In vivo biological performance of a novel highly bioactive glass-ceramic (Biosilicate®): A biomechanical and histomorphometric study in rat tibial defects

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Received 13 October 2010; revised 20 May 2010; accepted 9 June 2010
Published online 2 February 2011 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.b.31795

Abstract: This study aimed to investigate bone responses to a novel bioactive fully crystallized glass-ceramic of the quaternary system P_2O_5-Na_2O-CaO-SiO_2 (Biosilicate®). Although a previous study demonstrated positive effects of Biosilicate® on in vitro bone-like matrix formation, its *in vivo* effect was not studied yet. Male Wistar rats ($n = 40$) with tibial defects were used. Four experimental groups were designed to compare this novel biomaterial with a gold standard bioactive material (Bioglass® 45S5), unfilled defects and intact controls. A three-point bending test was performed 20 days after the surgical procedure, as well as the histomorphometric analysis in two regions of interest: cortical bone and medullary canal where the particulate biomaterial was implanted. The biomechanical test revealed a significant increase in the maximum load at failure and stiffness in the Biosilicate® group (vs. control defects), whose values were similar to uninjured bones. There were no differences in the cortical bone parameters in groups with bone defects, but a great deal of woven bone was present surrounding Biosilicate® and Bioglass® 45S5 particulate. Although both bioactive materials supported significant higher bone formation; Biosilicate® was superior to Bioglass® 45S5 in some histomorphometric parameters (bone volume and number of osteoblasts). Regarding bone resorption, Biosilicate® group showed significant higher number of osteoclasts per unit of tissue area than defect and intact controls, despite of the non-significant difference in the osteoclastic surface as percentage of bone surface. This study reveals that the fully crystallized Biosilicate® has good bone-forming and bone-bonding properties. © 2011 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 97B: 139–147, 2011.

Key Words: bioactive glass and glass-ceramic, particulate biomaterial, bone histomorphometry, mechanical properties, animal study

INTRODUCTION

Millions of bone fractures occur every year worldwide. Although bone regeneration is a well-organized biological process, delayed union, or nonunions may result from insufficient wound healing responses and cause significant pain and morbidity, specially considering that the traditional surgical method of treatment includes the harvesting of autogenous bone from the iliac crest through an additional open incision.[1] The donor site morbidity and the limited availability of autografts concern its long-established use also in other clinical situations involving bone loss, such as various diseases or tumor resection.[2]

In this context, there is a critical need for technologies that are able to enhance bone healing, devoid of the disadvantages of the conventional procedure.[2] One promising treatment is the use of bioactive glasses as bone graft substitutes due to their ability to bond and integrate with living bone by forming a biologically active bonelike apatite layer on their surfaces.[3,4] Osteoproduction, which is the proliferation of new bone within a bone defect site,[4] occurs as a consequence of rapid reactions on bioactive glass surface. Moreover, surface reactions release critical concentrations of soluble silicon, calcium, phosphorus and sodium ions that stimulate the attachment, proliferation and differentiation of osteoblasts (bone-forming cells).[4–8]

Bioglass® 45S5, a silica-based melt-derived glass (45% SiO_2, 24.5% Na_2O, 24.5% CaO, 6% P_2O_5), has been known for many years as the most bioactive composition among

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numerous bone-bonding glasses.[4] It was first introduced in the early 70s by Hench and, since then, it has been reached many clinical applications, including the repair of periodontal bone defects, maxillofacial defects reconstruction, spinal surgery, and bone replacement.[4]

Many works have proved that Bioglass® 45S5 has good bone bonding properties and a stimulatory effect on osteogenesis.[9–11] Human bone cell culture over the surface of Bioglass® 45S5 discs demonstrated enhanced formation of bone nodules, which are three-dimensional structures composed of mineralized extracellular matrix and cell aggregates with organizational complexity similar to natural bone grown *in vivo*.[6] In addition, the ionic products of Bioglass® 45S5 induced an increase in both human osteoblast proliferation and expression of insulin-like growth factor II (IGF-II), a potent osteoblast mitogenic growth factor.[7] Indeed, the mechanism for *in situ* tissue regeneration involves upregulation of seven families of genes that control the osteoblast cell cycle, mitosis, and differentiation.[12]

Despite its well-known stimulatory effects on osteogenesis, the use of monolithic Bioglass® 45S5 for bone engineering applications has been limited mainly due to its relatively poor mechanical properties.[13,14] Considering this important matter, our research group has developed nucleation and growth thermal treatments to obtain a novel fully crystallized bioactive glass-ceramic of the quaternary P_2O_5-Na_2O-CaO-SiO_2 system (Biosilicate®, patent application WO 2004/074199). Crystallinity significantly changes the fracture characteristics of glasses leading to glass-ceramics with improved mechanical properties. On the other hand, the introduction of crystalline phases could, in principle, decrease the bioactivity.[13,15] However, interestingly, experiments have demonstrated that Biosilicate® exhibits higher bone-like matrix formation than its parent glass and Bioglass® 45S5 in an osteogenic culture system,[16] while exhibiting improved mechanical properties.[17]

This important finding stimulated us to progress the understanding of the effects of this fully crystallized glass-ceramic on osteogenesis. Although Biosilicate® promotes enhanced *in vitro* bone-like matrix formation; its effect on *in vivo* bone growth has not been studied yet. In this context, this study aims to investigate and compare the effects of Biosilicate® and Bioglass® 45S5 (gold standard biomaterial) on the bone regenerative process of surgically created defects in rat tibia.

METHODOLOGY

Biomaterials

High purity silica and reagent grade calcium carbonate, sodium carbonate, and sodium phosphate were used to obtain the generic glass compositions: Bioglass® 45S5 (45% SiO_2, 24–25% Na_2O, 24–25% CaO, 6% P_2O_5; wt %)[4] and Biosilicate® parent glass (40–50% SiO_2, 20–25% Na_2O, 20–26% CaO, 3–7% P_2O_5; wt %). The chemicals were weighed and mixed for 30 min. Premixed batches were melted in Pt crucible at a temperature range of 1250 to 1380°C for 3 h in an electric furnace (Rapid Temp 1710 BL, CM Furnaces, Bloomfield, NJ) at the Vitreous Materials Laboratory of the Federal University of São Carlos (São Carlos, SP, Brazil). Immediately after, the liquid was rapidly cooled in cylindrical stainless steel molds in order to achieve vitreous plaques.

To obtain the fully crystallized Biosilicate® glass-ceramic, Biosilicate® parent glass cylinders underwent cycles of thermal treatment to promote their crystallization. The first thermal cycle was performed at a relatively low temperature, just above the glass transition temperature ($T_g \sim 550$°C) to promote volumetric nucleation of crystals. Afterward, the nucleated samples were submitted to further treatment at about 100°C above the nucleation temperatures to foster crystal growth. The detailed compositions and thermal treatment schedules to obtain the Biosilicate® glass-ceramic are described in the patent WO 2004/074199.[18]

Biosilicate® and Bioglass® 45S5 cylinders were crushed and the powders were sieved to select particles in the 180–210 μm range, which were used to fill bone defects in this study.

Experimental design

Forty male Wistar rats (aged 12 weeks and weighting 250–300 g) were used in this study. They were maintained under natural conditions of humidity and temperature, light-dark periods of 12 h, and with free access to water and commercial diet. All animal handling and surgical procedures were strictly conducted according the Guiding Principles for the Use of Laboratory Animals. This study was approved by the Animal Care Committee guidelines of the Federal University of São Carlos.

Rats were randomly distributed into four groups of 10 animals each:

- Control intact group (intact tibias without the surgical creation of bone defects)
- Control defect group (bone defects without any filler)
- Bioglass® 45S5 group (bone defects filled with the gold standard biomaterial)
- Biosilicate® group (bone defects filled with the biomaterial to be tested).

Surgical procedures

Bilateral noncritical size bone defects were surgically created at the upper third of the tibia (10 mm distal of the knee joint). Surgery was performed under sterile conditions and general anesthesia induced by intraperitoneal injection of Ketamine/Xylazine (80/10 mg/kg). The medial compartment of the tibia was exposed through a longitudinal incision on the shaved skin. A standardized 2.5-mm-diameter bone defect was created by using a motorized drill under copious irrigation with saline solution. Perforations were performed unicortically, throughout the entire depth of a single cortex. Holes were compressed with gauze for 5 min. Immediately afterward, bone cavities were completely filled with the corresponding biomaterial in the treated animal groups. After implantation, the cutaneous flap was replaced and sutured with resorbable polyglactin, and the skin was disinfected with povidone iodin. The health status of the rats was monitored daily.

Twenty days after the surgical procedure, animals were euthanized by anesthesia overdose and tibias were bilaterally harvested for analysis.

Mechanical test

Biomechanical properties of the left tibia were determined by a three-point bending test in an Instron® Universal Testing Machine (USA, 4444 model, 1 KN load cell).

Tibias were placed on a special holding device with two supports located at a distance of 21.7 mm. The load cell was perpendicularly positioned at the exact site of the bone defect, prior to submitting the medial surface (repair area) to traction. A 5-N preload was applied in order to avoid specimen sliding. Finally, the bending force was applied at a constant deformation rate of 5 mm/min until fracture occurred. From de load-deformation curve, the maximum load at failure (N), structural stiffness (N/mm), and energy absorption (J) were obtained.

Bone histomorphometric analysis

After harvesting the right tibias, the proximal segment was fixed in 70% ethanol, dehydrated, embedded in methylmetacrylate, and transversally sectioned using a Polycut S microtome (Leica, Heidelberg, Germany). Five-micrometer-thick sections were obtained from the centre of the bone defect site. Sections were stained with 0.1% toluidine blue at pH 6.4, and at least two nonconsecutive sections were examined for each sample. Data were obtained using the Osteomeasure semiautomatic image analyzer (Osteometrics, Atlanta, GA), with which each structure (mineralized and osteoid tissue) and cell (osteoblast and osteoclast) was first identified by a single expert and, afterwards, quantified by the software.

Measurements were first performed at the inner and outer shells of cortical bone. Static parameters of cortical volume (Ct.V/TV, %) and thickness (Ct.Th, μm) were obtained. Afterwards, measurements were performed at two standardized fields inside the medullary canal where biomaterial particles were placed. The mean tissue area (T.Ar) analyzed was 0.3392 ± 0.0004 mm^2. The static indices obtained were bone volume as a percentage of tissue volume (BV/TV, %), osteoid volume as a percentage of tissue volume (OV/TV, %), osteoid thickness (O.Th, μm), number of osteoblasts per unit of tissue area (N.Ob/T.Ar, /mm^2), osteoblast surface as a percentage of bone surface (Ob.S/BS,%), number of osteoclasts per unit of tissue area (N.Oc/T.Ar, /mm^2), and osteoclast surface as a percentage of bone surface (Oc.S/BS, %). Histomorphometric indices were reported according to the standard nomenclature recommended by the American Society of Bone and Mineral Research.[19] All measurements were performed by a single observer who had no knowledge of which experimental group the samples belonged.

Statistical analysis

The normality of all variable distribution was verified using Shapiro-Wilk's W test. For the variables that exhibited normal distributions (BV/TV, OV/TV, O.Th, N.Ob/T.Ar, Ob.S/BS, N.Oc/T.Ar, energy absorption), comparisons among groups were made using one-way analysis of variance (ANOVA), complemented by Tukey HSD post-test analysis. Kruskal-Wallis test was performed for the variables not exhibiting normal distributions (Ct.V/TV, Ct.Th, Oc.S/BS, maximum load, stiffness). STATISTICA version 7.0 (data analysis software system—StatSoft Inc.) was used to carry out the statistics analysis. Values of $p < 0.05$ were considered statistically significant. Results are all presented as mean ± standard error of the mean.

RESULTS

During the experiments, the particulate could easily pack within the defect site and stay in place, without problems with particulate migrating. The difficult of placing and retaining the particles was overcome by the cohesive mass formed when Biosilicate® and Bioglass® 45S5 particles were placed in bleeding sites. This cohesive mass was rapidly formed after the implantation, was accompanied by hemostasis and had an aspect of blood clot, suggesting that a gel layer was formed on the surface in contact with the moisture. Both particulate materials packed similarly in the defect sites. It was also not found discrepant differences in the irregular shape of Biosilicate® and Bioglass® 45S5 particles 20 days after the surgical procedure (Figure 1). Neither postoperative complications nor behavioral changes were observed. All animals recovered from the bilateral surgery and returned rapidly to their normal diet and movement pattern. The sacrifice time was reached without any adverse events.

Bone mechanical properties

Table I summarizes the data of the biomechanical test. Bones which had the created defects filled with Biosilicate® exhibited similar biomechanical properties than intact controls. In addition, they could abide a significant large load before failure occurrence and exhibited higher stiffness than unfilled bone defects (defect controls). No significant difference was found in the comparison between Bisilicate® and Bioglass® 45S5 groups, instead of the nonsignificant difference among bone defects filled with Bioglass® 45S5 and unfilled bones.

Bone histomorphometry

Cortical volume (Ct.V/TV) was significantly decreased in experimental groups submitted to the surgical creation of bone defects (control defect, Bioglass® 45S5 and Biosilicate® groups) in comparison with intact bones (Figure 2). The cortical thickness was also significantly higher in control intact group than in Biosilicate® and Bioglass® 45S5 groups. No significant difference was found among bone defects filled with Biosilicate®, Bioglass® 45S5, and unfilled bone defects (control defects).

However, a great deal of high cellularized woven bone was present circumjacent to the biomaterial particles (Figure 1) and the quantitative analysis demonstrated that bone volume (BV/TV) was significantly higher in the Biosilicate® and Bioglass® 45S5 groups than in both control groups (Figure 3), with defect controls also exhibiting osteogenic

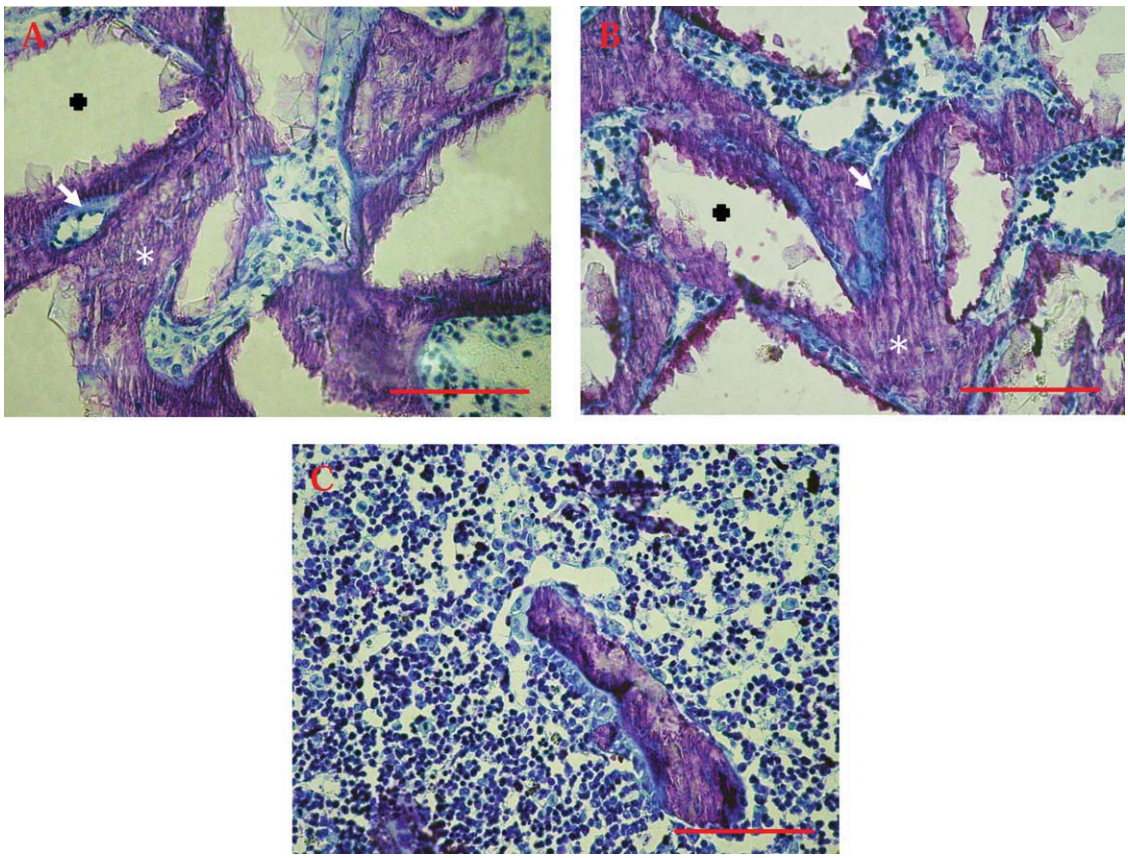

FIGURE 1. Biosilicate® (A) and Bioglass® 45S5 (B) particles (+) envolved by cellularized woven bone (*) that was parcially covered by osteoid deposits (↑) and rows of active osteoblasts. In C, osteogenic activity also verified in the control defect group. Toluidine blue staining (0.1%), bar = 100 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

activity in the medullary region. By comparing Biosilicate® with the gold standard biomaterial (Bioglass® 45S5), we found a significantly higher bone volume in the Biosilicate® group (Figure 3).

Newly formed bone was observed surrounding Biosilicate® and Bioglass® 45S5 particles, in a direct contact with the material surface (Figure 1). Moreover, interparticle spaces were also filled by new bone trabeculae, exhibiting trabeculae bridges formation between some particles.

Nonmineralized bone tissue (osteoid) deposits were present over the surface of the woven bone (Figure 1). Osteiod volume was significantly higher in the Biosilicate® group than in both control groups (Figure 4), and the osteoid thickness higher than in control intacts (Figure 5). Bioglass® 45S5 and control defect group also exhibited higher osteoid volume than control intact group, without differences regarding osteoid thickness.

Active cuboidal osteoblasts were present lining areas of osteoid tissue (Figure 1). Bone defects filled with Biosilicate® showed a significant higher number of osteoblasts per unit of tissue area (N.Ob/T.Ar) than defects filled with Bioglass® 45S5, unfilled defects and intact controls (Figure 6). The number of osteoblasts was also significantly higher in the Bioglass® 45S5 group than in intact and defect controls, instead of the greater osteoblast surface as a percentage of bone surface (Ob.S/BS) in control groups in

TABLE I. Mechanical Properties of Rat Tibias (Control Intact Group) After Surgically Created Bone Defects (Control Defect Group) Were Filled With Biomaterials (Bioglass® 45S5 and Biosilicate® Groups)

Indices	Control Intact	Control Defect	Bioglass® 45S5	Biosilicate®
Maximum Load (KN)	0.072 ± 0.003	0.05 ± 0.002*	0.054 ± 0.005*	0.076 ± 0.009**
Energy Absorption (J)	0.049 ± 0.004	0.023 ± 0.003*	0.034 ± 0.004	0.042 ± 0.006
Stiffness (N/m)	151 ± 12	107 ± 9*	122 ± 11	168 ± 16**

Data are expressed as means ± SEM.
* $p < 0.05$ versus control intact; ** $p < 0.05$ versus control defect.

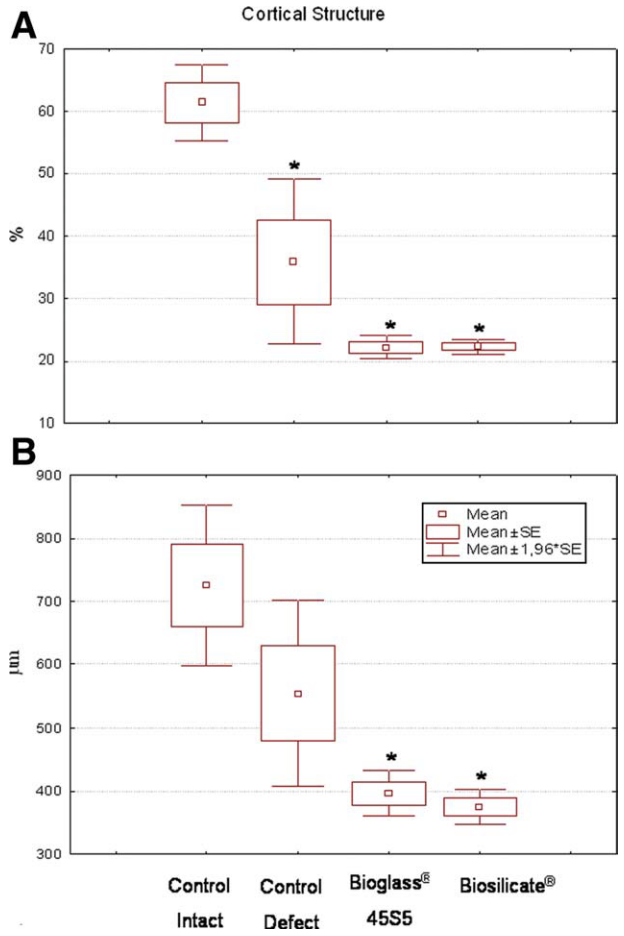

FIGURE 2. (A) Cortical Volume (Ct.V/TV, %) and (B) Cortical thickness (Ct.Th, μm) 20 days after intact tibias (control intact) had bone defects been surgically created (control defect) and filled with biomaterials (Bioglass® 45S5 and Biosilicate® groups). $p < 0.05$ vs. control intact. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

comparison with the two treated groups (Biosilicate® and Bioglass® 45S5) (Figure 7).

Regarding the histomorphometric parameters of bone resorption, the Biosilicate® group showed a significant higher number of osteoclasts per unit of tissue area than both control groups (Figure 8), instead of the nonsignificant difference in the osteoclast surface as a percentage of bone surface (Figure 9).

DISCUSSION

Since a previous *in vitro* study demonstrated the high osteogenic potential of Biosilicate®,[16] we hypothesized that this novel biomaterial could also greatly induce *in vivo* bone growth. Results from the present work showed that, instead of the incomplete cortical healing, bones which had the surgically created defects filled with Biosilicate® particulate exhibited significant larger volumes of newly formed bone tissue and greater mechanical properties.

The bone mass, as well as the quality and arrangement of its microstructural elements, influences bone mechanical

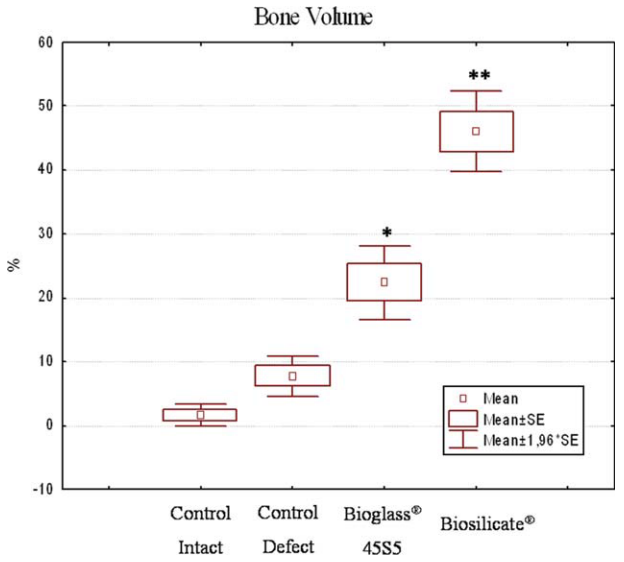

FIGURE 3. Bone volume as a percentage of tissue volume (BV/TV, %) 20 days after intact tibias (control intact) had bone defects been surgically created (control defect) and filled with biomaterials (Bioglass® 45S5 and Biosilicate® groups). *$p < 0.05$ vs. control intact and control defect. **$p < 0.05$ vs. control intact, control defect and Bioglass® 45S5. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

properties.[20] Therefore, the improved bone load-bearing capacity and stiffness stimulated by Biosilicate® probably mirror the huge amount and/or spatial distribution of newly formed bone into the defect site, the presence of the biomaterial particulate and the strong bond between them, which can be very useful during bone healing processes.

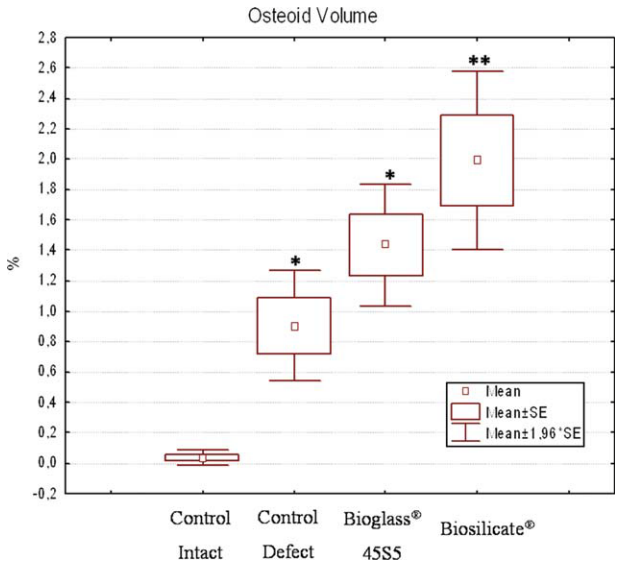

FIGURE 4. Osteoid volume as a percentage of tissue volume (OV/TV, %) 20 days after intact tibias (control intact) had bone defects been surgically created (control defect) and filled with biomaterials (Bioglass® 45S5 and Biosilicate® groups). *$p < 0.05$ vs. control intact **$p < 0.05$ vs. control intact and control defect. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

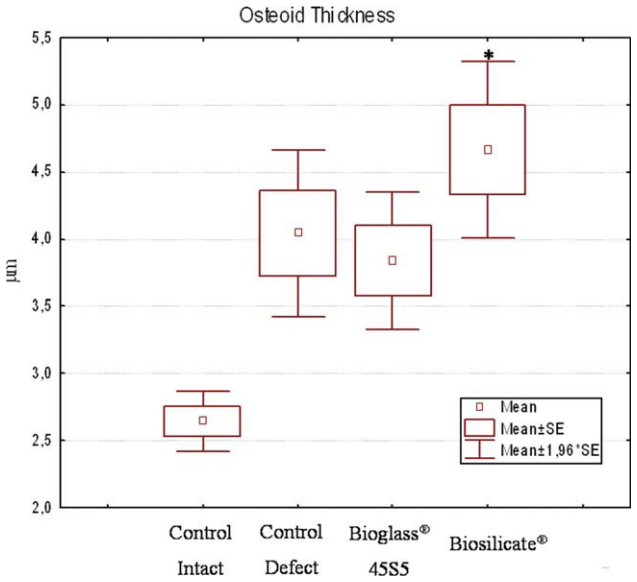

FIGURE 5. Osteoid thickness (O.Th, µm) 20 days after intact tibias (control intact) had bone defects been surgically created (control defect) and filled with biomaterials (Bioglass® 45S5 and Biosilicate® groups). $p < 0.05$ vs. control intact. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

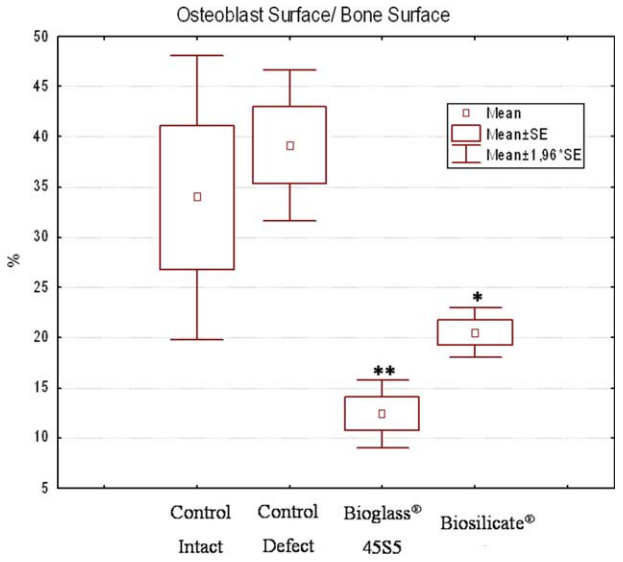

FIGURE 7. Osteoblast surface as a percentage of bone surface (Ob.S/BS,%) 20 days after intact tibias (control intact) had bone defects been surgically created (control defect) and filled with biomaterials (Bioglass® 45S5 and Biosilicate® groups). *$p < 0.05$ vs. control defect **$p < 0.05$ vs. control intact and control defect. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Newly formed bone was observed surrounding Biosilicate® and Bioglass® 45S5 particles, in a direct contact with the material surface. Moreover, interparticle spaces were also filled by new bone trabeculae, exhibiting trabeculae bridges formation between some particles.

Oonishi et al. demonstrated a progressive bone formation at the surface of Bioglass® 45S5 particles filling bone defects in rabbits.[21] According to these authors, the new bone had a trabecular architecture that incorporates the glass particles within the bone structure.

In this study, Biosilicate® particulate implantation resulted in both mineralized and osteiod matrix volumes augmentation. These findings uphold the working principle of bioactive glasses or glass ceramics, which is considered

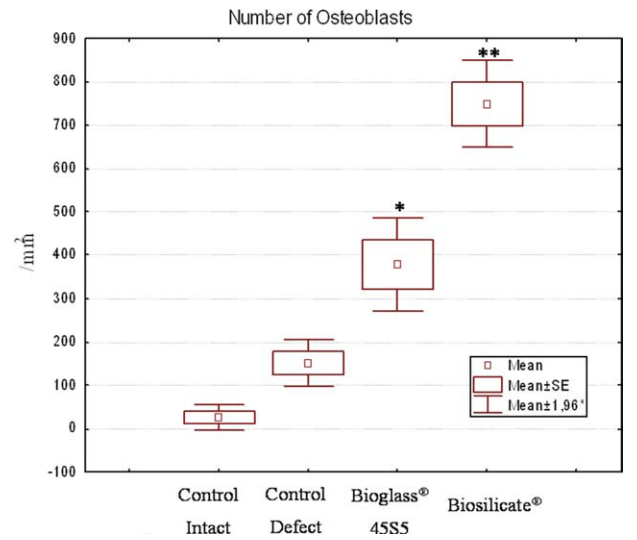

FIGURE 6. Number of osteoblasts per unit of tissue area (N.Ob/T.Ar, / mm^2) 20 days after intact tibias (control intact) had bone defects been surgically created (control defect) and filled with biomaterials (Bioglass® 45S5 and Biosilicate® groups). *$p < 0.05$ vs. control intact and control defect. **$p < 0.05$ vs. control intact, control defect and Bioglass® 45S5. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

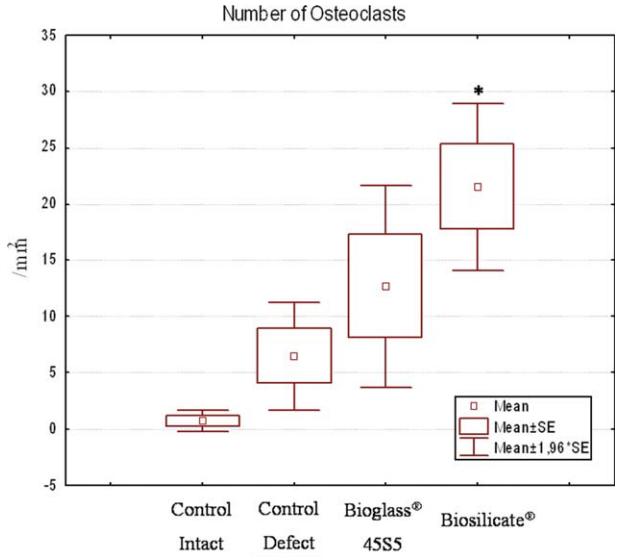

FIGURE 8. Number of osteoclasts per unit of tissue area (N.Oc/T.Ar, / mm^2) 20 days after intact tibias (control intact) had bone defects been surgically created (control defect) and filled with biomaterials (Bioglass® 45S5 and Biosilicate® groups). *$p < 0.05$ vs. control intact and control defect. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

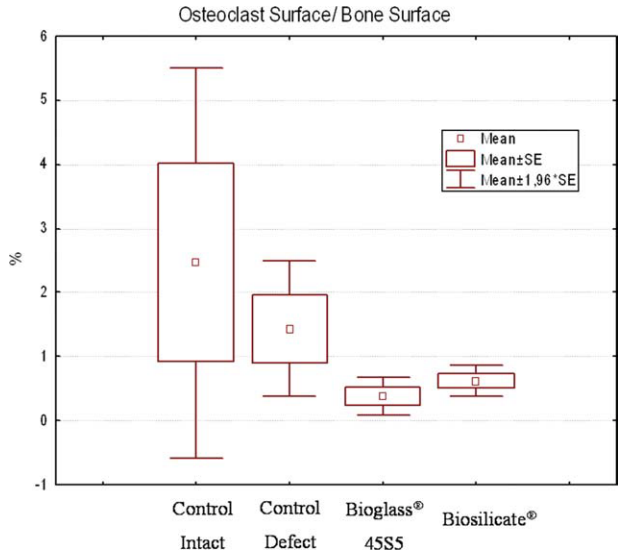

FIGURE 9. Osteoclast surface as a percentage of bone surface (Oc.S/BS,%) 20 days after intact tibias (control intact) had bone defects been surgically created (control defect) and filled with biomaterials (Bioglass® 45S5 and Biosilicate® groups). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

to be a rapidly deposition of a layer of hydroxycarbonate apatite (HCA) on the surface of the silica gel as a result of exchange between calcium and silica ions when bioactive glass came into contact with living tissues.[4,15] Entirely inorganic reactions, previously described by Hench,[4] lead to rapid growth of HCA bone mineral on the glass surface and to a rapid proliferation of new bone within a bone defect site, a process termed osteoproduction.

The size, crystal habit, orientation, and composition of the HCA phase are equivalent to that of the mineral phase of living bone. This phase provides binding site for osteoprogenitor cells attachment, proliferation and differentiation; hence cell function is favored and osteoid matrix is generated.[4] Indeed, collagen fibrils are incorporated within the mineralized layer of newly forming bone, as demonstrated by electron microscopy of the glass interface bonded to bone.[22]

However, the rate of the reactions that occurs on the material surface is highly sensitive to the material composition.[4] In the present study, comparisons among bone defects filled with Biosilicate® and Bioglass® 45S5, both belonging to the quaternary P_2O_5-Na_2O-CaO-SiO_2 system, demonstrated significant larger areas of mineralized matrix formation in Biosilicate® group on day 20 post-surgery. This *in vivo* finding corroborates previous *in vitro* results, which showed that, although Biosilicate® and Bioglass® 45S5 discs supported osteogenesis, significantly larger areas of calcified matrix were detected for the fully-crystallized glass-ceramic (Biosilicate®) after 17 days in an osteogenic culture system.[16]

Taken together, these results suggest that the special nucleation and growth thermal treatments developed to improve the mechanical properties of glasses may also alter other important properties of the material, such as the dissolution rate.[16] In other words, during the crystallization process of Biosilicate®, selective attachment of atoms on the crystal growth front may occur, resulting in the production of chemical gradient from the crystal centre to its border, contrarily to Bioglass® 45S5 that has a homogeneous glass matrix in which the concentration of chemical elements is supposed to be uniform all over the material.[16] This structural dissimilarity most probably favor the kinetics of the reaction stages on the Biosilicate® surface, thus leading to a higher bone-like tissue formation. Likewise, the ionic products of the higher kinetics of Biosilicate® dissolution may also result in more expressive cell recruitment; that, in turn, influence bone formation as well. In effect, the rate of bone formation is largely determined by the rate of osteoprogenitors replication; the number of active osteoblasts, which was indeed significantly higher in Biosilicate® group; and the life-span of these cells.[23,24]

In fact, recent research shows that the critical stage of reaction for bone regeneration is related with cell attachment and proliferation on the biomaterial surface,[3,4,8] mechanisms still beginning to be understood. It's believed that cellular responses constitute an essential requirement for the bioactive behavior.[25] Multiple studies have shown that an exposure of human primary osteoblasts to the soluble chemical extracts of Bioglass® 45S5 is responsible for activating several families of genes within few hours, including genes encoding nuclear transcription factors and potent growth factors.[5–8] These findings indicate that Class A bioactive glasses (i.e., glasses with composition between 42 to 52 mol% SiO_2, with rapid rates of reaction stages)[4] promote osteogenesis through a direct control over genes that regulate cell cycle induction and progression, which can explain the great number of osteoblasts per unit of area (N.Ob/T.Ar) in Biosilicate® and Bioglass® 45S5 groups in this study.

Conversely, the osteoblast surface as a percentage of bone surface was significantly higher in the control group. Therefore, despite the huge number of osteoblasts observed in bone defects filled with Biosilicate® and Bioglass® 45S5, the magnitude of the bone formation was higher than the increment in the number of cells. In fact, any cells or organic molecules are required for the rapid growth of HCA bone mineral on the bioactive glass or glass-ceramic surface.[4] However, isolating the physicochemical phenomena from the reactions affected by cellular activity would be a mechanistic explanation for the multiple and parallel interactions occurring at the material-tissue interface. Considering this important matter, we hypothesize that cellular activity and function may also be enhanced by the local chemical environment created by Biosilicate® and Bioglass® 45S5 particulate implantation. In this case, these bioactive materials could influence not only cell proliferation but also the osteogenic potential of these cells.

Motivated by the remarkable number of osteoblasts surrounding Biosilicate® granules found in the present work, further studies must be developed to investigate the influence of the inorganic ions released by this novel glass-

ceramic on the genetic control of the osteoblast cell cycle and function.

Moreover, once new bone matrix has been formed by osteoblasts, supplementary efforts should be spent on the investigation of its degradation caused by osteoclasts. The remodeling process, including bone-forming activity and bioresorption, is an important prerequisite to the obtainment of the desired complete reorganization of the implanted biomaterial into bone tissue.[26,27]

Although many studies have focused on bone formation from both mature and bone marrow-derived osteoblasts on various biomaterials, few investigations address osteoclasts role in bone tissue engineering.[26] Hamadouche et al. investigated the long-term *in vivo* bioactivity and degradability of bulk sol-gel-derived glasses (58S and 77S Bioglass®), using bulk 45S5 melt-derived Bioglass® as a control.[28] Absorbability was observed for both sol-gel-derived glasses starting 12 weeks after implantation, and reaching 40% after 52 weeks. The authors assumed that this absorbability was partially related to osteoclastic resorption as viable osteoclasts-like cells were found to be in direct contact with the glass surfaces. On the other hand, no degradation could be measured in the case of Bioglass® 45S5 within one year of implantation.

In this study, Biosilicate® group showed a significant higher number of osteoclasts per unit of tissue, instead of the nonsignificant difference in the osteoclastic surface as percentage of bone surface. Nevertheless, this study investigated a single stage of the bone regenerative process induced by a well-defined size range of the biomaterial particulate. Then, further studies should be developed to provide additional information concerning the dynamics of the Biosilicate® influence on bone remodeling and the structural changing behavior of this biomaterial. This supplementary investigation should focus on the final aim of bone induced-regeneration, which is the ability to restore the bone architecture with biological and mechanical properties similar to the uninjured one.

CONCLUSION

This study reveals that the fully crystallized Biosilicate® has good bone-forming properties. The novel bioactive glass-ceramic supported a great number of osteoblasts inside the defect site and enhanced new bone formation, as well as improved mechanical properties of the bone. Osteoproductive properties was evidenced for both materials analyzed in this study, however, Biosilicate® promoted enhanced bone formation and osteoblast recruitment in comparison with the gold standard bioactive glass, (Bioglass® 45S5); although these effects were not accompanied by significant differences regarding the mechanical properties of the bone. However, bone defects filled with Biosilicate® exhibited similar bone mechanical behavior than intact bones, which may be a crucial factor for therapeutic success, especially considering load-bearing situations. Although further long-term studies and clinical trials are required, the findings here presented point to a promising use of Biosilicate® for bone engineering applications.

ACKNOWLEDGMENTS

Authors would like to thank Keico Okino Monaka, Ph.D., from the Department of Physiology, Federal University of São Carlos. They are also grateful to Rosimeire Costa, for her Technical Assistance. This study was supported by grants from Capes, Brazil.

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