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# Histopathological, cytotoxicity and genotoxicity evaluation of Biosilicate® glass–ceramic scaffolds

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**Abstract:** This study evaluated the biocompatibility of Biosilicate® scaffolds by means of histopathological, cytotoxicity, and genotoxicity analysis. The histopathologic analysis of the biomaterial was performed using 65 male rats, distributed into the groups: control and Biosilicate®, evaluated at 7, 15, 30, 45, and 60 days after implantation. The cytotoxicity analysis was performed by the methyl thiazolyl tetrazolium (MTT) assay, with various concentrations of extracts from the biomaterial in culture of osteoblasts and fibroblasts after 24, 72, and 120 h. The genotoxicity analysis (comet assay) was performed in osteoblasts and fibroblasts after contact with the biomaterial during 24, 72, and 96 h. In the histopathology analysis, we observed a foreign body reaction, characterized by the presence of granulation tissue after 7 days of implantation of the biomaterial, and fibrosis connective tissue and multinucleated giant cells for longer periods. In the cytotoxicity analysis, extracts from the biomaterial did not inhibit the proliferation of osteoblasts and fibroblasts, and relatively low concentrations (12.5% and 25%) stimulated the proliferation of both cell types after 72 and 120 h. The analysis of genotoxicity showed that Biosilicate® did not induce DNA damage in both lineages tested in all periods. The results showed that the Biosilicate® scaffolds present *in vivo* and *in vitro* biocompatibility. © 2012 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 00A:000–000, 2012.

**Key Words:** biocompatibility, tissue response, bioactive material, scaffold, biomaterial

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## INTRODUCTION

Millions of bone fractures occur every year worldwide. Among them, 10–30% result in nonunions[1] and others exhibit delayed healing,[2] indicating that, in several cases, bone tissue cannot heal by itself.

In this context, there is a critical need to develop technologies and materials that are able to minimize the time of bone healing, as well as to decrease the chance of developing complications resulting from abnormal process of bone repair.[3] A promising treatment is the use of bioactive glasses, due to their ability to bond to the bone by forming a biologically active bone-like apatite layer on the tissue surface.[4,5] Due to these properties, many authors have demonstrated that bioactive glasses are able of inducing osteogenesis and stimulating fracture healing.[3–5]

Despite the stimulatory effects of bioactive glass on bone metabolism, the use of these biomaterials has been restricted due to their poor mechanical properties.[5,6] In this context, our research group has developed a novel fully crystallized bioactive glass–ceramic from the quaternary $P_2O_5$–$Na_2O$–CaO–$SiO_2$ system (Biosilicate®, patent application WO 2004/074199).[7] Significant crystallization of the original glass may lead to enhanced mechanical properties of monolithic pieces and less sharp and abrasive particles when the material is milled into a powder.

An *in vitro* study demonstrated that Biosilicate® enhances bone-like matrix formation in comparison to its parent glass and to Bioglass® 45S5 (which has been known as the golden standard bioactive glass, with the highest bioactivity index) in an osteogenic cell culture system.[5] By comparing the growth of osteogenic cells on Biosilicate® and Bioglass 45S5® disks for a period of up to 17 days, Moura et al.[5] found that, although no significant differences were detected in terms of protein content and alkaline phosphatase activity on days 11

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and 17, Biosilicate® induced significantly larger areas of calcified matrix on day 17. In addition, Granito et al.[8] found that Biosilicate® was capable of increasing the biomechanical properties, the bone volume and the number of osteoblasts at the site of bone callus of tibial defects in rats compared with the Bioglass® 45S5. In addition, other authors demonstrated the stimulatory effects of Biosilicate® on bone consolidation in normal rats[9] and osteoporotic rats.[10]

Although the positive effects of Biosilicate® on bone have been demonstrated by *in vitro* and *in vivo* studies, the mechanism by which this material acts on bone is not fully understood. Before this biomaterial can be used with confidence as a therapeutic modality, to definitively determine its safety and efficacy it is necessary to fully investigate the physical and chemical characteristics, as well as all the biological responses to this therapy.[11] Hence, the aim of this study was to evaluate the *in vivo* (histopathological) and *in vitro* (cytotoxicity and genotoxicity) biocompatibility of Biosilicate®.

## MATERIALS AND METHODS

### Preparation and characterization of Biosilicate® scaffolds

Biosilicate® was obtained by melting reagent grade raw materials ($Na_2CO_3$ – JT Baker, $CaCO_3$ – JT Baker, $Na_2HPO_4$ – JT Baker, and $SiO_2$ – Zetasil 2) in Pt crucible at 1450°C. The glass was splat cooled and heat treated until full crystallization. More details of the synthesis of Biosilicate® are described in the WO 2004/074199 patent.[7] Thus, the glass pieces were crushed in an agate and milled in a planetary ball mill at 550 rpm for 240 min.

In this study, Biosilicate® scaffolds were used, manufactured by a method based in the addition of a porogen agent.[12] Initially, we prepared 100 mL of a suspension containing 67 vol % of isopropyl alcohol anhydrous (QHEMIS), 3 vol % of polyvinyl butiral (Butvar B-98), 24 vol % of carbon black (CABOT BP-120), and 6 vol % of Biosilicate®, was prepared. Then isopropyl alcohol, PVB, and Biosilicate® were mixed in an agate jar containing 30 agate spheres ($\phi = 10$ mm) and mixed in a planetary ball mill (Pulverisette 6 – FRITSCH) at 550 rpm for 1 h. The agate spheres were removed from the suspension, and the presieved carbon black (300–600 μm) was added, being mixed for 5 min at 150 rpm. The suspension was poured into a plastic container and dried with a blower. The resulting granulated powder was passed in a nylon sieve (18 MESH) and pressed in two steps. In the first step, the powder was uniaxially pressed in a cylindrical stainless steel mould ($\phi = 12$ mm) at 20 MPa for 10 s. In the second step, the samples were isostatically pressed at 100 MPa for 10 s. Finally, the sintering of the samples was performed in four stages: (1) heating at 1°C $min^{-1}$ from room temperature up to 375°C, with a holding time of 2 h, for PVB removal; (2) heating at 1°C $min^{-1}$ from 35°C to 610°C, with a holding time of 2 h, for carbon black burnout; (3) heating at 1°C $min^{-1}$ from 610°C to 975°C, with a holding time of 5 h, for Biosilicate® sintering; and (4) cooling at 5°C $min^{-1}$ from 975°C to the room temperature. Scaffolds, with approximately 8 mm (diameter) × 2 mm (thickness) were obtained. After synthesis, these biomaterials were sterilized overnight in an electric oven at 130°C.

The total and apparent porosities were measured in 15 scaffold samples using the immersion technique based in the Archimedes's principle. The scaffold morphology was evaluated by stereomicroscopy (Leica MZ75).

### *In vivo* study

***Animals and experimental design.*** The subcutaneous test was used to evaluate the biocompatibility of the biomaterial *in vivo*. For this purpose, 65 male Wistar rats were used (aged 8 weeks and weighting 210–260 g), maintained under controlled conditions of temperature (24 ± 2°C), light–dark periods of 12 h and with free access to water and commercial diet. All animal's handling and surgical procedures were strictly conducted according to the Guiding Principles for the Use of Laboratory Animals. This study was approved by the Animal Care Committee guidelines of the São Carlos Federal University (protocol 045/2009). Rats were randomly distributed into two groups: control group ($n = 3$ animals per period) and Biosilicate® group ($n = 10$ animals per period), evaluated at 7, 15, 30, 45, and 60 days after surgery.

For the surgical procedure, the animals were anesthetized with Ketamine (80 mg/kg) and Xylazine (10 mg/kg). To insert the subcutaneous implants, rats were immobilized on their dorsal region, and the skin was shaved and disinfected with iodine. Following the median sagittal line, at 8 cm from the skull, a 2-cm incision was made and a subcutaneous pocket was created by blunt dissection. The implants were randomly placed ($n = 1$ per animal), and the skin was sutured with a 4-0 nylon monofilament (Shalon®). The animals were housed in pairs. In the initial postoperative period, the intake of water and food was monitored. Furthermore, the animals were observed for signs of pain, infection, and proper activity. Animals were sacrificed with a lethal dose of anesthetic (Ketamine/Xylazine) at the corresponding experimental set point, and the biomaterials were harvested with surrounding tissue for histopathological analysis.

***Histopathological analysis.*** The specimens were fixed in 10% buffered formalin (Merck, Darmstadt, Germany) for 24 h, followed by dehydration in a graded series of ethanol and embedding in paraffin. In the transverse axis to the implant, thin sections (5 μm) were prepared using a microtome (Leica Microsystems SP 1600, Nussloch, Germany). Three sections of each specimen were stained with hematoxylin and eosin (H.E. stain, Merck) and examined using optical microscopy (Olympus Optical Co., Tokyo, Japan). The tissue response to subcutaneous implants was analyzed qualitatively (inflammation, fibrosis, granulation tissue, necrotic tissue, and the presence of the biomaterial). This analysis was performed by an experienced pathologist in a blinded manner.

### *In vitro* study

***Cell culture.*** Neonatal murine calvarial osteoblastic (OSTEO-1) and murine fibroblasts (L929) were employed in this study. These lineages were chosen by staying in close contact with Biosilicate® scaffold in a possible therapeutic

application of biomaterial. In addition, L929 cells were chosen because they are widely used in analysis of biocompatibility.[13–15]

The cells from both lineages were cultured in Dulbecco's Modified Eagle Medium (DMEM, Vitrocell, Campinas, SP, Brazil) supplemented with 10% fetal bovine serum (Vitrocell, Campinas, SP, Brazil) and 1% antibiotic–antimycotic solution (Vitrocell, Campinas, SP, Brazil) at 37°C in a humidified atmosphere of 5% $CO_2$. Cells were maintained at densities subconfluents and subcultivated every 2–3 days until their use. Both lineages were used for the analysis of cytotoxicity and genotoxicity of biomaterials.

***Cytotoxicity analysis.*** The effect of the degradation products of Biosilicate® scaffolds on the cell proliferation was assessed by the colorimetric quantification of MTT.[16] Each cell line was distributed into two groups: control (cells cultivated with DMEM) and cells cultivated with extracts of Biosilicate® scaffolds. To obtain the extracts, the Biosilicate® scaffolds were thoroughly immersed and incubated in DMEM supplemented at 37°C for 7 days. After this period, the DMEM was discarded, and each scaffold was immersed in 2 mL of new DMEM for 24 h at 37°C. This new DMEM was considered concentrated 100%. Through all of it, various dilutions were performed (50%, 25%, and 12.5%)[17] to evaluate the influence of different concentrations of cell proliferation in two lineages tested. Cells from the control and treated groups were plated ($1 \times 10^3$ cells/well) for 24, 72, and 120 h in 96-well plates.

After each experimental period, the wells of culture plates were washed with phosphate buffered saline (PBS), received 50 μL of MTT solution (0.5 mg/mL) (Sigma–Aldrich, St. Louis, MO), and the cells were incubated in an atmosphere of 5% $CO_2$ at 37°C for 3 h. Then, 100 μL of isopropanol was added to each well to dissolve the formazan crystals. Absorbance was measured at 620 nm using a microplate reader (Anthos 2020, Anthos Labtec Instruments, Wals, Austria). Three independent experiments were always performed in quadruplicate.

***Genotoxicity analysis.*** The genotoxicity analysis was used to assess the damage in DNA of osteoblastic and fibroblastic cells grown in contact with Biosilicate® scaffolds, through an electrophoresis test in the single cell gel (comet assay).[18] For this, cells were counted and distributed in culture plates of 12 wells. Were used $2 \times 10^4$ cells in 2 mL of DMEM supplemented in each well of culture plate. Each cell line was distributed into two groups (control and Biosilicate®), evaluated at different periods of 24, 72, and 96 h. The control group followed with the cells grown in DMEM supplemented. The Biosilicate® group was grown in contact with Biosilicate® scaffolds (1 scaffold/well), previously conditioned for a period of 7 days in DMEM (1 scaffold/2 mL of DMEM), during all experimental periods. The experiment was performed in triplicate.

After each experimental period, the wells of culture plates were washed with PBS, trypsinized, and the cells were added to 50 mL tubes. The tubes were centrifuged at 1200 rpm for a period of 5 min. The DMEM was discarded, and cells resuspended in 1 mL of new DMEM. After taking this step, a volume of 100 μL of DMEM with the respective cells of each experimental group was added to 120 μL of 0.5% low-melting point agarose (Invitrogen Corporation, New York) at 37°C, layered onto a precoated slide with 1.5% regular agarose, and covered with a coverslip. After brief agarose solidification in the refrigerator, the coverslip was removed, and slides were immersed in lysis solution (2.5 *M* NaCl, 100 m*M* EDTA – Merck, St. Louis; 10 m*M* Tris–HCl buffer pH = 10 – Sigma–Aldrich, St. Louis; 1% sodium sarcosinate – Sigma–Aldrich, St. Louis; with 1% Triton X-100 – Sigma–Aldrich, St. Louis; and 10% dimethyl sulfoxide – Merck, St. Louis) for about 1 h. Before electrophoresis, the slides were left in alkaline buffer (0.3 m*M* NaOH, Merck, St. Louis; and 1 m*M* EDTA, Merck, St. Louis; pH > 13) for 20 min and electrophoresed for another 20 min, at 25 V (0.86 V/cm) and 300 mA. After electrophoresis, the slides were neutralized in 0.4 *M* Tris–HCl (pH = 7.5), fixed in absolute ethanol (Merck, Darmstadt, Germany), stained with 100 μL ethidium bromide (50 mg/mL), and stored at room temperature until analysis. To minimize extraneous DNA damage from environment ultraviolet radiation, all steps were performed with reduced illumination. A total of 50 randomly captured comets per treatment/period[19] were blindly examined at 400× magnification using a fluorescence microscope (Olympus, Orangeburg) connected through a black and white camera and to an image analysis system (Comet Assay II, Perceptive Instruments, Sufolk, Haverhill, UK). To quantify the DNA damage, the tail moment was evaluated. Tail moment was calculated as the product of the tail length and the fraction of DNA in the comet's tail. The comet tail moment is positively correlated with the level of DNA breakage in a cell. The mean value of the tail moment in a particular sample was taken as the index of DNA damage in this sample.

### Statistical analysis

Data were expressed as mean values and standard deviations for each sample group. The normal distribution of all variables was checked using the Shapiro–Wilk's *W* test. For parametric samples, the one-way analysis of variance test was used, followed by the Tukey test. For nonparametric samples, the Mann–Whitney test was used. Analyses were performed on Excel (2007) and STATISTICA software (version 7.0). For all the tests, the significance level of 5% ($p \leq 0.05$) was considered.

## RESULTS

### Scaffolds characterization

The scaffolds obtained via addition of carbon black as a porogen agent are highly porous, as can be seen in the image obtained via stereomicroscopy (Fig. 1). They show a total porosity of 82 ± 2% and an apparent porosity of 77 ± 2%, indicating that only approximately 5% of the total porosity is composed of unwanted closed micropores. These scaffolds showed enough mechanical strength for handling and placing inside the incision site.

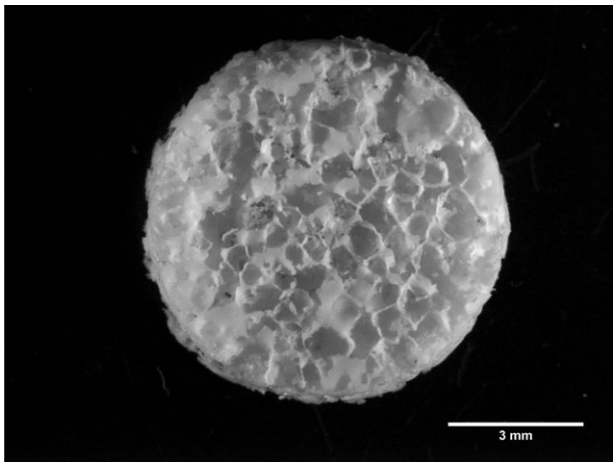


**FIGURE 1.** Image of the Biosilicate® scaffold obtained by the stereomicroscope (Leica MZ75).

### *In vivo* results

***Histopathological analysis.*** During the experimental period, the animals showed no postoperative complications. They quickly returned to their normal diet and showed no loss of body mass. Furthermore, no animal died during the experiment and infection in the injured area was not detected.

Control animals presented no morphological differences throughout the different experimental periods. No presence of inflammatory process, necrotic tissue or fibrous tissue was observed in any sample [Fig. 2(A)].

Seven days after implantation, the presence of granulation tissue around the Biosilicate® scaffolds [Fig. 2(B)] was noticed. Multinucleated giant cells, a chronic inflammatory process and a thin fibrotic capsule surrounding the implants were observed 15 days after implantation [Fig. 2(C)]. Within 30 days, postsurgery was observed a chronic inflammatory process with fibrous tissue surrounding the scaffolds and

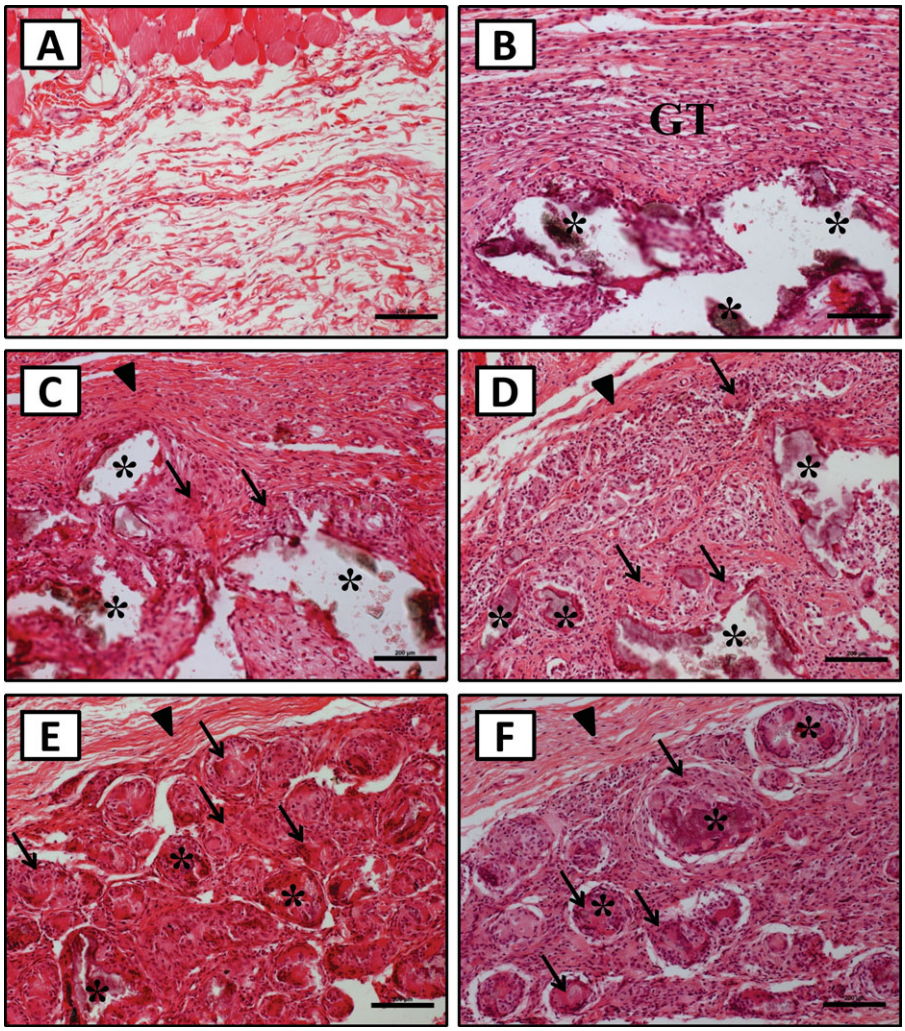


**FIGURE 2.** Photomicrograph of subcutaneous rat tissue demonstrating the absence of inflammation in the control group (A), presence granulation tissue (GT) 7 days after implantation of the biomaterial (B), and a chronic inflammation after 15 (C), 30 (D), 45 (E), and 60 days of implantation (F), characterized by the presence of fibrous tissue (▼), multinucleated giant cells (arrows) isolated or close to the biomaterial (*). H.E. stain, bar = 200 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

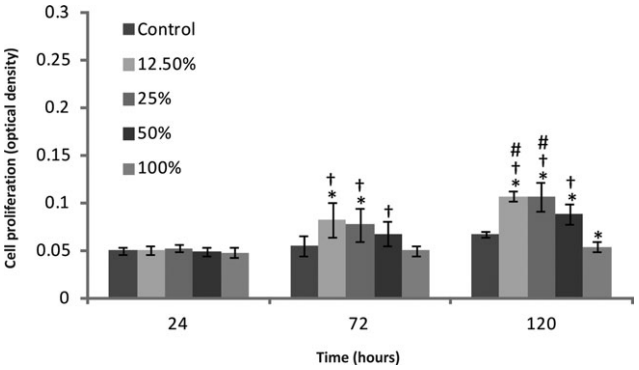


**FIGURE 3.** Proliferation of osteoblastic cell line grown in solutions containing different concentrations of extract of Biosilicate® scaffolds (12.5%, 25%, 50%, and 100%) at different times of cultivation (24, 72, and 120 h). *$p \leq 0.05$ versus control, †$p \leq 0.05$ versus 100%, and #$p \leq 0.05$ versus 50%.

an increased presence of multinucleated giant cells close to the particles of the biomaterial [Fig. 2(D)]. At 45 days after implantation, the qualitative histological evaluation demonstrated the presence of a chronic inflammatory process, a dense fibrotic capsule surrounding the biomaterial particles and the presence of multinucleated giant cells [Fig. 2(E)]. At the last experimental period, a higher amount of multinuclear giant cells and a thicker fibrotic capsule around the biomaterial particles were observed comparing the histological findings at 45 days of implantation [Fig. 2(F)]. Moreover, some particles of the biomaterial still could be observed. In all groups, it was not evidenced the presence of necrotic tissue (Fig. 2).

### *In vitro* results

***Cytotoxicity analysis.*** Osteoblast proliferation assay showed no statistically significant difference among the groups Repetitious 24 h after the seeding (Fig. 3). After 72 h, the control group showed a statistically significant difference when compared with groups with 12.5% and 25% of biomaterial extract concentrations. However, when comparing the control group with the concentrations of 50% and 100%, no statistical difference was observed. The groups with concentration of 12.5%, 25%, and 50% had significantly higher values of cell proliferation compared with the 100% biomaterial extract concentration. No statistical difference was observed between the 12.5%, 25%, and 50% groups (Fig. 3). After 120 h, the control group showed statistically lower values when compared with the 12.5%, 25%, and 50% biomaterial extract concentration. However, the control group showed a significantly higher cell proliferation compared with the 100% concentration. The groups with concentration of 12.5% and 25% presented significantly higher values compared with the groups with 50 and 100% concentration. Moreover, a statistically significant difference was observed between the groups with 50% and 100% concentrations (Fig. 3).

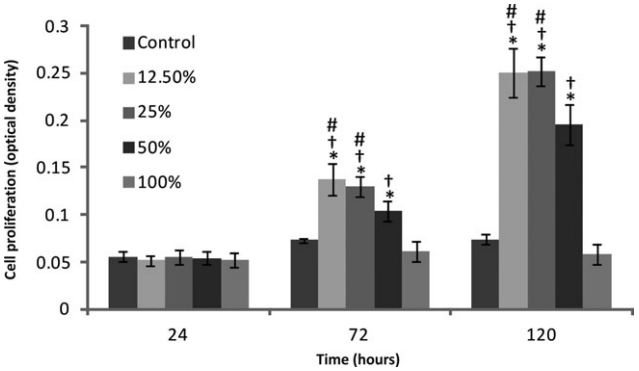


**FIGURE 4.** Proliferation of fibroblastic cell line grown in solutions containing different concentrations of extract of Biosilicate® scaffolds (12.5%, 25%, 50%, and 100%) at different times of cultivation (24, 72, and 120 h). *$p \leq 0.05$ versus control, †$p \leq 0.05$ versus 100%, and #$p \leq 0.05$ versus 50%.

**TABLE I. Mean and Standard Deviation of DNA Damage (Tail Moment) in Osteoblastic Cell Line Exposed to Biosilicate® Scaffolds**

| | Experimental Groups | |
|---|---|---|
| Time (h) | Control[a] | Biosilicate® |
| 24 | 0.4 ± 0.3 | 0.6 ± 0.2 |
| 72 | 0.7 ± 0.3 | 0.7 ± 0.5 |
| 96 | 0.6 ± 0.3 | 0.5 ± 0.2 |

[a] DMEM.

No statistically significant difference was found in the fibroblast cell proliferation assay in any group analyzed 24 h after seeding (Fig. 4). After 72 h, the control group presented statistically lower values compared with the groups with the concentration of 12.5%, 25%, and 50%. No difference was observed comparing the results found in the control and 100% biomaterial extract concentration groups. In addition, the groups with concentrations of 12.5% and 25% showed significantly higher values of fibroblast cell proliferation compared with the groups with the 50% and 100% (Fig. 4). After 120 h, the control group presented statistically lower values when compared with the groups containing 12.5%, 25%, and 50% biomaterial extract concentration. No statistically significant difference was found between the control group and 100% extract concentration. Fibroblast cell proliferation of the groups with the concentration of 12.5% and 25% was significantly higher in averages compared with groups with the concentration of 50 and 100%. A statistically significant difference was observed between the groups with 50% and 100% concentration (Fig. 4).

***Genotoxicity analysis.*** The single cell gel (comet) assay was used to measure DNA damage in osteoblastic and fibroblastic cells *in vitro*. DNA strand breaks were represented by the mean tail moment for 50 comets/sample. As seen in Table I, the Biosilicate® scaffolds did not induce DNA strand breaks in osteoblastic cells at any period evaluated in this study. The same was found for the experiments with fibroblastic cells (Table II).

## DISCUSSION

The use of biomaterials might be a promising approach to improve bone healing in patients with large defects and

**TABLE II. Mean and Standard Deviation of DNA Damage (Tail Moment) in Fibroblastic Cell Line Exposed to Biosilicate® Scaffolds**

| Time (h) | Experimental Groups | |
|---|---|---|
| | Control[a] | Biosilicate® |
| 24 | 0.8 ± 0.3 | 0.6 ± 0.3 |
| 72 | 0.6 ± 0.1 | 0.4 ± 0.1 |
| 96 | 0.7 ± 0.4 | 0.7 ± 0.3 |

[a] DMEM.

compromised bone repair potential.[20] Recently, many treatments have been proposed based on ceramics, osteogenic bioglasses, and laser therapy for recalcitrant bone nonunions.[21] In this context, the aim of this study was to evaluate the *in vivo* and *in vitro* biocompatibility of the Biosilicate®. To the best of our knowledge, this approach has not been addressed so far.

The results concerning the histopathological analysis showed that the implantation of Biosilicate® scaffolds produced a mild tissue response, with the development of an acute inflammatory process and the presence of granulation tissue at the first period evaluated (7 days) progressing to a chronic inflammatory process with the presence of fibrous tissue and multinucleated giant cells after 15 days. This morphological description, called foreign body reaction,[22] was observed during the other experimental periods (30, 45, and 60 days), with a greater presence of fibrous tissue and multinucleated giant cells during those periods.

The characteristics of the biomaterials used may dictate the intensity of foreign body reaction. Biomaterials with greater surface area, such as biomaterials in the form of particles or scaffolds, contribute to an increased recruitment of macrophages, giant cells, and development of fibrous tissue in relation to materials with smaller surface area.[22] Vogel et al.[23] found a higher concentration of multinucleated giant cells in around the Bioglass® 45S5 implants compared with other tested glass compositions (52*s* and 55*s*), suggesting that this event is due to a higher dissolution rate of these materials. Misra et al.[24] observed that the degradation of a scaffold of polymer base coated with layer of bioactive glass (Bioglass® 45S5) led to the formation of smaller particles of the biomaterial, inducing to the development of foreign body reaction.

In summary, the results obtained in the histopathological analysis showed that the Biosilicate® scaffolds did not induce tissue necrosis, or the development of infections, but promoted a foreign body reaction, which can also be observed with the use other biocompatible materials. Therefore, these results indicate that Biosilicate® scaffolds are biocompatible materials in this setting.

The results also demonstrated that the Biosilicate® scaffolds did not present cytotoxic potential after 24 h, because there was no inhibition of proliferation of osteoblast and fibroblast cell in this period. Similarly, Ferraz et al.[25] observed that a biomaterial containing a particulated bioactive ceramic (hidroxyapatite) did not promote any cytotoxic effects in culture of human osteosarcoma cells. Quan et al.[26] also evidenced that hidroxyapatite-based materials have no cytotoxic potential after 24 h of culturing in fibroblastic cells.

Moreover, 72 and 120 h after the seeding, Biosilicate® scaffolds at the concentrations of 12.5% and 25% produced a significant increase in cell proliferation in both lineages. These results corroborate those of Xynos et al.,[27] who also observed an increase in osteoblast cell proliferation exposed to Bioglass® 45S5. Interestingly, osteoblast and fibroblast proliferation was reduced in the cells exposed to high concentration of the biomaterial (100%). This may be related to the possible higher pH of the medium in the samples containing the higher concentration of Biosilicate® extracts.[28] Liu et al.[17] observed that the pH may be a determining factor in cell proliferation. These researchers showed that the highest concentration of extracts of a bioactive borosilicate glass scaffold (100%) presented a culture medium with high pH, and this induced a greater inhibition of proliferation of goat bone marrow stroma cells.

The analysis of genotoxicity showed that the single cell gel test (comet), under the experimental conditions used, did not detect the presence of DNA damage in osteoblastic and fibroblastic cells cultured on Biosilicate® scaffolds in all periods tested. These results demonstrated that this biomaterial has no genotoxic potential. The same has been observed by others authors using *in vitro* genotoxicity tests of different ceramic compounds. For instance, Noushad et al.[29] investigating a dental porcelain, observed that these materials have no genotoxic potential when cultured with fibroblastic cells after 24 h. Ribeiro et al.[30] tested a mineral trioxide aggregate in cultured mouse lymphoma cells for a period of 3 h. The authors also evidenced that these materials do not have genotoxic potential, corroborating with the findings of this study.

The use of subcutaneous implantation of biomaterials in rats was chosen due to the effectiveness shown in the literature.[24,31] The *in vitro* methodologies used in this study are adequate to evaluate the cytotoxicity[16] and genotoxicity[18] of biomaterials. The information from *in vitro* assays, together with the histopathological information, is important for understanding the behavior of biomaterials in biological systems. Although the biocompatibility of the Biosilicate® scaffolds is evidenced in this study, other studies would be welcome to validate the behavior of these biomaterials in prolonged periods, or in other experimental models.

## CONCLUSIONS

The results clearly indicate that Biosilicate® scaffolds are biocompatible and noncytotoxic. These are promising findings because they convey important information about the behavior of this novel biomaterial in different biological systems and highlight the possible clinical application of Biosilicate® scaffolds.

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