Histomorphometric Analysis of Tissue Responses to Bioactive Glass Implants in Critical Defects in Rat Calvaria

Ana Karina M.V. Cardoso a, Aryon de Almeida Barbosa, Jr. c, Fúlvio Borges Miguel a, Elcio Marcantonio, Jr. d, Marcos Farina e, Glória Dulce de Almeida Soares f, Fabiana Paim Rosa b

a Dentistry Faculty and b Health Sciences Institute, Biointeraction Department, Federal University of Bahia and c Gonzalo Moniz Research Center, Oswaldo Cruz Foundation (FIOCRUZ), Salvador, d Department of Periodontology, Dental School at Araraquara, State University of São Paulo (UNESP), Araraquara, and e Biomedical Sciences Institute and f Metallurgical and Materials Engineering, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Key Words
Bioactive glass · Bone regeneration · Biomaterials · Aloplastic graft

Abstract
The aim of this study was to evaluate the osteogenic behavior of two chemically similar bioactive glass products (Biogran® and Perioglas®) implanted in critical bone defects in rat calvaria. Thirty-six transfixed bone defects of 8 mm diameter were made surgically in adult male Wistar rats. The animals were distributed equally into three groups: Biogran (GI), Perioglas (GII) and without implant material (control; GIII). The morphology and composition of both bioactive glasses were analyzed by scanning electron microscopy and energy-dispersive spectrometry. Tissue specimens were analyzed at the biological time points of 15, 30 and 60 days by optical microscopy and morphometry, demonstrating biocompatibility for the tested materials with moderate chronic inflammation involving their particles. Bone neoformation resulted only as a reparative reaction to an intentionally produced defect and was limited to the defect’s edges. No statistically sig-

Abbreviations used in this paper

AM amorphous matter
ANOVA one-way analysis of variance
BE bone edge
CI chronic inflammation
DCT dense connective tissue
EDS energy-dispersive spectrometry
F fibroblasts
FS fibrous septation areas
GI group I: bioglass implant – Biogran®
GII group II: bioglass implant – Perioglas®
GIII group III: control
HE hematoxylin-eosin
HP hyalinization in plates
LR linear regression
MP mineralization plate
NB bone neoformation
P particles
PIFG sirius red
SCT slack connective tissue
SEM scanning electron microscopy
VC vascular congestion

© 2007 S. Karger AG, Basel
1422–6405/06/1844–0128$23.50/0
Accessible online at: www.karger.com/cto
significant differences among the groups were observed. At the scar interstice, abundant deposits of collagenous fibers enveloping the particles were noted. The present results indicated that the bioactive glasses, under the experimental conditions analyzed, did not show osteogenic behavior.

Copyright © 2007 S. Karger AG, Basel

Introduction

Bone tissue engineering offers a potential strategy to restore lost bone tissue. Currently, there are a variety of biomaterials that may be used clinically as bone substitutes; among them are alloplastic grafts [Furusawa and Mizunuma, 1997], which are prepared in laboratories with natural (e.g., collagen) or synthetic materials (e.g., metallics, biopolymerics, bioceramics). Bioceramics are divided into three categories [Oonishi et al, 1999]: (1) bioinert ceramics (alumina), (2) resorbable bioactive ceramics (α-tricalcium phosphate, β-tricalcium phosphate, tetracalcium phosphate and octacalcium phosphate) and (3) surface-bioactive ceramics (sintered hydroxyapatite and bioactive glasses). These materials have varying degrees of osteoconductive behavior [Fujishiro and Hench, 1997]. When granules of these bioceramics are implanted, growth behavior at the bone site and reaction to the bone are expected to be different owing to the variation in materials and the size of the granules [Oonishi et al., 1999].

Bioceramics derived from calcium phosphate such as hydroxyapatites and β-tricalcium phosphate are both osteophilic and biocompatible materials, most likely because they have a similar composition and structure to the mineral part of the bone tissue [Rosa et al., 1998]. Calcium-based glasses (bioactive glasses) were developed in the 1970s and represent another ceramic option [Hench, 1991].

The bioactive glasses have been outstanding as osteoconductor materials, with bioactive properties superior to hydroxyapatite and validated by the interaction of the interface between the implant surface and the adjacent tissues [Misch and Dietsh, 1993; Vrouwenvelder et al., 1993; Chan et al., 2002]. Moreover, bioactive glass has been shown to be easy to manipulate and hemostatic, besides being more stable at a bleeding site than the hydroxyapatite. When the bioactive glass particles are mixed in vivo with saline or blood, they rapidly form a cohesive mass because of the gel layer that forms on the surface in contact with body fluids. Consequently, the particles pack easily into a defect and stay in place, even when the site is bleeding [Oonishi et al., 1997]. They have the peculiar characteristic of having silica in their composition, making them highly bioactive. They are structurally reactive when in contact with tissue fluids, through a series of chemical reactions favoring resorption and the formation of new bone tissue [Schepers et al., 1991, 1993; Furusawa and Mizunuma, 1997; Schepers and Ducheyne, 1997; Wheeler et al., 1997, Oonishi et al., 1999].

Bone neoformation generally depends on the size and type of the defect [Takagi and Urist, 1982; Schmitz and Höllinger, 1986; Chesmel et al., 1998, Miguel et al., 2006], the species of the animal and the implant site. However, repairing defects of critical dimensions is still very difficult, because under these conditions, there is not sufficient formation or organization of blood clots [Frame, 1980], which is essential for bone regeneration. Furthermore, in defects with critical dimensions, it becomes necessary to use biomaterial that, by filling the surgical wound, can act as a scaffold for bone formation, while it stimulates the migration and proliferation of osteogenic cells, necessary for the bone matrix synthesis [Schepers et al., 1998; Piattelli et al., 2000].

The objective of the present work was to assess the osteogenic behavior of two bioactive glasses when implanted in surgically created transfixed defects of critical dimensions (of 8 mm in diameter) in rat calvaria.

Materials and Methods

After approval by the Ethical Research Committee on Animals of the Dentistry Faculty of the Federal University of Bahia (UFBA), 36 adult male Wistar rats with body mass ranging between 400 and 450 g were used, kept in the Experimental Animal Laboratory (LEA) of the Dentistry Faculty of the Federal University of Bahia. The animals were fed solid chow and water ad libitum, before and throughout the entire experimental period.

The materials used in this study were bioactive glasses: Biogran® and Perioglas®. Morphology and composition of both bioactive glass granules prior to the implantation were evaluated using scanning electron microscopy (SEM). For this, a JEOL JSM-6460LV scanning electron microscope, operating at 15 kV, with an energy-dispersive spectrometer (EDS; NORAN system SIX, model 200®) and a digital image processing system software (Global Lab®) were used. Elemental compositions of grafts were estimated by using an EDS detector. Granules were deposited on a conductive carbon tape and four images of the sample of each granule were acquired in order to verify size (axis ratio, meaning the ratio between minor and major axis) and roundness [ratio between minimum and maximum radius (perimeter)/4π(area)]. At least 50 particles of each sample were used for the quantification. However, according to the manufacturers, these bioactive glasses differ only with regard to their particle size, with Biogran having diameters from 350 to 355 μm and Perioglas ranging from 90 to 710 μm, but they had similar
chemical compositions and specifications, containing 45% silica (SiO$_2$), 24.5% calcium oxide (CaO), 24.5% sodium oxide (Na$_2$O) and 6% of phosphorus pentoxide (P$_2$O$_5$).

The animals were randomly selected, divided into three groups, and assessed for three postoperative periods (15, 30 and 60 days). A total of 36 bone defects were made, organized equally in the following manner: bioactive glass implant – Biogran (group I, GI), bioactive glass implant – Perioglas (group II, GII), and control – without implant material (group III, GIII).

Total anesthesia was achieved by intramuscular injection of ketamine chloride in the proportion of 0.08 ml/100 g of body mass, and sedation plus analgesia with intramuscular injection of a single dose of xylazine chloride in the proportion of 0.04 ml/100 g of body mass without any postoperative medication.

The surgical technique employed in this work was described by Takagi and Urist [1982]. The surgical access to the portion of the calvaria was obtained with an incision of approximately 3 cm to expose bone tissue. The tissue was raised until the periosteum was exposed and then incised and removed.

Using a trephine milling cutter of 8 mm diameter circular bone defects were made in the middle portion of the calvaria; they measured 8 mm in diameter and were approximately 1.5 mm deep, corresponding to an area of approximately 50 mm$^2$; they were mounted on a counter-angle device with a reduction of 16:1, coupled to a 1,500 rpm motor for implant, and were under constant irrigation with saline solution. Subsequently, each material was implanted in the bone defect with the exception of GIII. After implantation the materials mixed with blood of the bleeding site, forming a cohesive mass, and remained stable in place. The planes were sutured with silk thread 4.0.

After 15, 30 and 60 postoperative days, the animals were sacrificed by sulfuric ether inhalation. The specimens were fixed in 10% formalin. The tissues were decalcified in 5% nitric acid for a period of 72 h, and sent for routine laboratory processing for inclusion in paraffin. The blocks were sectioned at 5 μm thickness, in the transversal direction of the cranial portion, and stained with hematoxylin-eosin (HE) and sirius red (PFIG).

For morphometric assessment, the Leica QWin Image Processing Analysis System 2.6 was used (Leica Microsystems Imaging Solutions, Cambridge, UK) attached to an optical microscope (Leica Microstar IV). The following parameters were measured: (1) total neoformed mineralized sectional area, (2) linear extension of the primary defect – the measurement obtained between points identified in the central area of the bone edges, and (3) percentage of linear filling of the defect by neoformed mineralized tissue. For statistical analysis the Software Graph Pad Prism version 3.0® was used. The differences between the means were assessed using the following tests: analysis of variance (ANOVA, one way), $χ^2$ table of contingency for proportions and linear regression (LR), which used a level of significance of $α = 5\% (p ≤ 0.05)$.

**Results**

**Morphology and Composition of the Bioactive Glasses**

The measured axis ratio (0.47 ± 0.15 and 0.54 ± 0.18 for Biogran and Perioglas, respectively) and roundness (0.60 ± 0.15 and 0.63 ± 0.15 for Biogran and Perioglas, respectively) indicate that the particles had irregular shapes compatible with SEM images (fig. 1a, b). When observed with higher magnification, Biogran granules seemed to have more microfissures than Perioglas material (fig. 2a, b). EDS spectra (fig. 3a, b) showed the presence of oxygen, sodium, silicon, phosphorus and calcium. For both materials, semiquantitative analysis of chemical composition exhibited a reasonable match with the data of the manufacturer.

**Histomorphological Analysis**

At the following postoperative periods of time a histomorphological analysis was done:

**15 Days**

The bone edges of the defect presented an irregular morphological appearance, in the majority of cases in bevel, with small areas of reparative bone neoformation in all the groups (fig. 4a, b).

In GI and GII, mesenchymal fusiform cells of the fibroblastic and macrophagic type were seen, surrounding the whole particles of the implanted material. In GIII bone neoformation was seen (fig. 4c), with abundant proliferation of osteoblasts at the periphery, associated with intense and active vascular proliferation throughout the
entire extent of the defect. However, in two cases in which one of the remaining edges was bevel-shaped, there was bone neoformation in the form of thin plates in the subdural region.

In GI, stroma with edema and the presence of collagenous fibers exhibiting a denser appearance among the particles were observed, while in GII there was mild edema showing thin fibroses and in GIII there was abundant

Tissue Responses to Bioactive Glass Implants in Rat Calvaria

Fig. 2. Surface of bioactive glass particles observed by SEM: Biogran (a) and Perioglas (b).

Fig. 3. EDS spectrum of Biogran granule (a) and Perioglas granule (b).
deposition of collagenous fibers constituting dense fibrous tissue. In both groups, GI and GII, a chronic granulomatous reaction was noted around the particles, which were generally presented and distributed in the tissue section. Only occasionally in GI, did the particles appear fragmented with an outline of fibrous septation, in which areas of mineralization in plates were noted, without the involvement of the particles located over the supradural portion (fig. 4a).

30 Days
At the bone edges of the defect, bone neoformation with limited expansion in direct contact with the particles of the material was noted in GI and GII. In the fibrous and organized stroma there were vascular proliferations, and a large number of undifferentiated mesenchymal fusiform cells, fibroblasts and macrophages involving the implanted particles with circumjacent moderate granulomatous reaction (fig. 5a). The particles of the material frequently presented complete and incomplete fibrous septa and there was deposition of noncollagenous amorphous material on them (fig. 5b). In GI, in a few cases there were areas of mineralization in plates in the supradural region and focal mineralization nuclei among the particles of the material, seen only in the deeper areas of the defect whereas such findings were not encountered in GII.

In GIII, the bone edges of the defect had a regular morphological appearance without osteoblastic proliferation and bone neoformation. At the interstice, there was more organized granulation tissue with mild chronic inflammation, without fibroblastic proliferation, but with hyalinization plates and absence of mineralization areas (fig. 5c).

60 Days
In GI, restricted bone neoformation was observed when the bone edges of the defect were regular. However, in cases in which the edges were bevel-shaped, there was bone neoformation around the material which eventually enveloped its particles, while in GII, bone neoformation was observed with limited expansion in direct contact with the particles.

A large number of mesenchymal fusiform cells, fibroblasts and macrophages were seen in GI and GII among the particles of the implanted material, accompanied by abundant vascularization and a greater amount of dense, organized connective tissue (fig. 6a–c).

At this time, the particles appeared to be more fragmented. In a few cases, there were areas of mineralization only in supradural linear plates which did not depend on the particles of the implanted material. However, in GII, small irregular areas of calcification dystrophy were noted. There were no cells similar to osteoblasts.

In GIII, the bone edges showed no osteoblastic proliferation, but neoformed lamella bone was observed, with a disorganized arrangement. In the proximities of the neoformed bone, there were mild edema and chronic inflammation in regression, as well as denser connective tissue. In the central areas of the defect, the connective tissue appeared to be thinner with a smaller number of collagen fibers and a complete absence of mineralization areas (fig. 6d).

**Morphometric Analysis**
The morphometric data assessed is shown in tables 1 and 2. The measurement of the neoformed sectional area of the mineralized zone in GI and GII showed a very mild

<table>
<thead>
<tr>
<th>Group</th>
<th>Period</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 days</td>
<td>30 days</td>
</tr>
<tr>
<td>GI</td>
<td>µm²</td>
<td>%</td>
</tr>
<tr>
<td>µm²</td>
<td>46.1 ± 40.8</td>
<td>9.2 ± 8.1</td>
</tr>
<tr>
<td>%</td>
<td>LR, p = 0.13, NS; ANOVA, p = 0.59, NS</td>
<td></td>
</tr>
<tr>
<td>GII</td>
<td>µm²</td>
<td>%</td>
</tr>
<tr>
<td>µm²</td>
<td>34.8 ± 32.6</td>
<td>6.9 ± 6.4</td>
</tr>
<tr>
<td>%</td>
<td>LR, p = 0.09, NS; ANOVA, p = 0.47, NS</td>
<td></td>
</tr>
<tr>
<td>GIII</td>
<td>µm²</td>
<td>%</td>
</tr>
<tr>
<td>µm²</td>
<td>51.7 ± 29.0</td>
<td>10.3 ± 5.8</td>
</tr>
<tr>
<td>%</td>
<td>LR, p = 0.55, NS; ANOVA, p = 0.57, NS</td>
<td></td>
</tr>
</tbody>
</table>

NS = Not statistically significant.
Fig. 4. 5 days. a GI Biogran: Remaining bone edge at bevel (BE). Reparative bone neoformation area (NB). Interstice filled with particles with regular dimensions. Linear mineralization plate in the supradural portion (MP). PIFG. b GII Perioglas: Bone edge of defect at bevel. Interstice filled with particles of varying dimensions covered by dense connective tissue (DCT). HE. c GIII control: Remaining bone edge with irregular appearance and at bevel with bone neoformation. SCT = Slack connective tissue. HE.

Fig. 5. 30 days. a GI Biogran: Giant cells (G) covering the particles of the material (P). DCT = Dense collagenous tissue; F = numerous fibroblasts. HE. b GII Perioglas: Particle with fragmented appearance, presenting fibrous septation areas (FS). Deposition of dense connective tissue in these areas. Chronic inflammation close to the particles (CI). HE. c GIII control: Areas of hyalinization in plates (HP). VC = Vascular congestion. HE.

Fig. 6. 60 days. a GI Biogran: Dense connective tissue (DCT) covering the particles. Fibrous septation (FS) and deposition of noncollagenous amorphous matter (AM). PIFG. b GII Perioglas: Particles of fragmented material (P) with fibrous septation. Deposition of noncollagenous amorphous material on particles. Presence of dense connective tissue organized among the particles. PIFG. c SEM image. d GIII control: Interstitial space completely filled with dense connective tissue. HE.
tendency to increase. The LR for GI (p = 0.13) and ANOVA (p = 0.59) and the LR for GII (p = 0.09) and ANOVA (p = 0.47) did not differ significantly. In the control group the neoformed mineralized area did not change after the initial increase (15 days) and remained stable until the end of the experiment (LR: p = 0.55; ANOVA: p = 0.57). The statistical tests used did not show significant differences between the means of the groups for the studied parameters above.

The measurements referring to the linear extension of the primary defect did not present any statistically significant differences between the means of groups GI and GII at any of the time points observed. The comparison with the control group also did not show differences in the neomineralized area (ANOVA: p = 0.17) or in the percentage of the linear filling of the defect (χ²: p = 0.33).

**Discussion**

In some reports in the literature [Schepers et al., 1998; Piattelli et al., 2000], bioglasses are presented as bone substitutes with osteostimulating and osteoconductor potential, enabling the migration and proliferation of osteogenic cells and bone matrix synthesis. The osteoconductor properties of this biomaterial were occasionally observed in our study, though only in the area of the reparative bone and were limited to the edges of the defect.

Bone growth behavior is related to the size of the granules and to the variation in material composition and characteristics (e.g. chemical composition, presence of impurities, crystallinity, density, porosity and micro-/macroporosity) [Oonishi et al, 1999].

Composition of both bioactive granules used in this work was very similar. However, their morphology showed minor differences, such as irregular shapes and the number of microfissures, as demonstrated by SEM. There has been speculation about the relevance of the size of the bioactive glass particles for their reparative potential. The particles with a diameter between 300 and 355 μm present better reparative potential compared to the smaller- and the larger-sized particles, because they enable the formation of space between the particles for tissue infiltration and bone regeneration [Schepers et al., 1993]. However, Oonishi et al. [1999] observed new bone formation between the particles of Bioglass® with a diameter ranging from 100 to 300 μm, when implanted in femoral defects of 6 mm in rabbits. Moreover, Wheeler et al. [1998] compared the effect of the particle size on bone repair in rabbit femurs, implanting the two biomaterials (Perioglas and Biogran) and obtaining better osteogenic behavior in the defects filled with particles of 90–710 μm (Perioglas) than with those of a more uniform size of 300–355 μm (Biogran). In contrast, Norton and Wilson [2002] did not confirm any clinical or histological difference when these two (Perioglas and Biogran) bioactive glasses were used. In nonhealing calvarial defects in the rabbit model [Bergman and Litkowski, 1995], the particulate Bioglass 110–310 μm showed little, if any, bone in-fill at all time intervals after placement, in contrast to the spherical Bioglass particles. In their study, the combination of particulate Bioglass, 90–710 μm and 50% autogenous calvarial corti-

**Table 2.** Linear extension of primary defect and percentage of linear filling by neoformed mineralized tissue according to the time period and group

<table>
<thead>
<tr>
<th>Group</th>
<th>Period</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>15 days</td>
<td>4.2 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>30 days</td>
<td>5.1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>60 days</td>
<td>6.0 ± 0.9</td>
</tr>
<tr>
<td>%</td>
<td>10.8 ± 7.2</td>
<td>17.4 ± 11.3</td>
</tr>
<tr>
<td></td>
<td>23.2 ± 11.9</td>
<td></td>
</tr>
<tr>
<td>GII</td>
<td>15 days</td>
<td>5.4 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>30 days</td>
<td>4.8 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>60 days</td>
<td>5.2 ± 0.9</td>
</tr>
<tr>
<td>%</td>
<td>14.3 ± 2.1</td>
<td>17.2 ± 9.7</td>
</tr>
<tr>
<td></td>
<td>32.2 ± 6.2</td>
<td></td>
</tr>
<tr>
<td>GIII</td>
<td>15 days</td>
<td>6.8 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>30 days</td>
<td>6.0 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>60 days</td>
<td>6.8 ± 1.6</td>
</tr>
<tr>
<td>%</td>
<td>16.7 ± 10</td>
<td>17.3 ± 13</td>
</tr>
<tr>
<td></td>
<td>15.0 ± 4</td>
<td></td>
</tr>
</tbody>
</table>

NS = Not statistically significant.
can profoundly influence cell metabolism and function. Therefore, as previously mentioned [Oonishi et al., 1999], the comparison between the discordant published results should consider the different experimental and analytical conditions used.

Recent findings show that controlled release of the ionic dissolution products from bioactive glasses results in the regeneration of tissues. For the design of osteoinductive biomaterials, with the inclusion of Si, some dissolution product concentrations have a beneficial effect, but at higher concentrations could cause programmed cell death [Gough et al., 2004]. Previous work demonstrated that certain concentrations of Si cause nodule formation and apoptosis [Anderson, 2001; Xynos et al., 2001]. Calcium has been shown to have an effect on apoptosis induction, but the levels of Si concentration may be more important in apoptosis induction. The mechanism for in situ tissue regeneration involves upregulation of seven families of genes that control the osteoblast cell cycle, mitosis and differentiation. In the presence of critical concentrations of Si and Ca ions, osteoblasts which are capable of differentiating into a mature osteocyte phenotype begin to proliferate and regenerate new bone within 48 h [Hench et al., 2004]. Osteoblasts that are not in the correct phase of the cell cycle and unable to proceed towards differentiation are switched into apoptosis by the ionic dissolution products. A controlled release of soluble Ca and Si from bioactive glass-resorbable polymer composites leads to vascularization of soft tissue regeneration [Hench et al., 2004].

In our study the neoformed bone involving the particles of the biomaterials was observed in GI and GII. Reactive bone neoformation was noted only when they were close to the bone edges of the defect. The other particles, both in GI and in GII, were seen at 60 days, surrounded by fragmented fibrous connective tissue, migrating progressively towards the center of the defect.

A recent study [Bosetti et al., 2002] which focused on osteoblast cells described an increase of the collagen production by osteoblast culture in the presence of bioactive glasses. Moreover, Silver et al. [2001] reported that this alkalization of the medium increases the metabolism of osteoblastic cells, while those metabolic effects were smaller in fibroblasts. An interesting and, from the biological standpoint, important property of Bioglass and similar materials [Ducheyne, 1998] is that, upon exposure to physiological solutions or body fluids, they rapidly release soluble silicon into the environment in the form of silicic acid (due to ion exchange with H+ and H2O) and cause external alkalization. Changes in [H+] can profoundly influence cell metabolism and function [Busa and Nuccitelli, 1984] and several consequences of a moderate rise in pH (i.e., alkalization) are either harmless or potentially beneficial.

In our study, both processes of biomaterial resorption [as described by Oonishi et al., 1999] were addressed from both a solution-mediated and a cell-mediated perspective. When employing the solution-mediated perspective, the appearance of amorphous, noncollagenous material in central areas of the particles could be explained by the solubilization of this material as a result of the physical-chemical reaction with tissue fluids, while from the cell-mediated perspective, these aspects were associated with granulomatous inflammatory activity. However, we cannot completely discard the hypothesis that at least part of the fragmented regions observed in the particle’s thin sections corresponded with their irregular topography observed under the cutting plane of microtomy (e.g. fig. 1 shows these particles with very irregular contours).

The regenerative potential of bone tissue is intrinsically correlated to the size and morphology of the defect, with a different behavior for each implant site [Schmitz and Höllinger, 1986; Schmid et al., 1997; Cancian et al., 1999]. The morphology and size of the bone defect are some of the important factors to be considered in bone repair [Schmid et al., 1997]. In our study the morphological shape of the defect produced was chosen because it enabled adequate standardization and reproducibility, presented a low risk of fracture, and did not repair spontaneously [Takagi and Urist, 1982]. Additionally, it favored the assessment of the osteogenic behavior of the biomaterial [Bosch et al., 1998]. Critical defects could be defined as the smallest defects that do not enable the bone tissue to spontaneously regenerate throughout the animal’s entire life [Schmitz and Höllinger, 1986]. They are histologically characterized as being filled with fibrous connective tissue. Such aspects are compatible with our results, in which there was only reparative bone neoformation limited to the bone edges of the defect and scar repair in its central areas. This could be supported in the control group, which was kept without biomaterial implant and filled with fibrous connective tissue.

Angiogenesis should also be considered an important additional factor in critical bone defects, since there is an intimate spatial relation between angiogenesis and bone repair. The strict connection, both physical and biochemical, between blood vessels and bone has long been recognized. Genetic, biochemical and pharmacological studies have identified and characterized factors involved during...
bone formation and repair [Carano and Filvaroff, 2003]. Angiogenesis precedes bone formation because the loose perivascular connective tissue that accompanies the blood capillaries in proliferation constitutes a source of osteoprogenitor cells [Schmid et al., 1997; Aza et al., 2003]. Angiogenesis is a multistep process, which speeds up the differentiation and/or maturation of infiltrating osteoblasts and osteoblast precursor cells during neo-bone development, perhaps providing a conduit for the delivery of osteoinductive soluble signals [Murphy et al., 2004]. In adult rat calvaria the source of osteoblastic cells is extremely limited. Therefore, it is believed that the undifferentiated mesenchymal cells are recruited from the perivascular connective tissue of the dura mater to complete bone regeneration [Takagi and Urist, 1982]. We frequently observed abundant vascularization as well as a large number of mesenchymal fusiform cells, fibroblasts and macrophages among the particles of the implanted materials, accompanied by a great amount of dense, organized connective tissue, but no bone matrix synthesis.

In conclusion, the bioactive glasses used (Biogran and Perioglas), when implanted in critical defects under the experimental conditions studied, did not present osteogenic behavior, and acted only as a biomaterial for filling. These findings are probably correlated with the dissolution product concentrations, blood supply and quantities and viability of the osteogenic cells. Thus, further studies are necessary to elucidate these points.

References


