department of Surgery and Integrated Clinic, Dental School of Araçatuba, University of State of São Paulo "Júlio de Mesquita Filho"–UNESP, Araçatuba, São Paulo,Brazil.

Correspondence to: Dr Luciana Liarte Gasparini Ribeiro, Rua Assis Chateaubriand, 399, CEP: 16020-230 Araçatuba, SP, Brazil. Fax: + 55 18 36225580. E-mail: luribeiro@terra.com.br

The purpose of this study was to histologically analyze the influence of bioactive glass and/or acellular dermal matrix on bone healing in surgically created defects in rat tibiae.

MATERIALS AND METHODS

Experimental Model

Sixty-four male rats (*Rattus norvegicus, albinus,* Wistar), weighing 350 to 400 grams (University of the State of São Paulo – UNESP, Dental School of Araçatuba, Animal Care Unit) were used in this study. The animals were kept in a room with a 12-hour cycle of light and darkness and a temperature from 22°C to 24°C; they received food and water ad libitum. The protocol of the study was approved by the Dental School of Araçatuba Institutional Animal Care and Use Committee (UNESP). The rats were divided into 4 study groups: surgical defect filled with a blood clot only (group C); surgical defect filled with bioactive glass particles (size, 300 µm to 355 µm; group BG); surgical defect without filling material and covered with the acellular dermal matrix (group ADM), and surgical defect filled with bioactive glass and covered with the acellular dermal matrix (group BG/ADM).

Surgical Procedure

Anesthesia was accomplished by intraperitoneal injection of ketamine (10 mg/kg) and xylazine (3 mg/kg). After shaving and aseptic preparation of the operative site, a linear 15-mm incision was made on the tibia, and full-thickness flaps were reflected. Sterile carbide round burs were used in a high-speed handpiece under continuous sterile saline irrigation to create a 4 × 3-mm unicortical defect on the anterolateral surface of the tibia.

In group C, the surgical defect was filled with a blood clot only. In group BG, the surgical defect was filled with bioactive glass (BioGran; Orthovita, Malvern, PA). In group ADM, the surgical defect was filled with a blood clot and covered with acellular dermal matrix (AlloDerm, Life Cell, The Woodlands, TX). The base of the membrane was turned toward the bone, and the connective side of the membrane was turned toward the muscle tissue, 1 mm beyond the perimeter of the surgical defect. The acellular dermal matrix was stabilized with a U-shaped compressive suture supported by the muscles (polyvicryl 5.0; Ethicon, São Paulo, Brazil). In group BG/ADM, the surgical defect was filled with bioactive glass and covered with the acellular dermal matrix. The soft tissues were then repositioned carefully and sutured (deep and superficial sutures) to achieve primary closure (polyvicryl 5.0 and silk 4.0; Ethicon, São Paulo, Brazil).

Tissue Processing

Animals were sacrificed at either 10 or 30 days postoperatively. The tibiae were removed and fixed in 10% formalin, decalcified, processed, and embedded in paraffin. Histologic serial sections were then prepared. These were 6 µm thick and were made as longitudinal sections of the specimens. The sections were stained with either hematoxylin-eosin (H&E) or Masson's trichrome for analysis by light microscopy.

Histomorphometric Analysis

ImageLab 2000 software (Diracom Bio Informática; Vargem Grande do Sul, SP, Brazil) was used for the histomorphometric analysis. The most central histologic section of each surgical defect was selected for the statistical evaluation. As suggested by Melo et al,[13] the following criteria were used to standardize the histomorphometric analysis of the digital image:

1. The total area (TA) to be analyzed was delineated on the digital image. It corresponded to the cortical region of the tibia where the surgical defect had been previously created.
2. The TA, calculated in μm^2, was considered 100% of the area to be analyzed. The newly formed bone area (NFBA) and the areas of the remnants of the implanted materials, named the bioactive glass area (BGA), were delineated within the confines of the TA.
3. The NFBA and the BGA were also measured in μm^2 and calculated as a percentage of TA according to the following formulas:

- NFBA (μm^2) / TA (μm^2) × 100
- BGA (μm^2) / TA (μm^2) × 100

Statistical Analysis

The values of NFBA represent the mean percentage of the 8 histologic sections per group. The significance of differences between groups in relation to NFBA was determined by an analysis of variance (ANOVA), followed by a post-hoc Tukey test when the ANOVA suggested a significant difference between groups ($P < .05$). The values of BGA for each animal in groups BG and BG/ADM were represented by the mean percentage of the 8 histologic sections. The significance of differences between groups in relation to BGA was determined by an ANOVA, followed by a post-hoc Tukey test when the ANOVA suggested a significant difference between groups ($P < .05$).

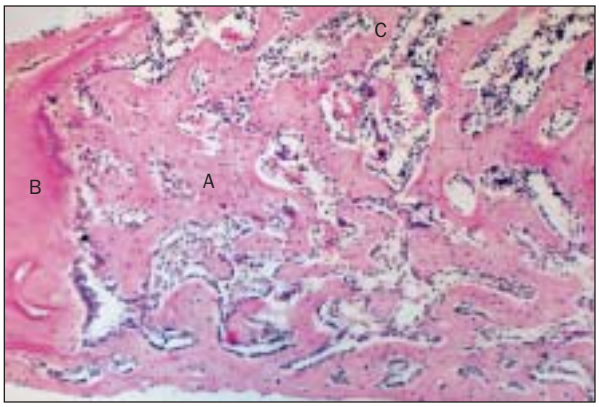

Fig 1 Group C 10 days postoperatively. Surgically created defect filled with thin bone trabeculae *(A)*. Adjacent cortical bone *(B)*. Woven bone in the medullary canal *(C)* (H&E, original magnification ×63).

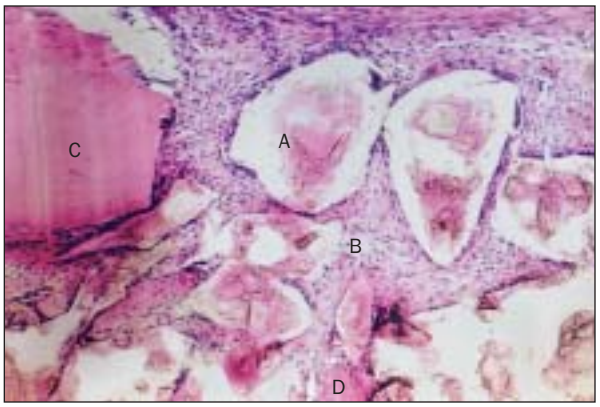

Fig 2 Group BG 10 days postoperatively. Surgically created defect filled with bioactive glass *(A)*, with presence of fibroblast-rich connective tissue *(B)*. Adjacent cortical bone *(C)*. Woven bone in the medullary canal *(D)* (H&E, original magnification ×63).

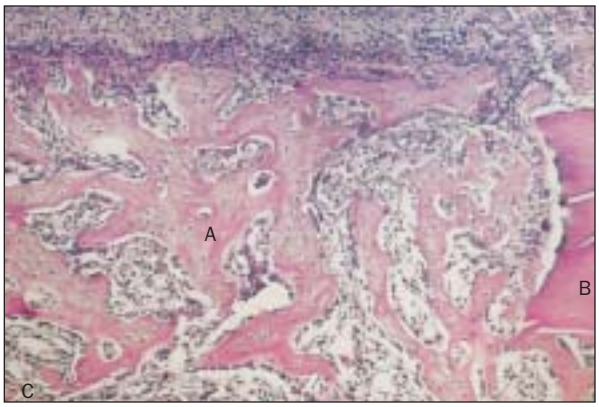

Fig 3 Group ADM (acellular dermal matrix) 10 days postoperatively. Immature bone tissue with large intertrabecular spaces *(A)*. Adjacent cortical bone *(B)*. Woven bone in the medullary canal *(C)* (H&E, original magnification ×63).

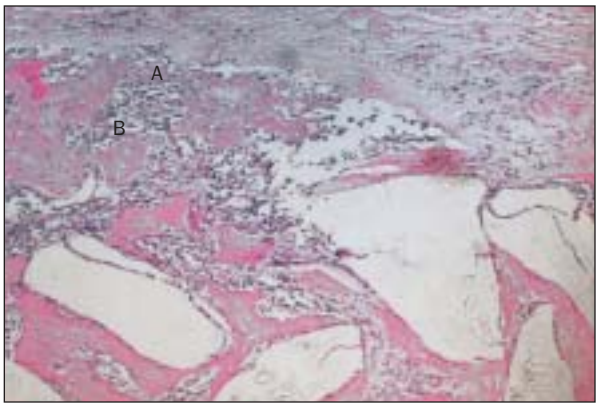

Fig 4 Group BG/ADM (bioactive glass/acellular dermal matrix) 10 days postoperatively. Areas of membrane resorption *(A)*; replacement by newly formed bone tissue *(B)*. (H&E, original magnification ×63).

RESULTS

Qualitative Histologic Analysis—10 Days

In group C, the control group, woven bone with large intertrabecular spaces was observed in the surgical defect in all specimens (Fig 1). At the level of the subjacent medullary canal, woven bone was seen in all specimens. The connective tissue between the trabeculae was well-vascularized and rich in fibro-blasts. Many osteoblasts were observed adjacent to the borders of the newly formed bone tissue.

In the BG group, in most specimens, the bioactive glass completely filled the surgical defect and was in contact with newly formed bone tissue or fibroblast-rich connective tissue (Fig 2). There was new bone formation or new connective tissue formation beyond the upper limit of the surgical defect with the implanted material in some specimens. In the connective tissue adjacent to the bioactive glass particles, moderate numbers of fibroblasts, macrophages, and lymphocytes were present. Internal erosion of the bioactive glass particles was not observed in any of the specimens. Woven bone was seen in the medullary canal.

In all specimens in group ADM, the surgical defect was filled with woven bone with a wide intertrabecular space (Fig 3). Well-vascularized, fibroblast-rich connective tissue was observed between the trabeculae. It was possible to observe small areas of membrane resorption close to the newly formed bone. Woven bone was observed in the medullary canal.

In most specimens in group ADM/BG, the bioactive glass completely filled the surgical defect and was in contact with newly formed bone tissue or fibroblast-rich connective tissue. Woven bone was observed beyond the upper limit of the surgical defect, and the bone tissue was in contact with the acellular dermal matrix, which displayed signs of partial resorption (Fig 4). Woven bone was also seen in the medullary canal.

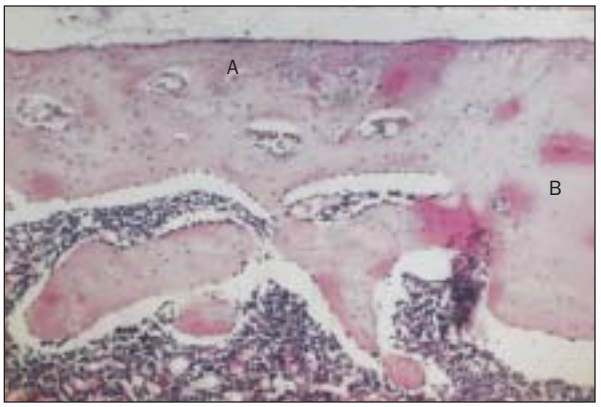

Fig 5 Group C 30 days postoperatively. Repaired surgically created defect with reduction in the thickness of the bone tissue *(A)*. Adjacent cortical bone *(B)* (H&E, original magnification ×63).

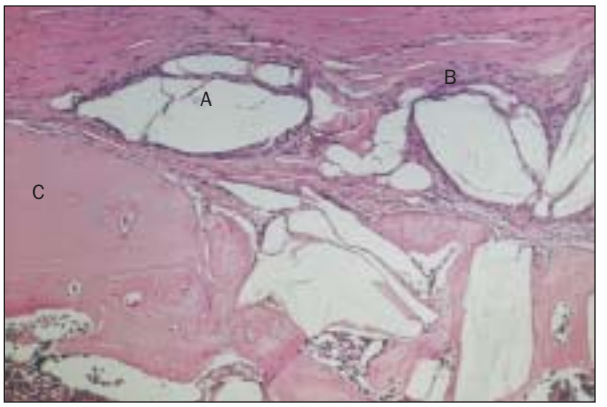

Fig 6 Group BG 30 days postoperatively. Part of the implant beyond the upper limit of the surgically created defect *(A)* with the presence of well-developed connective tissue *(B)*. Adjacent cortical bone *(C)* (H&E, original magnification ×63).

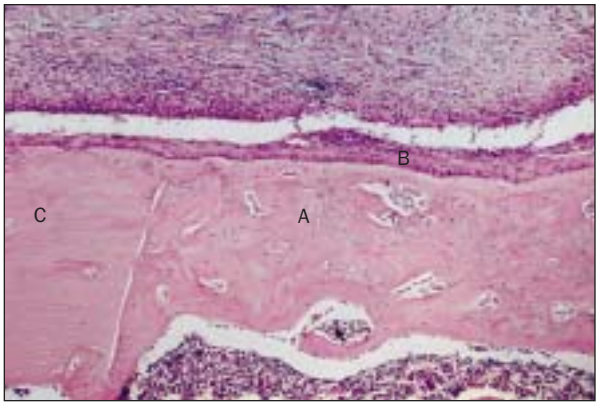

Fig 7 Group ADM 30 days postoperatively. Completely repaired bone wall *(A)* could be observed separated from the membrane by undifferentiated connective tissue *(B)*. Adjacent cortical bone *(C)* (H&E, original magnification ×63).

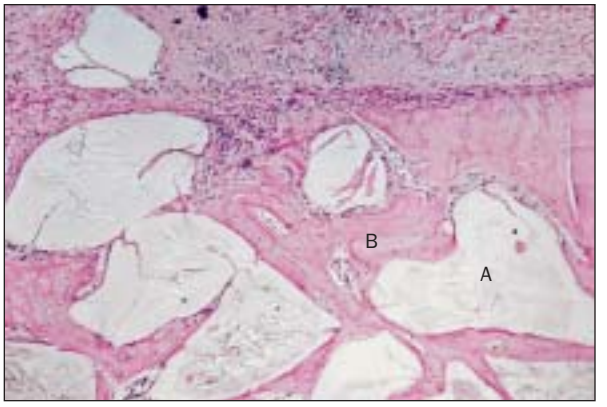

Fig 8 Group BG/ADM 30 days postoperatively. Implant *(A)* separated by well-developed bone tissue *(B)* can be observed (H&E, original magnification ×63).

Qualitative Histological Analysis—30 Days

In group C, the surgical defect was completely repaired by well-organized bone tissue in all specimens. In most specimens, the newly formed bone was thicker close to the border of the surgical defect and thinner in the central area. The woven bone seen at the level of medullary canal in the 10-day specimens had been completely resorbed (Fig 5).

In group BG, the repair of the surgical defect was minimal in all specimens. There was a significant amount of the implanted material (bioactive glass) in all cases. Well-differentiated bone trabeculae were observed between the particles of the material. A large amount of connective tissue without bone differentiation was also seen (Fig 6). Small numbers of fibroblasts, lymphocytes, and macrophages were seen within this connective tissue. Internal erosion of the bioactive glass particles was not observed in any of the specimens.

In group ADM, the surgical defects of most of the specimens were repaired by well-organized bone tissue, with less developed bone trabeculae in some instances. A thin layer of connective tissue was observed between the newly formed bone tissue and the membrane (Fig 7). At the area close to the surgical defect, the membrane seemed to undergo gradual resorption and replacement by connective tissue.

In group BG/ADM, the repair of the surgical defect was minimal in all specimens. The surgical defects were completely filled by the material, which was in contact with well-organized bone tissue or connective tissue (Fig 8). A thin layer of connective tissue was seen between the bioactive glass and the acellular dermal matrix. The membrane often displayed signs of resorption on the borders close to the filling material, being replaced by connective tissue.

Table 1 Mean Percentage of Newly Formed Bone Area (NFBA) in the Region Corresponding to the Surgical Defect 10 and 30 Days Postoperatively

Treatment group	n	NFBA at 10 d (%)		NFBA at 30 d (%)	
		Mean	SD	Mean	SD
C	8	39.65 aB	5.63	63.34 aA	5.22
ADM	8	38.12 aB	5.53	58.96 aA	7.05
BG	8	13.10 bB	6.29	29.50 bA	5.56
BG/ADM	8	20.72 bA	8.31	24.19 bA	6.69

Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ by Tukey test ($P < .05$).

Table 2 Mean Percentage of Bioglass Particle (BG) in the Region Corresponding to the Surgical Defect 10 and 30 Days Postoperatively

Treatment group	n	NFBA at 10 d (%)		NFBA at 30 d (%)	
		Mean	SD	Mean	SD
BG	8	58.9 aB	14.5	55.6 aB	10.2
BG/ADM	8	43.6 bB	10.4	46.0 aB	14.4

Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ by Tukey test ($P < .05$).

Histometric and Statistical Analysis

The means and standard deviations of NFBA (as a percentage) for each group at both 10 and 30 days postoperatively are depicted in Table 1, as well as the results of the comparison among the groups. Table 2 demonstrates the lack of resorption of BG particles between 10 and 30 days postoperatively in groups BG and BG/ADM.

DISCUSSION

This study was designed to histologically analyze the influence of bioactive glass particles of uniform size (300 to 355 μm; BioGran) and/or an acellular dermal matrix membrane on bone healing in surgically created defects in rat tibiae. The newly formed bone in the cortical region of the surgical defect was evaluated by both histologic and histometric analyses. At 10 days postoperatively, the control group (39.65%) and group ADM (38.12%) presented significantly more bone formation than group BG (13.10%) or group BG/ADM (20.72%). No significant differences were found between the last 2 experimental groups. At 30 days postoperatively, the histologic and histometric analyses showed that the control group (63.34%) and group ADM (58.96%) also presented significantly more bone formation than group BG (29.50%) and group BG/ADM (24.19%). These groups exhibited statistically similar new bone formation. However, group BG/ADM did not present a significant increase in bone formation between the 2 time points studied as opposed to the other groups.

In the present study, the control areas presented significantly more bone formation than the areas that received implantation of graft materials. This was probably due to the rapidity of the physiologic bone turnover and healing response at 10 and 30 days postoperatively in the animal selected as the experimental model. Also, the control defects were completely repaired by bone at 30 days post-op demonstrating that they were not "critical size" defects. According to Schmitz and Hollinger,[14] critical-size defects are defined as defects of a size that precludes spontaneous bone regeneration/healing during the lifetime of the animal. The defects surgically created in the present study measured 4 mm in length and 3 mm in width. This experimental model was based on the work of Lewandrowski et al[15] in another animal study. According to the authors, 3-mm-diameter defects surgically created in rat tibiae did not heal spontaneously in the control group throughout the experimental period (up to 7 weeks postoperatively). They stated that these were critical-size defects. Based on their observations, Lewandrowski et al[15] stated that both the animal model selected and the defect surgically created were appropriate for comparative histologic evaluations. Although surgically created defects with greater dimensions than those used by Lewandrowski et al were used in the present study,[15] the defects of the control group in this study were completely repaired by bone at 30 days postoperatively. One possible explanation for this could be that a different rat species was used. These observations and the fact that the control group healed spontaneously without a barrier and a grafting material lead to the hypothesis that when acellular dermal matrix and bioglass are used in non–critical-size defects they cause a delay in healing. However, if the defect was a critical-size defect, the use of acellular dermal matrix as a membrane and bioglass as a grafting material would probably enable a better and faster healing pattern.

The areas that received the bioactive glass showed bone differentiation intimately associated with the bioactive glass particles, demonstrating the osteoconductive properties of the material. These histologic findings corroborated a study by Schepers et al[16] that evaluated the implantation of bioactive glass (BioGran) and 2 forms of hydroxylapatite in surgically created defects in mandibles of dogs. After 1

month of implantation, a considerable number of bioactive glass particles near the wall of the cavity were already intimately associated with bone tissue. According to the authors, the bone growth around these particles was enhanced by the osteoconductive properties of the bioactive glass. Subsequent studies conducted by Schepers et al[5,17] have confirmed the findings of this initial study. However, the osteostimulatory effect described in the literature[5,16,17] was not seen in any of the specimens of the present study at either 10 or 30 days postoperatively. Perhaps the time of the analysis was not long enough for this phenomenon to have occurred.

Although osteconductive bone growth could be observed coming from the walls of the defect in groups BG and BG/ADM, there were no signs of complete cortical repair. These results could be explained, in part, by the long period necessary for the resorption of bioactive glass particles.[18,19] Bone graft particles were observed in groups BG and BG/ADM at both time points, confirming the slow process of resorption of this material observed in histologic studies in animals[17,18] and humans.[19,20] Schepers and Ducheyne[17] observed the presence of BG particles up to 24 months after their implantation in surgically created bony defects in mandibles of dogs. According to MacNeill et al,[3] graft materials that require extended time periods for complete resorption will reduce the total amount of newly formed bone due to their continued presence. The lack of resorption of the bone graft particles probably accounted for the greater amount of newly formed bone observed in groups C and ADM when compared to groups BG and BG/ADM.

Evaluation of the group ADM reveals that acellular dermal matrix performed satisfactorily as a membrane. At 30 days, complete healing of the cortical bone was observed in all specimens in this group. However, qualitative analysis of group ADM demonstrated a mild delay in the process of bone maturation when compared to group C. This delay may be related to the role of mechanical barrier played by the membrane, which impairs the bilateral blood supply in the beginning of the healing process. This result might be different in cases of larger defects, where, even if the membrane led to a delay in the initial healing process, the inability of other tissues to participate in long-term bone regeneration might lead to more new bone formation than if no membrane was used.

In the specimens where the acellular dermal matrix was used to protect the bioactive glass particles (Group BG/ADM), even greater delay in the healing process was shown. This group did not present a significant increase in bone formation between the 2 time points studied. This result might be related to the combined effects of the mechanical barrier played by the acellular dermal matrix and the lack of resorption of the bioactive glass particles. Another phenomenon that might have occurred would be the difficulty of the membrane to adapt over the bioactive glass particles at the borders of the defect. This would allow easier penetration of connective tissue and, consequently, less bone formation, since the acellular dermal matrix does not seem to allow the penetration of any connective tissue through its structure.

Several investigators have used the acellular dermal matrix as a membrane on bone tissue to be regenerated. They also demonstrated the ability of the dermal matrix to protect bone grafts and blood clots, even when exposed to the oral cavity.[2,11,12] Novaes et al[21] used an acellular dermal matrix as a membrane barrier to cover an immediate implant in extraction socket, and a bioactive glass was used as a grafting material. According to the authors, the materials fulfilled the expectations. The bioglass functioned well as a spacer marker and scaffolding material to allow mineralized tissue formation, and acellular dermal matrix performed well as a membrane. Further, these materials probably would perform well when used together in sinus floor elevation. Bioglass has already been used as a grafting material in this treatment, and acellular dermal matrix could be used as a membrane to close the defect, protecting the grafting material.

Some studies stated that the acellular dermal matrix is not resorbed, but rather incorporated to the receptor site. Incorporation is thought to occur because there is revascularization of the matrix through the vascular canals of the allograft and repopulation of the fibroblasts and keratinocytes, as well as elastin fibers not common in the oral cavity, even 1 year after implantation of the material.[6,7,9] The present study revealed partial resorption and replacement of the matrix by granulated tissue at 30 days. These findings are in agreement with the study of Richardson and Maynard,[22] who performed a biopsy of the portion of acellular dermal matrix placed subepithelially and in contact with the tooth root. Those researchers observed that the most apical portion of this allograft was resorbed and replaced by connective tissue.

Within the limits of this study, it can be concluded that ADM acted as a membrane for isolation of bone defect. BG showed osteoconductive potential, as demonstrated by the close contact between the particles and the new bone. In summary, all of the materials used in this study delayed bone healing in non–critical-size defects.

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