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Biosilicate® — A multipurpose, highly bioactive glass-ceramic. *In vitro*, *in vivo* and clinical trials

Murilo C. Crovace *, Marina T. Souza, Clever R. Chinaglia, Oscar Peitl, Edgar D. Zanotto

Vitreous Materials Laboratory, Department of Materials Engineering, Center for Research, Education and Technology in Vitreous Materials (CeRTEV), Federal University of São Carlos (UFSCar), SP, Brazil

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ABSTRACT

In this review we critically analyze 28 theses and dissertations and over 30 scientific papers that tested Biosilicate®, a highly bioactive glass-ceramic, in a number of applications throughout the past 20 years. Biosilicate® presents a combination of positive features for bone tissue regeneration: it is highly bioactive, osteoconductive, osteoinductive, non-cytotoxic, non-genotoxic and has antibacterial properties. In addition, in the monolithic form, it is quite strong and tough. Its in vitro bioactivity is similar to that of the gold standard Bioglass 45S5. Biosilicate® has shown to be a very versatile, multipurpose biomaterial. It can be applied in powder, monolithic and 3D scaffold forms that could be easily machined during surgical procedures. This material has been successfully tested in a number of in vitro, in vivo and clinical studies, and several trials are ongoing. Biosilicate® is indeed a great option for a wide range of tissue engineering applications.

1. Introduction

The use of different materials to improve the performance of or to repair damaged bones, teeth and other tissues due to disease or trauma has been performed for millennia. Initially these materials were limited to those available in nature. Only in the early twentieth century, with the development of new materials and processes, a considerable increase in the number of individuals using biomaterials has occurred [1].

The word "biomaterial" is comprehensive and defines a portion of nearly three hundred thousand healthcare products [2]. These are materials that directly interact with biological systems to treat, augment or replace a tissue, organ or a function of the body [3]. The first studies in this area originated from the fields of materials science and engineering and were focused on the mechanical performance of implant devices. At that time, the negative reactions of the body to these materials were poorly understood; hence, the selection of "biomaterials" was limited to those that were considered "inert". Early studies and discoveries, some of them informal, that linked the biological response of the organism to the chemical composition of these biomaterials have provided a rational basis for the development of biologically inert substrates, the first-generation biomaterials, and have also provided the basis for the development of second- and third-generation biomaterials. Second-generation biomaterials were defined by Hench [4] as those which "…*could elicit a controlled action and reaction in the physiological environment*", whereas those from the third generation could "…*stimulate specific cellular responses at the molecular level...*" and "…*activate genes that stimulate regeneration of living tissues*". For example, third-generation biomaterials could bind effectively to bone [5] and also stimulate osteoblast differentiation and proliferation (an osteoinductive material) [6,7].

Bioactive glasses and glass-ceramics belong to the third generation of biomaterials. Among all synthetic biomaterials available for the treatment of bone lesions, bone substitution or any other application where bone regeneration is needed, bioactive glasses show the best clinical outcome. The best known bioactive glass is Bioglass® 45S5, developed by Larry Hench in the late 1960s, which has a composition of 24.5Na_2O–24.5CaO–45SiO_2–6P_2O_5 (wt.%). This glass exhibits the highest bioactivity index ($I_B = 12.5$) and is still considered the gold standard of bioactive materials.

After the development of Bioglass®, several other compositions of bioactive silicate glasses were developed [8–12]. There are several other types of bioactive glasses, including phosphate [13,14], borate [15,16] and invert glasses [17,18], i.e., when the amount of network former elements is lower than that of the modifiers.

When in contact with body fluids, bioactive silicate glasses undergo five-stage reactions, which lead to the formation of a hydroxycarbonate

* Corresponding author.
E-mail address: murilocc@yahoo.com.br (M.C. Crovace).
URL's: http://www.certev.ufscar.br (M.C. Crovace), http://www.certev.ufscar.br (M.T. Souza), http://www.certev.ufscar.br (C.R. Chinaglia), http://www.certev.ufscar.br (O. Peitl), http://www.certev.ufscar.br (E.D. Zanotto).

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Table 1
Bioactivity index and mechanical properties of bioactive materials used in clinical procedures [19,24,25,29,34].

Bioceramics	Bioglass® 4555	Bioglass® 52S4.6	Cerabone® A/W	Ceravital®	Bioverit® I	HAs	Biolox® Forte	Biosilicate® (monolithic)	Cortical bone (longitudinal)	Cancellous bone
Bioactivity index (BI)	12,5	10,5	6	5,6	IB < 8	3,1	0	IB > 8	–	–
Flexural strength (MPa)	40	40	215	100–150	140–180	50–200	466	120–210	–	–
Compressive strength (MPa)	?	?	1080	500	500	500–100	4400	?	100–133	1.5–7.5
Young's modulus (GPa)	60	60	120	100–160	70–90	80–110	380	70–80	12–17.7	0.2–0.6
Structure	Glass	Glass	(1) β-CaSiO3 + apatite + glass	(1) + devitrite apatite + glass	(1) mica + apatite + glass	(2) apatite	α-Al2O3	1N2C3S	Apatite + organic material	Apatite + organic material
Machinability	Poor	Poor	Poor	Poor	Good	Poor	Poor	Moderate	–	–

?: not found data. Cerabone® A/W: glass-ceramic/β-CaSiO3: beta-wollastonite/(1): undefined composition. Ceravital®: glass-ceramic/(1): undefined composition. Bioverit® I: glass-ceramic/(1): undefined composition. Has: synthetic hydroxyapatite/(2): $(Ca)_{10}(PO_4)_6(OH)_2$. Biosilicate®: glass-ceramic 100% crystallized. 1N2C3S: Sodium Calcium Silicate (1 N = sodium; 2C = calcium; 3S = silicon).

apatite layer (HCA) on its surface. This set of reactions was proposed by Hench [19]. In stage I, alkali and alkali earth ions are released into the fluid and are replaced in the glass structure by H^+ or H_3O^+ ions from the fluid. This reaction causes an increase in the local pH, resulting in the rupture of Si–O–Si bonds. Then, silicon is released into the fluid in the form of silanols ($Si(OH)_4$) (stage II). If the local pH is lower than 9.5, $Si(OH)_4$ condensates, forming a polymerized silica gel layer on the surface of the glass (stage III). The open structure of silica gel allows the continuity of ionic exchange between the glass and the fluid. Calcium and phosphate ions diffuse from the glass and, in conjunction with the calcium and phosphate ions from the fluid, form an amorphous calcium phosphate layer over the silica gel (stage IV). After the thickness increase due to both the silica gel and the amorphous calcium phosphate layer, the incorporated carbonate species in the latter begins to crystallize into HCA. The HCA is chemically and structurally similar to the mineral apatite phase found in bone tissue.

The formation of a HCA layer at the material/tissue interface is accepted as a necessary condition for the formation of a chemical bond between the material and bone [19]. The development of HCA is a desired characteristic in all inorganic materials used in bone tissue repair, bone substitution and orthopedic implants. The formation of HCA is intimately associated with osteoconduction. Bioactive glasses show a higher osteoconductivity than bioactive ceramics, such as hydroxyapatite (HA). According to Jones et al. [20], the reason for this higher osteoconductivity is related to the rate of superficial HCA formed; the higher the rate of HCA formation, the faster the material will bond to bone, increasing its osteoconductivity.

After a HCA layer is formed, the subsequent stages of cellular interaction are still not well understood. It is known that certain types of proteins adhere to the HCA layer. Finally, the attachment of osteoprogenitor cells occurs; these cells undergo a differentiation process and begin to synthesize bone matrix [21]. However, the exact mechanism is difficult to follow using *in vitro* and *in vivo* tests [8].

The mechanism of osteoinduction is more complex than the mechanism of osteoconduction. As a bioactive glass degrades, ions such as silicon (in the form of silanol), calcium, sodium and phosphate are

Fig. 1. Onset time for HCA formation as a function of the crystallized volume fraction (1.07N2C3S = $18.5Na_2O$–$31.3CaO$–$50.3SiO_2$; SS = $24.8Na_2O$–$24.8CaO$–$50.5SiO_2$; SSP2 = $24.2Na_2O$–$24.2CaO$–$49.5SiO_2$–$2P_2O_5$; SSP4 = $23.8Na_2O$–$23.8CaO$–$48.5SiO_2$–$4P_2O_5$; SSP6 = $23.2Na_2O$–$23.2CaO$–$47.5SiO_2$–$6P_2O_5$ — wt.%) [28].

released into the physiological medium. It is believed that a combination of such ions stimulates cells to produce new bone tissue, specially calcium and silicon [4]. Molecular biology studies have shown that seven gene families related to osteogenesis are activated by the dissolution products of bioactive glasses [22].

Although bioactive glasses show both osteoconduction and osteoinduction, bioactive ceramics based on calcium phosphate also show osteoconduction but have reduced or absent osteoinduction [20]. In an *in vivo* study conducted by Oonishi et al. [23], the effects of Bioglass 45S5 and hydroxyapatite (HA) on the regeneration of bone defects were compared. Bioglass allowed the complete recovery of bone after only two weeks, whereas HA took approximately twelve weeks to produce a comparable response.

However, despite its excellent bioactive properties, the major disadvantages of bioactive glasses are their low mechanical strength and low fracture toughness (bending strength of approximately 70 MPa, as shown in Table 1 [24], and K_{IC} of 0.5 MPa·$m^{1/2}$). These characteristics restrict their use to a few applications. To improve the mechanical performance, bioactive glass-ceramics have been developed.

The most well-known bioactive glass-ceramics are Ceravital®, Bioverit® and A/W Cerabone® (developed by Kokubo in the late 1980s). Ceravital® is composed of a glassy phase, devitrite and apatite crystals. The main application of this glass-ceramic is as substitute of the ossicular chain in the middle ear. In addition to the glassy phase, Bioverit® is composed of apatite and mica crystals, which are responsible for its excellent machinability. Bioverit® pieces have been implanted in more than 850 orthopedic surgeries up to 1992, such as in spinal spacers and in head and neck surgery [25]. The A/W Cerabone® glass-ceramic is composed of an apatite matrix reinforced by needle-like wollastonite crystals, which yields a K_{IC} of 2 MPa·$m^{1/2}$, the highest value among all of the bioactive glass-ceramics. Among other applications, A/W Cerabone® has been used in the substitution of the iliac bone crest. This glass-ceramic was produced from 1991 to 2000 and applied with success in more than 60,000 patients [25].

All of the three glass-ceramics cited are composed of an apatite-like crystalline phase, which is much less soluble than Bioglass® 45S5. Although these glass-ceramics exhibit better mechanical performance than any glass, their bioactivity level is relatively low and is comparable to that of traditional calcium phosphate ceramics.

2. Biosilicate®, a 20-year history

In the mid 1990s, a great challenge was the development of a new material that could combine both high bioactivity, as observed in Bioglass® 45S5, and the good mechanical strength and toughness of some glass-ceramics, such as A/W Cerabone®. A straightforward strategy to achieve this goal was to improve the mechanical strength of Bioglass® 45S5 or any other bioactive glass through controlled crystallization. However, two questions arose: 1) Does crystallization impair bioactivity? 2) Can crystallization of such bioactive glasses significantly improve their mechanical properties?

Li et al. [26] reported in 1992 that the rate of HCA formation *in vitro* on the bioactive glass $20Na_2O–22.5CaO–48SiO_2–9.5P_2O_5$ (wt.%) significantly decreased as the crystallized volume fraction increased. The poor bioactivity was attributed to the low amount of the residual glass phase. For a crystallized volume fraction of 95%, the formation of HCA was completely inhibited. Then, crystallization of this glass transformed it into an inert material. At that stage, the bioactive glass community became very distressed and assumed that crystallization of any glass would impair bioactivity.

However, a few years later, in 1996, Peitl et al. [27] published a groundbreaking report that the crystallization of Bioglass 45S5 slightly

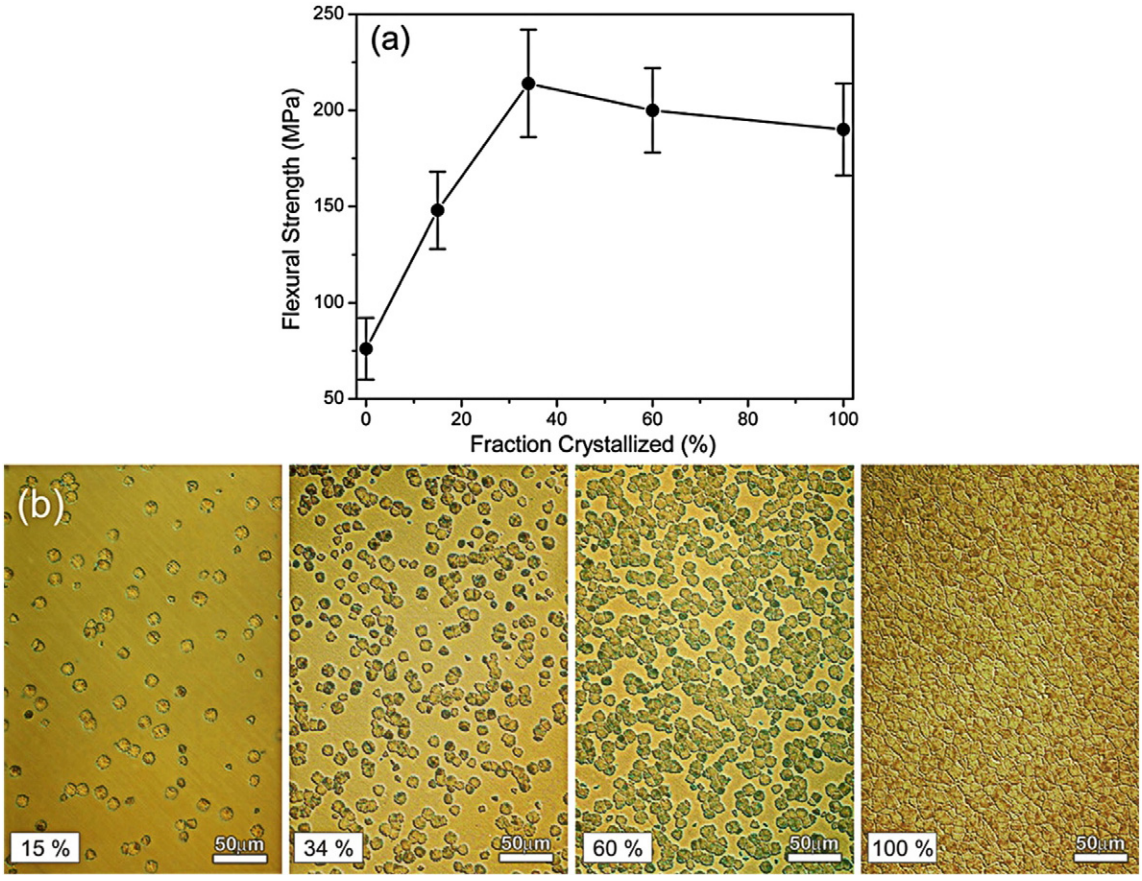

Fig. 2. (a) 3-point or 4-point bending strength versus crystalline volume fraction for a $23.8Na_2O–23.8CaO–48.5SiO_2–4P_2O_5$ (wt.%) glass-ceramic with a constant crystal size of 13 μm; (b) optical micrographs of the corresponding microstructures for 15%, 34%, 60% and fully crystallized samples (b) [29].

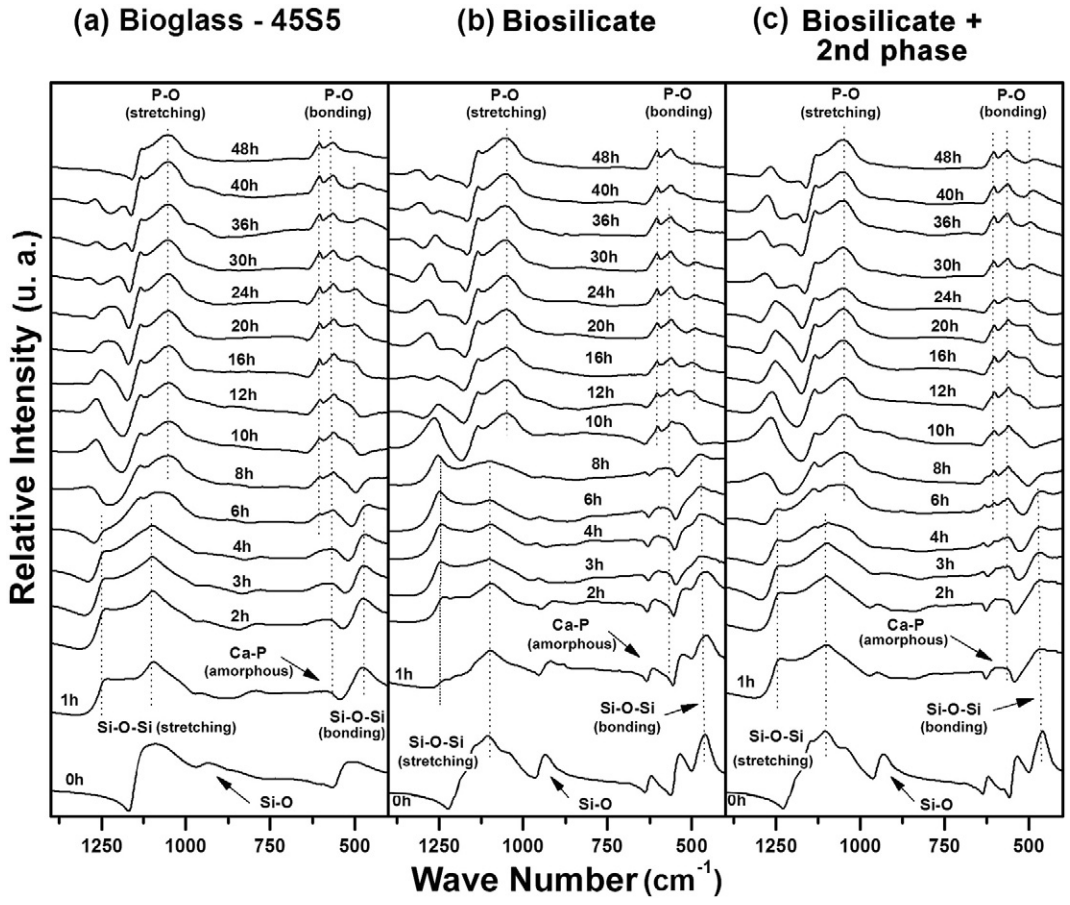

Fig. 3. FTIR spectra for Bioglass 45S5 (a), Biosilicate® (1P) (b) and Biosilicate® containing two crystal phases (2P) exposed to SBF-K9 solution from 1 h to 48 h [33].

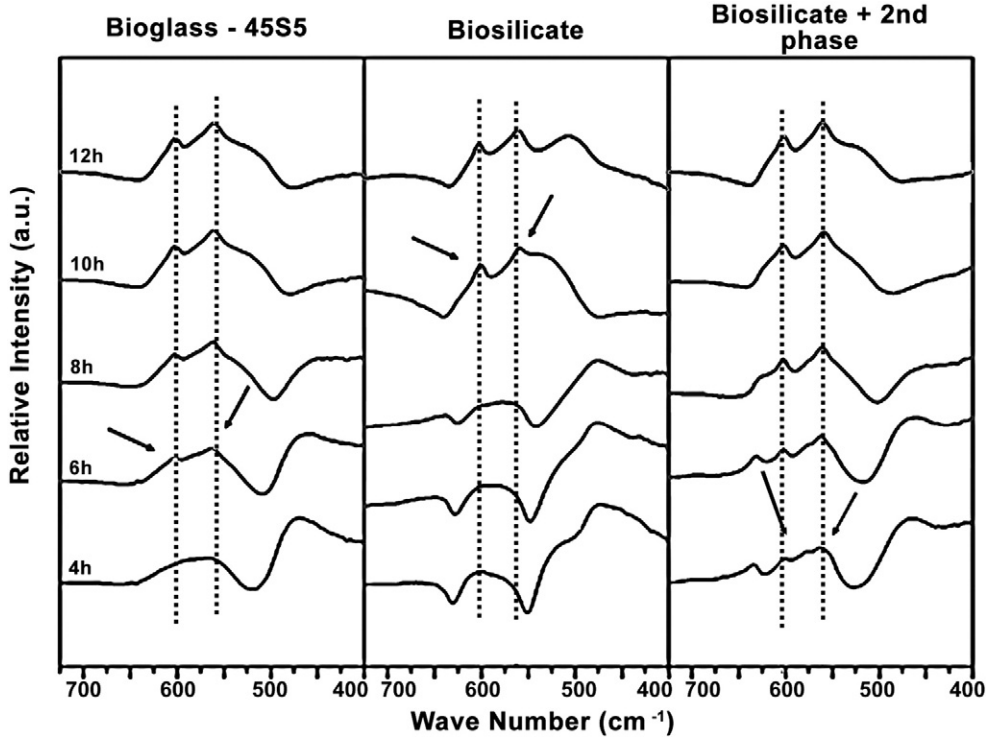

Fig. 4. Magnification of the FTIR spectra for Bioglass 45S5, Biosilicate® (1P) and Biosilicate® containing two crystal phases (2P) exposed to SBF for periods of time varying between 4 and 12 h [33].

decreased the kinetics of HCA formation but did not inhibit its formation, even in the case of "full" crystallization. Later, Peitl et al. [28] tested the in vitro bioactivity of glass-ceramics with different compositions and crystallized fractions within the Na_2O–CaO–SiO_2–P_2O_5 system. The result was similar: crystallization did not hinder HCA formation for glasses of this system, as can be observed in Fig. 1. The fast formation of a HCA layer on these glass-ceramics was attributed to the combination of two mechanisms: the presence of a soluble non-phosphate crystal phase (1N2C3S) and phosphorus ions in solid solution being rapidly released to the medium in a similar way to Bioglass 45S5 [27,28]. However, even the stoichiometric composition $1Na_2O$–$2CaO$–$3SiO_2$, without P_2O_5, was found to be bioactive when fully crystallized.

Peitl et al. [29] later showed that controlled crystallization of glasses of this system could increase their average 4-point bending strength by a factor of 2.8 when compared with that of the parent glass (from 75 MPa to 210 MPa, Fig. 2). This value is similar to that which is found for the A/W glass-ceramic (215 MPa), which exhibits the best mechanical performance among the commercial bioactive glass-ceramics [25].

The elastic modulus also underwent a small increase, from 60 to 80 GPa, but it is still the closest value to that of human cortical bone (~20 GPa) among the commercial bioactive glass-ceramics. This characteristic is important to minimize "stress-shielding" effects. In addition, the fracture toughness increased by 60% due to a crack deflection mechanism.

These results were collected in the patent WO 97/41079 [30] entitled "*Bioactive ceramics and method for preparing bioactive ceramics*" that was granted in 1997. This patent claims the use of glass-ceramic compositions in the Na_2O–CaO–SiO_2–P_2O_5 system with crystallized fractions from 34 to 60% in dentistry and orthopedic applications, as well as the process to prepare these glass-ceramics, which involves double-stage heat treatments.

Further research led to the development of a new fully crystalline glass-ceramic in the same system but with some compositional changes, named Biosilicate®. In one of the first clinical tests, powdered Biosilicate® was found to occlude dentin tubules, rapidly reacting with saliva and the surrounding tissue and successfully eliminating the cause of dentin hypersensitivity, as will be shown later. A new patent (WO 2004074199 A1) entitled "*Process and compositions for preparing particulate, bioactive or resorbable biosilicates for use in the treatment of oral ailments*" was granted in 2007 [31].

Biosilicate® is the designation of the particular composition 23.75Na_2O–23.75CaO–48.5SiO_2–4P_2O_5 (wt.%). Under controlled double-stage heat treatments, this material can be engineered to compose one (1P) or two crystalline phases (2P): a sodium–calcium silicate ($Na_2CaSi_2O_6$) or both $Na_2CaSi_2O_6$ and a sodium–calcium phosphate ($NaCaPO_4$) phase. Biosilicate® was found to exhibit fair machinability because it is relatively easy to cut and drill, which is an important feature that allows the fabrication of implants with different shapes for specific purposes. Before testing the effectiveness of Biosilicate® in clinical trials,

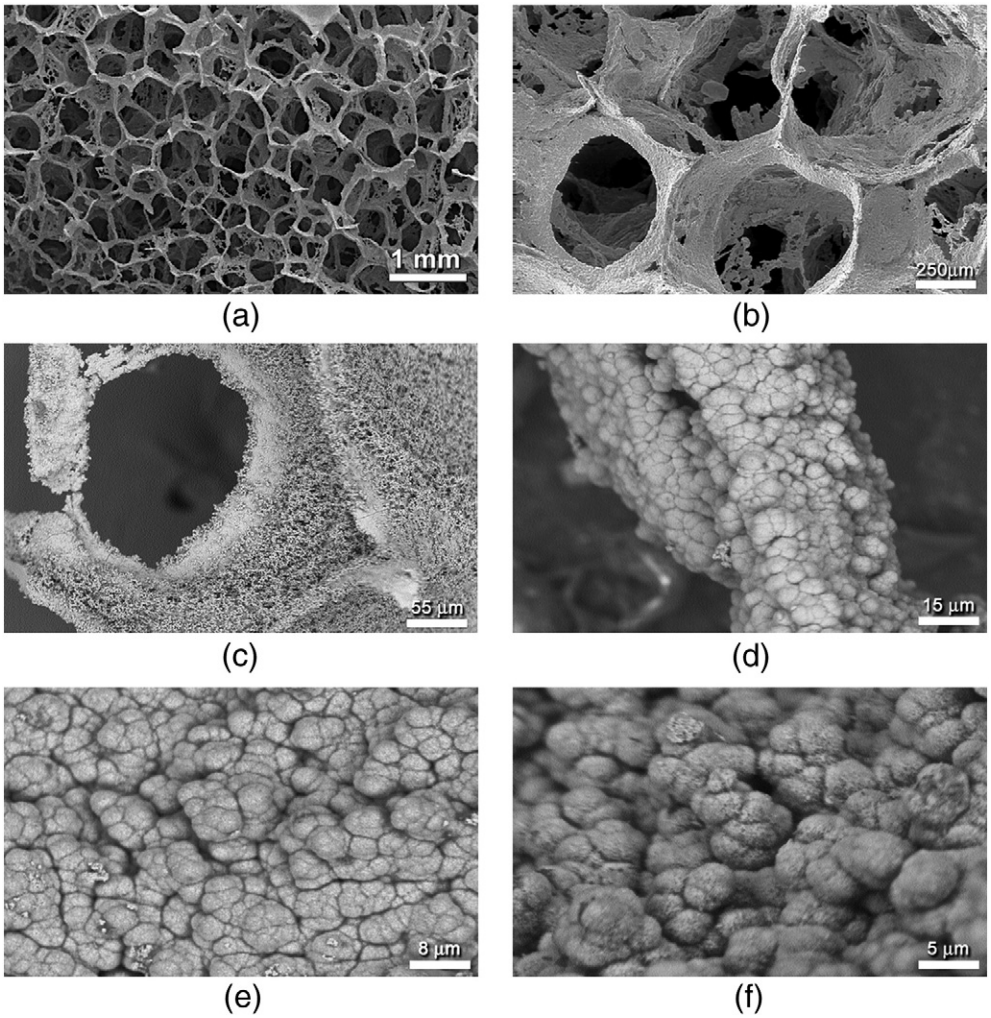

Fig. 5. SEM micrographs of the Biosilicate® (2P) scaffold as-sintered (a-b) and immersed in SBF-K9 solution for 1 day (c), 3 days (d), 7 days (e) and 10 days (f) [35].

it underwent a series of in vitro and in vivo studies, which are described in the next sections.

3. In vitro studies

3.1. Tests using simulated body fluid (SBF)

Inorganic *in vitro* bioactivity tests consist of exposing the material to an acellular solution, e.g., the one developed by Kokubo et al. [32] and named "simulated body fluid" (SBF), which contains several ions in concentrations close to that found in human blood plasma. This test provides information regarding the kinetics of hydroxycarbonate apatite (HCA) formation on the surface of the material under study. This test is preliminary, selective and inexpensive; therefore, it is conducted prior to other in vitro and in vivo tests which involve higher costs and specialized professionals for breeding, implantation and sacrificing of animals to collect samples for analysis. Additionally, in vitro tests are more practical and can be performed in much shorter times.

After exposure to SBF solution for different lengths of time, the surface of the material is generally analyzed using FTIR. The spectra in Fig. 3 [33] illustrate the evolution of surface changes as a function of exposure time for Bioglass 45S5 (a), Biosilicate® (1P) (b) and Biosilicate® containing two crystalline phases (2P). The transformation sequence was the same for all of the materials; the only difference was the kinetics with which these reactions occur. This sequence followed stages III–V of HCA formation proposed by Hench [34] and observed by Peitl et al. [28] for glass-ceramics. Stages I and II occur faster and could not be followed using FTIR.

After only 1 h of exposure to SBF, a silica gel rich layer (corresponding to stage III) was identified in the spectra of the three materials. The presence of the silica gel rich layer was confirmed by two peaks corresponding to the stretching of the Si–O–Si bond at 1250 and 1095 cm^{-1} and by the sharpening of the band at 470 cm^{-1}. The intensity of the peak related to the Si–O bond at 930 cm^{-1} is reduced due to the polymerization process.

Within 1 h, it was also possible to identify the formation of an amorphous calcium phosphate phase (Ca–P) corresponding to stage IV of HCA formation. This phase was confirmed by a broad peak at 560 cm^{-1}.

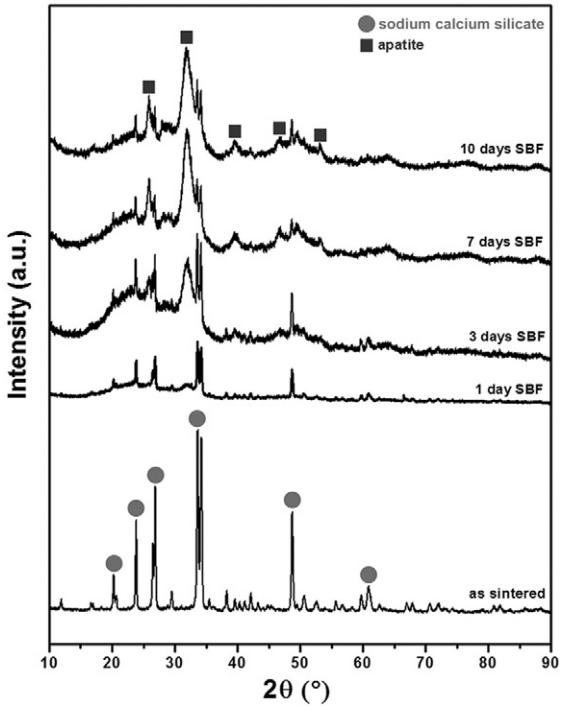

Fig. 6. XRD spectra of the Biosilicate® (2P) scaffolds immersed in SBF-K9 for periods between 1 and 10 days, showing the development of the apatite phase [35].

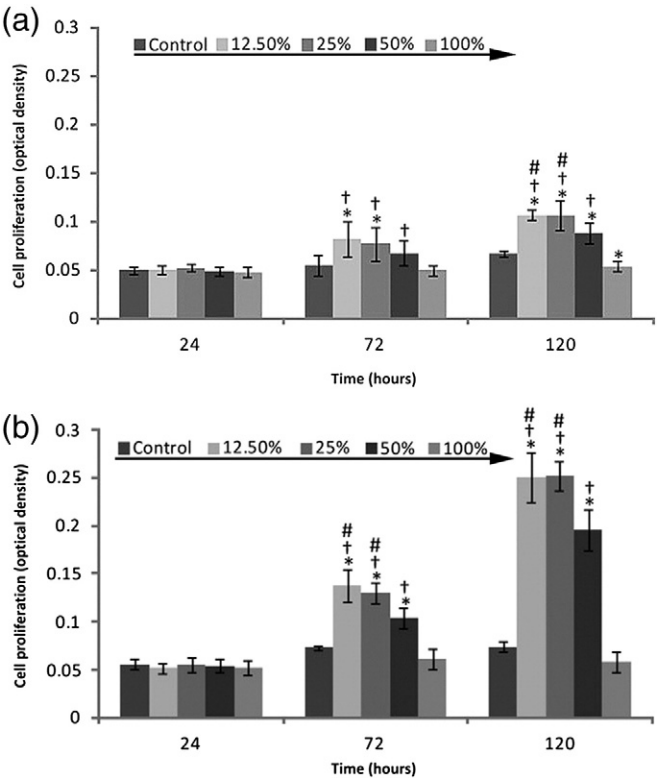

Fig. 7. (a) Proliferation of the osteoblastic cell line grown in solutions containing different concentrations of the Biosilicate® scaffold extract (12.5%, 25%, 50%, and 100%) at different times of cultivation (24, 72, and 120 h). *p ≤ 0.05 versus control, †p ≤ 0.05 versus 100%, and #p ≤ 0.05 versus 50%. (b) Proliferation of the fibroblastic cell line grown in solutions containing different concentrations of Biosilicate® scaffold extract (12.5%, 25%, 50%, and 100%) at different times of cultivation (24, 72, and 120 h). *p ≤ 0.05 versus control, †p ≤ 0.05 versus 100%, and #p ≤ 0.05 versus 50% [39].

As the reaction time increased, the broad peak at 560 cm^{-1} sharpened. Subsequently, it divided into two peaks at 602 and 560 cm^{-1}. These two peaks were attributed to the P–O bond, and their appearance indicated that the amorphous Ca–P phase crystallized into HCA. Simultaneously, another peak at 1050 cm^{-1} that was attributed to the stretching of the P–O bond appears.

As the HCA layer grew, these three peaks (at 560, 602 and 1050 cm^{-1}) became sharper and tended to dominate the spectra. The peaks attributed to the silica gel layer gradually decreased in intensity and disappeared after 16 h for Biosilicate® and after 8 h for Bioglass and Biosilicate® containing two phases. Then, a third peak corresponding to the P–O bond at 490 cm^{-1} increased, evidencing a well-crystallized HCA layer.

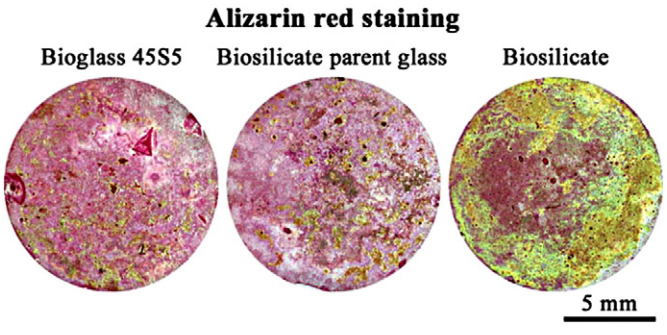

Fig. 8. Macroscopic images of osteogenic cultures grown on Bioglass 45S5, Biosilicate® parent glass and Biosilicate® (1P) glass-ceramic and stained with Alizarin red at day 17. Biosilicate® supports larger amounts of bone-like matrix formation (yellowish areas in a reddish background) [40].

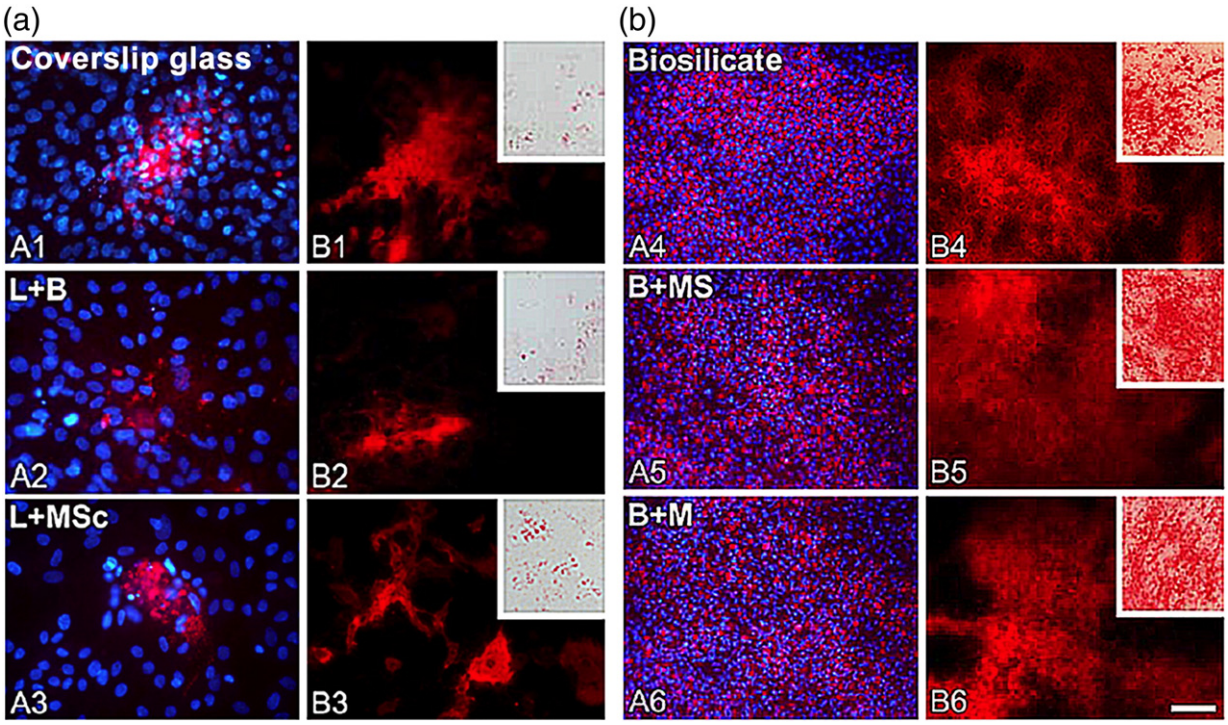

Fig. 9. Epifluorescence of osteogenic cultures of coverslip glass (1–3) and Biosilicate® (4–6) for different conditions in 7 days. The red fluorescence (A) indicates markup for BSP and ALP activity is B (scale A1-3 = 50 μm and A4-6 and B1-6 = 100 μm) [41].

The third peak appeared after 10 h for Bioglass and after 12 h for both Biosilicates (1P and 2P). Subsequently, only the peak related to HCA was observed in the spectra.

Peitl et al. [28] defined the onset time for HCA formation as the appearance of the peak at 602 cm^{-1}. Using this definition, HCA begins to crystallize after 6 h for Bioglass, 10 h for Biosilicate® 1P and only 4 h for Biosilicate® 2P. This result is shown by the spectra in Fig. 4 [33]. The formation of HCA on Biosilicate® was much faster than hydroxyapatite ceramics (HA) and commercial glass-ceramics, such as Ceravital®, Bioverit® and A/W Cerabone®, for which the onset time is at least seven days.

Biosilicate® (2P) was also employed in powder form to synthesize scaffolds using the replication technique [35], also known as the "polymer sponge method" or "foam replica method". In this method, polyurethane (PU) foams were soaked in a suspension consisting of 57 wt.% of isopropyl alcohol (99.5% J.T. Baker), 3 wt.% of polyvinyl butyral (PVB, Butvar B98) and 40 wt.% of Biosilicate® powder (average particle size of 3 μm). After sintering, highly porous scaffolds were obtained, which exhibited a total porosity of 92% and pores between 400 and 900 μm (Fig. 5a and b). To evaluate their comparative apatite formation ability, SBF-K9 in vitro tests were conducted from 1 to 10 days. For longer exposure periods (7 and 10 days), the solution was replaced twice per week.

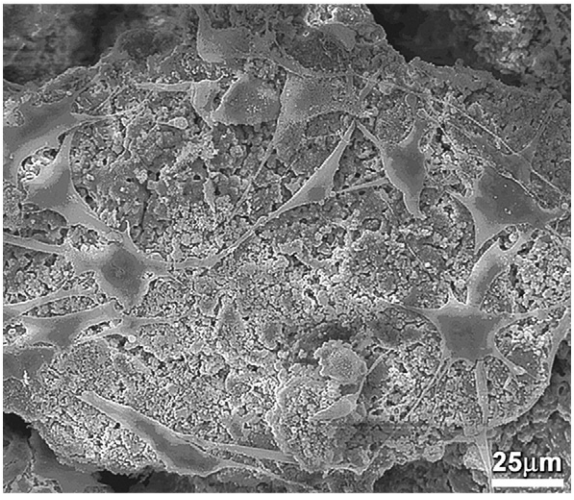

Fig. 10. Osteoblasts on a Biosilicate® scaffold [42].

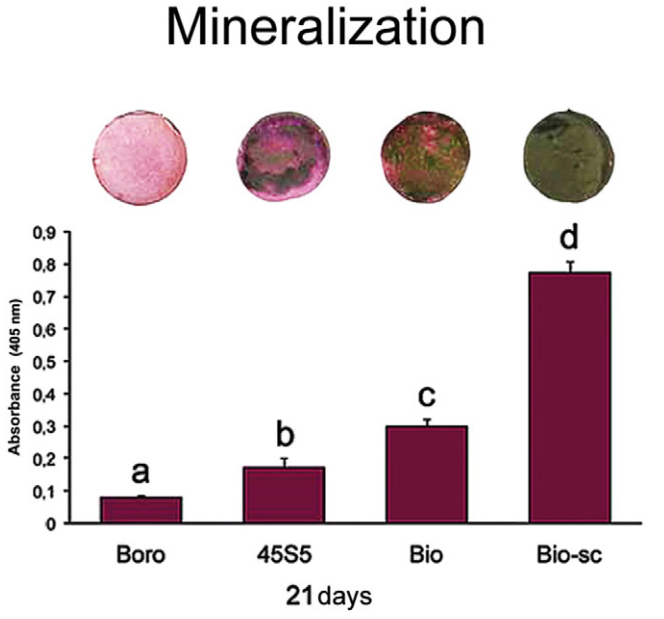

Fig. 11. Macroscopic view of MC3T3-E1 cells cultured on Borosilicate, Bioglass 45S5, Biosilicate® 1P (Bio) and Biosilicate® 2P (Bio-sc). The samples were stained with Alizarin red, which indicated the highest osteogenic potential for Bio and Bio-sc. Letters represent the statistical difference between groups [43].

After only 3 days of immersion, the scaffold struts were fully covered with apatite crystals. The size of the HCA globular-like structures increased for longer immersion periods (Fig. 5e and f).

As observed in the XRD spectra (Fig. 6), the crystallinity of the sintered scaffolds decreased with increasing SBF immersion time. Eventually, small peaks of the sodium calcium silicate phase disappeared from the XRD spectrum after 7 days of immersion. In addition to the crystallinity decrease, growing peaks of a HA-like phase appeared in the spectra of the soaked samples. A typical broad halo produced by an amorphous phase was also detected in all of the spectra after 1 day of immersion in SBF; this broad halo persisted for up to 10 days. Biosilicate® was gradually converted into an amorphous phase and in an apatite-like phase. A similar behavior was observed in the case of Bioglass 45S5-based scaffolds [36].

Due to the high porosity and pore size, the mechanical strength of scaffolds for bone tissue engineering is always of concern. The mechanical integrity is a key issue for the scaffolds to be easily handled by surgeons and to allow their use in load-bearing sites. More recently, Desimone et al. [37] showed that the compressive strength of Biosilicate® scaffolds obtained by the foam replica method can be improved by a factor of 10 by applying a gelatin coating, without significant reduction of the pore interconnectivity or scaffold bioactivity in SBF.

The in vitro bioactivity tests using SBF showed that the crystallization of Biosilicate® parent glass, whether in solid or scaffold form, does not significantly affect the formation of HCA. Instead, it can be even improved depending on the crystalline phases present. This result makes Biosilicate® a potential candidate for applications in which bone-bonding or bone regeneration is desired.

3.2. Cell culture

Although *in vitro* tests using SBF are indicative of the high bioactivity of Biosilicate®, they are considered insufficient to predict the in vivo performance [38]. To better evaluate the osteogenic potential of Biosilicate® several *in vitro* tests using cells were performed.

3.2.1. Cytotoxicity and genotoxicity

Cytotoxicity tests are performed to evaluate if the dissolution products of a given biomaterial and the resulting pH of the solution are toxic to cells. Kido et al. [39] analyzed the effect of the dissolution products of Biosilicate® (2P) scaffolds in osteoblast and fibroblast cell cultures. In this study, cells were not cultivated directly in the scaffold. Instead, the liquid extracted from the scaffolds was immersed in Dulbecco's modified Eagle's medium (DMEM, Vitrocell). To obtain the extracts, Biosilicate® (2P) scaffolds were thoroughly immersed and incubated in supplemented DMEM 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 100% concentrated. Throughout the experiment, various dilutions were performed (50%, 25%, and 12.5%) to evaluate the influence of different concentrations on the cell proliferation of both lineages. The proliferation was assessed using colorimetric quantification of MTT (methyl thiazolyl tetrazolium).

The results demonstrated that the Biosilicate® (2P) scaffolds did not present cytotoxic potential after 24 h because the proliferation of osteoblast and fibroblast cells was not inhibited (Fig. 7). No statistically significant difference was found in the cell proliferation assay in any group analyzed 24 h after seeding. Moreover, after 72 and 120 h after the seeding, Biosilicate® scaffolds at the concentrations of 12.5%, 25% and 50% produced a significant increase in cell proliferation in both lineages. Interestingly, the osteoblast and fibroblast proliferation was reduced in the cells exposed to a high concentration (100%) of the biomaterial (Fig. 7). This result may be related to the possible higher pH of the medium in the samples containing the highest concentration of Biosilicate® (2P) extracts.

The aim of genotoxicity tests is to detect if a material can cause damage to genes within a cell and thereby cause mutations. This evaluation is important because DNA mutation in cells may lead to cancer. Kido et al. [39] also tested the *in vitro* genotoxicity of both osteoblasts and fibroblasts grown in contact with Biosilicate® (2P) scaffolds for 24, 72 and 96 h. In this test, the cells were extracted and analyzed using electrophoresis (single cell gel, i.e., a "comet assay"). The results showed that Biosilicate® scaffolds did not induce DNA damage in both cell lineages tested.

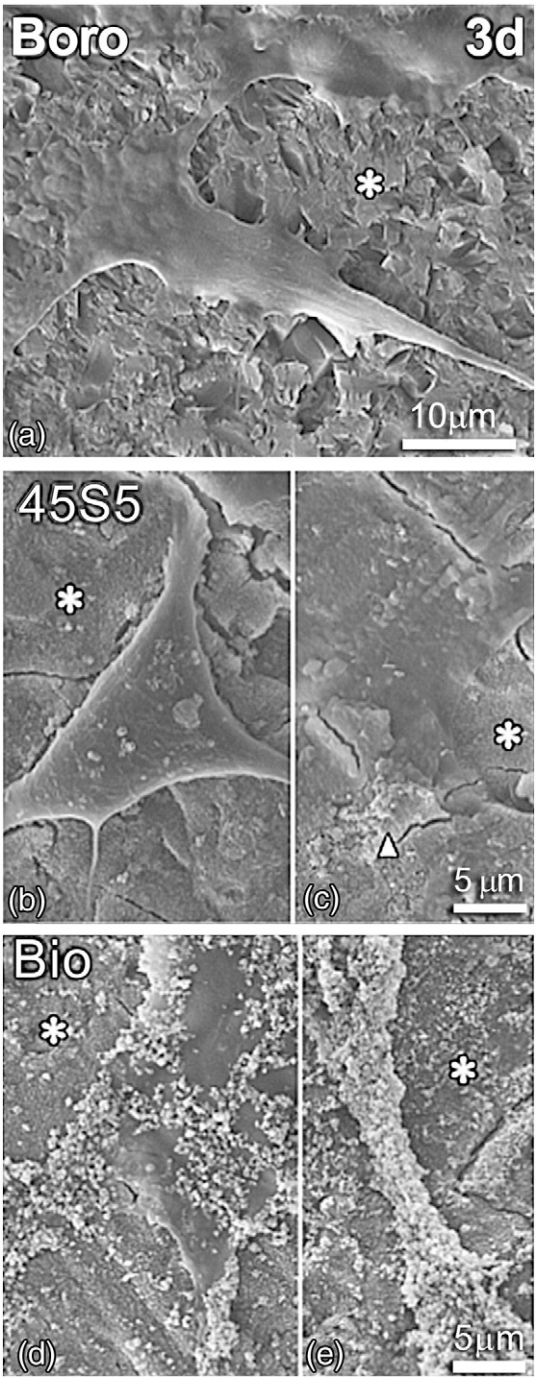

Fig. 12. SEM micrographs of osteogenic cells on material surfaces after 3 days. a) Boro: borosilicate, scale: 20 μm; b, c) Bioglass 45S5, scale: 10 μm; d, e) Bio: Biosilicate®, scale: 10 μm. It is possible to observe precipitated material on the cells for Biosilicate® samples. (* — substrate) [44].

3.2.2. Osteogenic cells

Moura et al., in 2006 [40], analyzed the potential of Biosilicate® (1P) on various key parameters of *in vitro* osteogenesis using rat calvaria bone cells seeded on the surface of glass-ceramic solid discs for up to 17 days. The results showed that, when compared with Bioglass 45S5 and Biosilicate® parent glass, no significant differences were observed in terms of total protein content and alkaline phosphatase activity at days 11 and 17. However, Biosilicate® supported a significant enhancement of calcified tissue areas and calcified matrix at day 17 compared with the other biomaterials (Fig. 8) [40]. The authors attributed this behavior to the different dissolution kinetics of Biosilicate® when compared to its parent glass or Bioglass 45S5 [40].

Another *in vitro* study evaluating the effect of Biosilicate® (1P) surface conditioning on osteogenic cells was conducted by Raucci in 2009 [41]. Rat calvaria-derived osteogenic cells were plated on Biosilicate® discs that were pre-conditioned either with supplemented culture medium or serum-free medium for different periods of time.

The results showed that the conditioning of Biosilicate® surfaces with culture medium prior to cell plating supports key aspects of cell–substrate interactions, increasing and/or accelerating expression of the osteoblastic cell phenotype. The cell viability was significantly higher for the conditioned Biosilicate® surfaces at 1, 3, and 7 days. After 7 days, the expression levels of RUNX2, alkaline phosphatase (ALP) and bone sialoprotein (BSP) mRNAs were also significantly higher for the conditioned Biosilicate® group, as seen in Fig. 9. This study also showed that, after 14 days, the conditioned Biosilicate® group presented significantly more areas of matrix mineralization.

3.2.2.1. Osteogenic cells and laser irradiation. In 2010, Renno et al. [42] studied the development of a method to seed osteoblast cells onto Biosilicate® (2P) scaffolds and evaluated the effect of laser phototherapy at 830 nm on the osteoblast proliferation on the same material. This work was conducted for 7 days, and the results showed that osteoblastic MC3T3 cells could successfully grow on discs composed of the glass-ceramic composite (Fig. 10). However, laser phototherapy irradiation resulted in a reduction in cell growth compared with non-irradiated controls.

3.2.2.2. Gene expression. Regarded as one of the most interesting effects of a biomaterial, the influence of Biosilicate® on gene expression was analyzed in two studies. One study evaluated the osteogenic phenotype in osteoblastic cell cultures and another studied mRNA and protein expression levels in osteogenic cell cultures.

The first study was conducted by Alves in 2012 [43] in which Biosilicate® (1P and 2P) in the form of discs were qualitatively and quantitatively evaluated *in vitro*. The development of the osteogenic phenotype in osteoblastic cell cultures (MC3T3-E1) was investigated for up to 21 days. At days 7, 12, and 21 post-plating, the cell morphology, the mineralized matrix formation and the expression profile of genes associated with osteogenesis were also evaluated for Bioglass 45S5, Borosilicate

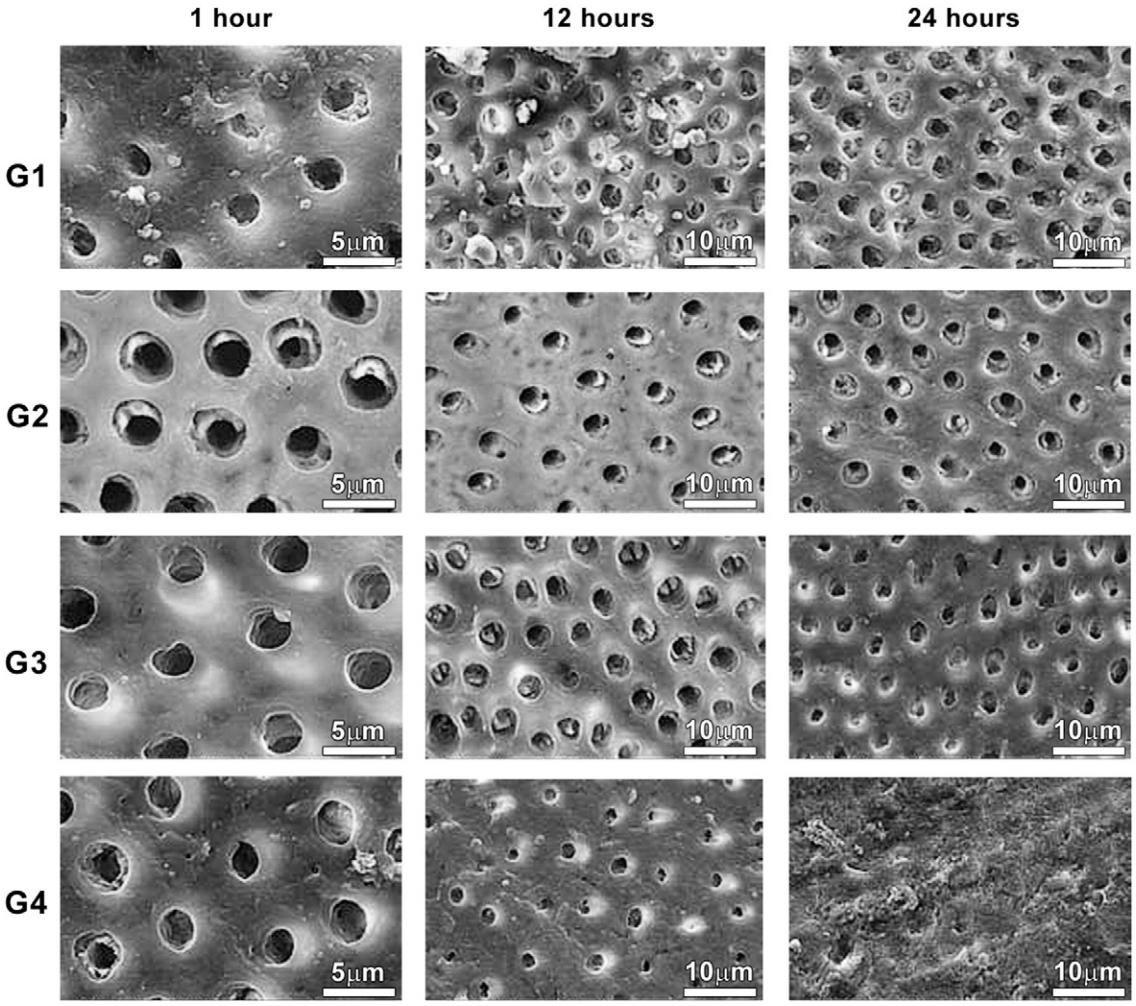

Fig. 13. SEM micrographs of dentin samples immersed in artificial saliva and treated for 1, 12 and 24 h following the treatment protocol: G1 — Dentifrice with potassium nitrate and fluoride; G2 — Two-step calcium phosphate precipitation treatment; G3 — Water-free gel containing Biosilicate® particles (1%); G4 — Biosilicate® particles mixed with distilled water in a 1:10 ratio [48].

(Pyrex® — Corning) and Biosilicate® 1P (Bio) and 2P (Bio-sc) in disc form. From the obtained results, it was inferred that changes in chemical characteristics between the glass and the glass-ceramic might positively impact the gene expression of osteoblastic cells in vitro. Biosilicate® 2P presented the highest osteogenic activity among all of the studied materials (Fig. 11).

The other study was conducted by Martins, also in 2012 [44], which aimed to evaluate whether changes in the labeling patterns for the cytoskeletal proteins actin and tubulin in osteogenic cells cultured on Bioglass 45S5 and Biosilicate® (1P) were due to altered mRNA and protein expression levels. The qualitative epifluorescence, quantitative mRNA expression and quantitative actin and tubulin expression were analyzed, and the cell morphology was verified at 3 and 7 days in Bioglass 45S5, Biosilicate® and borosilicate samples. At days 3 and 7, cultures exposed to the bioactive materials presented significant changes in actin and tubulin mRNA expression. Additionally, a positive correlation was observed between mRNA, protein expression levels and epifluorescence imaging.

In conclusion, Bioglass 45S5 and Biosilicate® affected osteogenic cell cultures regarding their actin and tubulin mRNA levels but did not influence the corresponding protein expression. Therefore, the variations in the labeling pattern for these proteins could be attributed, at least in part, to the precipitation of materials, likely calcium phosphate, on the surface of the cells (Fig. 12).

3.3. Antimicrobial activity

Another interesting property analyzed was the antibacterial capacity of Biosilicate®. Martins et al. [45] studied the antimicrobial activity against anaerobic, microaerophilic and facultative anaerobic microorganisms using three different methods. Aiming to explore the spectra of Biosilicate® (1P) in powder form (particles of 0.1 to 20 μm were used), agar diffusion, direct contact and minimal inhibitory concentration (MIC) techniques were performed using 20 different microorganisms.

For the agar diffusion technique, inhibition halos were observed ranging from 9 ± 1 to 22 ± 2 mm against various microorganisms and 14 out of 20 bacteria were inhibited by Biosilicate®. Regarding aerobic and facultative anaerobic microorganisms, Biosilicate® was most active against the standard strain *Candida albicans* (17 ± 1 mm). For microaerophilic microorganisms, the best activity was obtained against *Aggregatibacter actinomycetemcomitans* (22 ± 2 mm) and *Streptococcus mutans* (19 ± 2 mm). Among the anaerobic bacteria, *Prevotella nigrescens* (19 ± 3 mm) was the most sensitive to the tested agent [45].

Using the direct contact technique, Biosilicate® exhibited antimicrobial activity against all of the tested microorganisms except for *Staphylococcus aureus*. In this test, 11 bacteria died within the first 10 min in contact with the material, whereas the other microorganisms subsided after 60 min [45].

Minimum inhibitory concentration tests showed that the microorganism development was inhibited by Biosilicate®, exhibiting MIC values ranging from ≤2.5 to 20 mg/ml. The best MIC results were obtained for oral microorganisms such as *C. albicans* (7.5 mg/ml), *Kocuria rhizophila*, and *Enterococcus faecalis* (15 mg/ml). *S. aureus*, *Salmonella choleraesuis*, and *Pseudomonas aeruginosa* exhibited increased resistance to Biosilicate®, displaying MIC values of 20 mg/ml [45].

These results were found to be remarkable, considering that no antimicrobial elements as silver and gallium are present on Biosilicate® (both 1P and 2P) composition. The antimicrobial activity of bioactive glasses and bioactive glass-ceramics is not fully understood and could be linked to a series of factors. However, their highly reactive surface, when in powder form, and the ability to increase the pH of aqueous suspensions, due to the leaching of Na^+ and Ca^{2+} ions, are likely important characteristics that lead to the inhibition of bacteria growth [46].

3.4. Dentin hypersensitivity treatment — in vitro investigations

Dentin hypersensitivity is a very common condition that affects approximately 1/3 of the adult population. Currently, there is no ideal product or treatment protocol. *In vitro* and clinical studies [47–49] were conducted using Biosilicate® as a mineralizing agent and potential treatment product. All of the studies reported very fast and satisfying results regarding the obliteration of the dentinal tubules.

Some of the first studies in this area using biomaterials were made by Tirapelli et al. in 2007 [47] and in 2010 [48]. In these two studies, the authors evaluated the effectiveness of powdered Biosilicate® (1P) in occluding open dentinal tubules in dentin disc models. The results showed that Biosilicate® required only 24 h to induce the precipitation of a homogeneous HCA layer covering the entire dentin surface, as shown in Fig. 13, whereas the other experimental groups did not provide

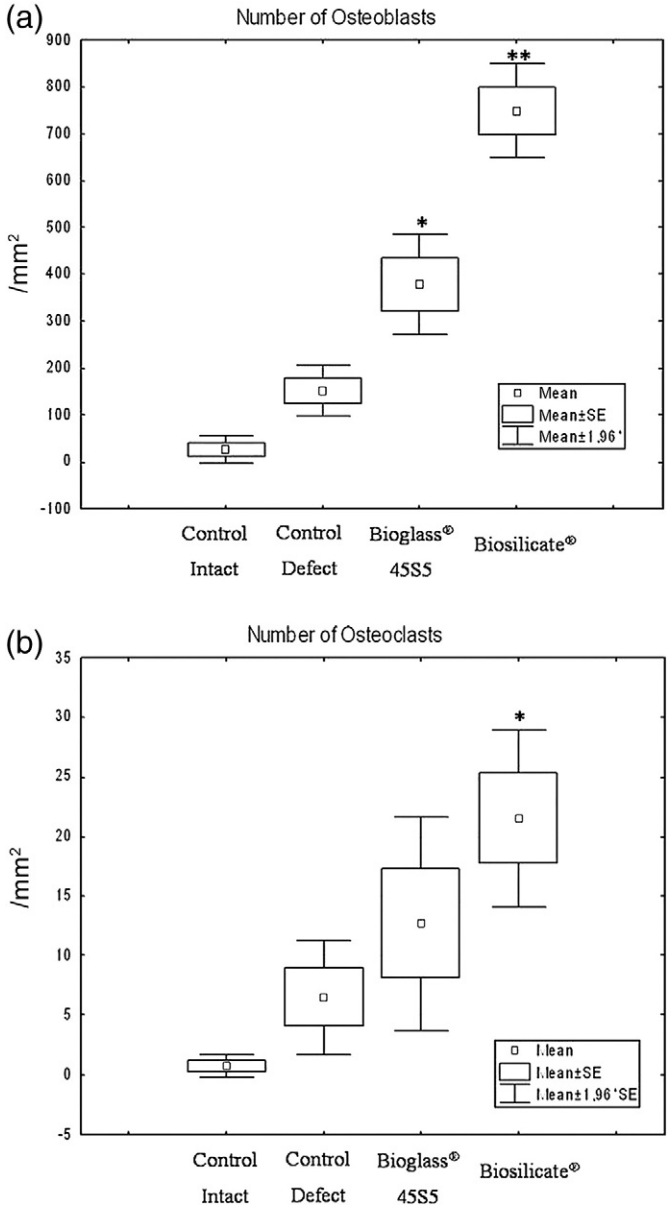

Fig. 14. Number of osteoblasts (a) and osteoclasts (b) per unit of tissue area (N.Ob/T.Ar,/mm^2) 20 days after intact tibias (control intact) had bone defects surgically created (control defect) and filled with a biomaterial (Bioglass 45S5 and Biosilicate® (1P) groups). *$p < 0.05$ vs. control intact and control defect. **$p < 0.05$ vs. control intact, control defect and Bioglass 45S5 [51].

the same effect. This result suggests that Biosilicate® could offer an efficient alternative for clinically treating dentin hypersensitivity.

Another *in vitro* study investigated the in situ influence of Biosilicate® (1P) on whitened enamel and dentin surfaces using the Knoop hardness test (KHN). The results indicated that, when combined with the whitening gel, the biomaterial benefited the tooth hardness and morphology, preventing demineralization [49].

4. In vivo studies

4.1. Bone grafting

4.1.1. Tibial defects in rats

4.1.1.1. Particulate form. To investigate the *in vivo* effects of Biosilicate® in powder form, Granito et al. [50,51] conducted two different studies.

The first study aimed to investigate the in vivo performance of Biosilicate® (1P) in tibia bone defects in rats. Fifty male Wistar rats were divided into 5 groups that underwent surgical procedures for the implantation of Biosilicate® and Bioglass of two different particle sizes (180–212 μm or 300–355 μm). Bone defects of non-critical size were made bilaterally at the upper third of the tibia. The control group did not receive any treatment.

After 15 days post-surgery, all of the animals were sacrificed, and histomorphometric and biomechanical analyses were carried-out. No significant differences were found for the morphometric analysis and for the maximal load, energy absorption and structural stiffness values. Histopathological analysis showed that the amount of fully formed bone was higher in specimens treated with Biosilicate® having particle sizes ranging from 300 to 355 μm. Such findings suggested that Biosilicate® presented a higher osteogenic activity compared with Bioglass 45S5 under subjective histopathological analysis.

From these positive results, Granito et al. [51] continued investigating the effects of Biosilicate® (1P) *in vivo*. In the second study, 40 male Wistar rats with tibial defects were randomly divided into four groups to compare Biosilicate®, Bioglass 45S5 (180–210 μm particle range), unfilled defects and intact controls. Twenty days after the surgical procedure, mechanical tests and histomorphometric analysis were performed. Compared with the control group, the biomechanical tests showed significant increases in the maximum load at failure and stiffness in the Biosilicate® group. It was possible to observe that the smaller-sized Biosilicate® particles presented partial reabsorption, which resulted in more pronounced osteogenic activity within the period of the experiment. Although both bioactive materials supported a significant increase in bone formation, Biosilicate® was superior to Bioglass 45S5 in some histomorphometric parameters such as bone volume and number of osteoblasts and osteoclasts (Fig. 14).

This second study revealed that Biosilicate® is a superior bone-forming biomaterial and has excellent bone-bonding properties. In addition, it can improve the mechanical properties of bone, as well as promote enhanced bone formation and osteoblast recruitment.

4.1.1.2. Scaffold form. To test the biocompatibility, Kido et al. [39] implanted highly porous (total porosity of approximately 82%) Biosilicate® (2P) scaffolds obtained by the porogen agent addition method in the dorsal subcutaneous tissue of 65 male rats. After 7, 15, 30, 45 and 60 days of implantation, the scaffolds were harvested, and histopathological analysis was performed in the surrounding tissue. Although the implantation of Biosilicate® scaffolds led to a mild tissue response, it did not induce tissue necrosis or the development of infections.

4.1.2. Femur defects in rabbits

Aiming to study the histological and histomorphometrical bone response to Biosilicate® in rabbits, Azenha et al. [52] investigated the effect of Biosilicate® parent glass, Biosilicate® (1P) and Biosilicate® (2P) in the form of rod implants in rabbit femur bone defects. Sixteen animals underwent the surgical procedure, and biomaterial rods with dimensions of 2.2 × 4.0 mm were bilaterally implanted. Bioglass 45S5 was used as a control material. After 8 and 12 weeks, histological and histomorphometrical analyses were performed. The histological

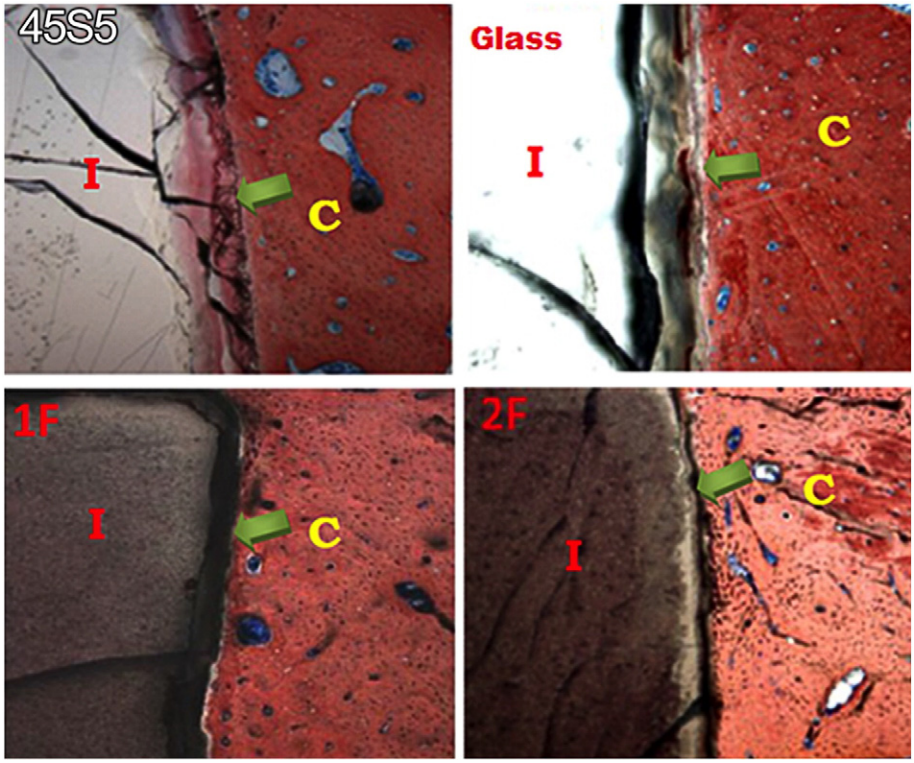

Fig. 15. Histological analysis showing the direct contact between the implants (I) and the cortical bone (C) after 12 weeks (HE — 10×) [52]. 1F = Biosilicate® 1P and 2F = Biosilicate® 2P.

examination did not reveal persistent inflammation or foreign body reactions at the implantation sites in all of the experimental periods.

After 8 weeks, 1F = Biosilicate® 1P and 2F = Biosilicate® 2P. presented higher cortical bone formation compared with Bioglass 45S5 and Biosilicate® parent glass ($p = 0.02$). All of the tested materials were considered biocompatible, demonstrating surface bone formation and satisfactory behavior in this particular biological environment (Fig. 15).

4.1.3. Biosilicate® coupled with laser therapy

4.1.3.1. Healthy rats. In the past decade, low-level laser therapy (LLLT) has emerged as a promising alternative for the treatment of bone lesions. Some studies have shown its positive effect on bone tissue metabolism and on fracture consolidation [53,54]. When laser light is applied to bone tissue, it is absorbed by chromophore photoreceptors located in the cells. Once absorbed, the light can modulate biochemical reactions and stimulate mitochondrial activity, producing molecular oxygen and adenosine triphosphate (ATP).

To accelerate the process of bone repair, Pinto et al. [55] studied the *in vivo* tissue response to Biosilicate® (2P) scaffolds and LLLT in tibial bone defects in rats. In this study, 90 Wistar rats were randomly divided into three groups: control group (CG) — empty non-critical size defect of 3 mm, Biosilicate® scaffold group (BG) — bone defect containing Biosilicate® scaffold, and bone defect containing Biosilicate® scaffold and irradiated with laser group (BLG). In the BLG group, a low-energy

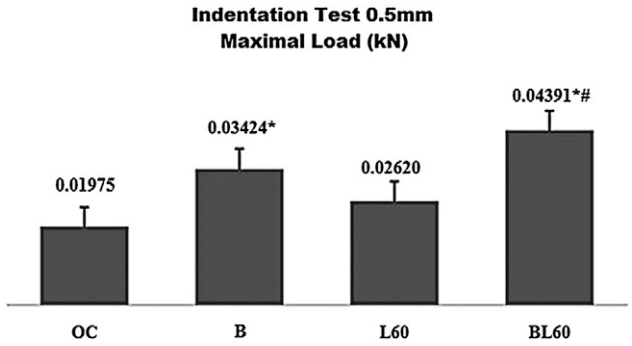

Fig. 17. Maximal load variation of indentations tests (0.5 mm depth), for the control group (OC), Biosilicate® group (B), laser therapy group (L60) and Biosilicate® + laser therapy group (BL60) ($p < 0.05$, * vs. OC, # vs. L60) [62].

(wavelength: 830 nm; power: 100 mW; fluence: 120 J/cm^2) Ga–Al–As laser (Teralaser®, DMC) was irradiated above the injury area every 48 h. Each group was divided into three sub-groups: 15, 30 and 45 days post-surgery.

The histological analysis revealed that all of the experimental groups showed inflammatory infiltrate and granulation tissue at the defect area at day 15. After 30 days, the CG still showed granulation tissue and bone ingrowth. Both Biosilicate® groups presented newly formed bone and

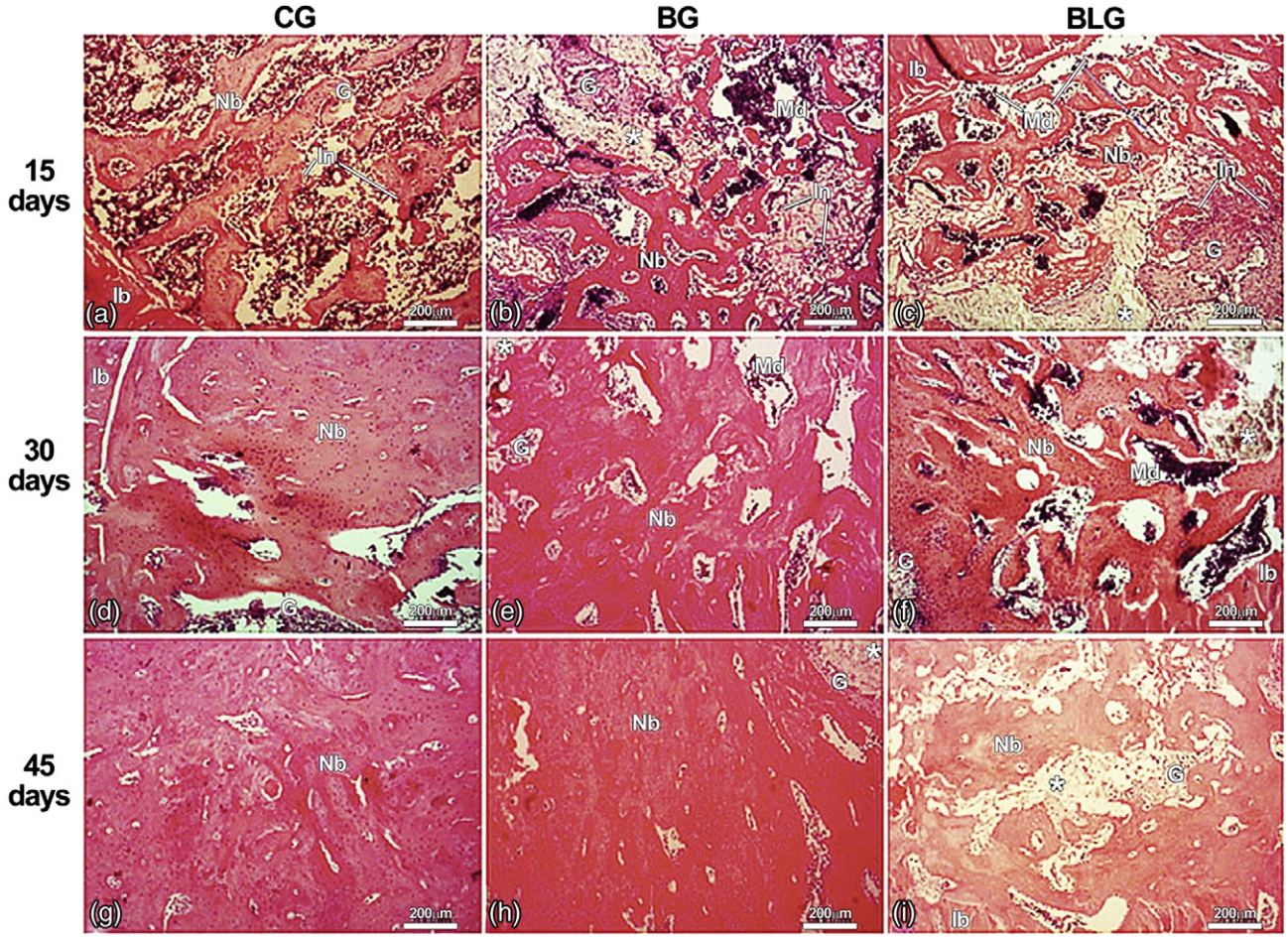

Fig. 16. Representative histological sections of the experimental groups. Intact bone (Ib), newly formed bone (Nb), granulation tissue (G), inflammatory infiltrate (In), medullar tissue (Md). Biosilicate® (asterisk). (a) Control group at 15 days. (b) Biosilicate® group at 15 days. (c) Biosilicate® + low-level laser therapy (LLLT) group at 15 days. (d) Control group at 30 days. (e) Biosilicate® group at 30 days. (f) Biosilicate® + LLLT group at 30 days. (g) Control group at 45 days. (h) Biosilicate® group at 45 days. (i) Biosilicate® + LLLT group at 45 days (hematoxylin and eosin [H.E.] stain) [55].

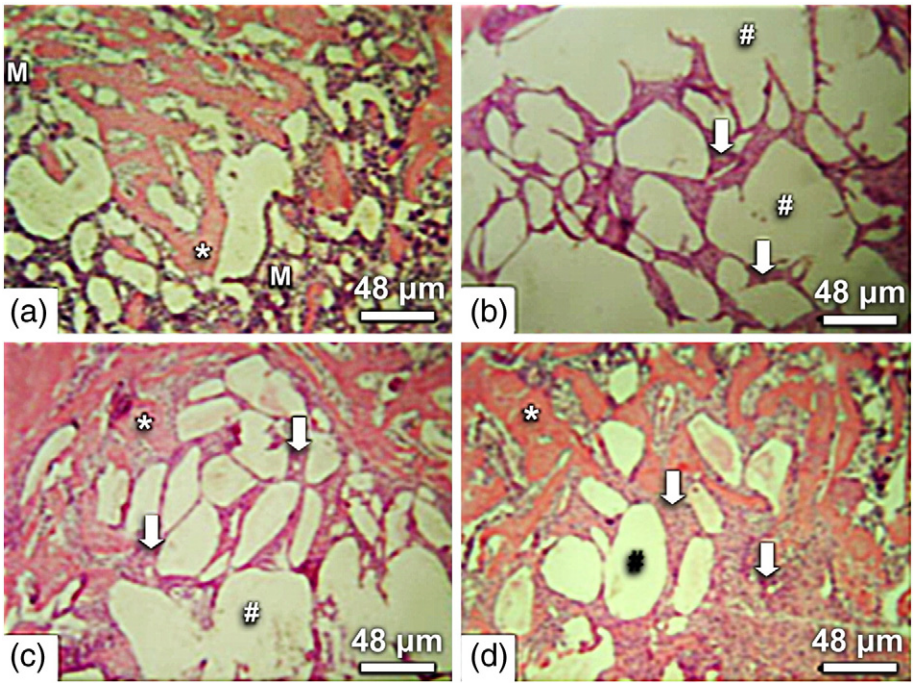

Fig. 18. Bone defects after 14 days in the CG rats (a) (∗) high cellularized woven formed and medullar region(M). (b) BG showing the biomaterial (#) and granulation tissue (arrow). (c) BG60 presenting formed bone (∗), granulation tissue (arrow) and the presence of the biomaterial (#). (d) BG120 showing woven bone (∗), the biomaterial (#) and granulation tissue (arrow). Samples stained with H&E Scale = 48 μm [63].

interconnected bone trabeculae. At 45 days, the CG showed immature newly formed bone. A more mature newly formed bone was observed in the BG and BLG (Fig. 16).

Pinto et al. [55] also performed immunohistochemistry analysis. COX-2 (polyclonal primary antibodies anti-cyclooxygenase), BMP-9 (anti-bone morphogenetic protein), and RANKL (anti-receptor activator of nuclear factor kappa-B ligand) immunoexpressions were evaluated both qualitatively (presence of the immunomarkers) and semi-quantitatively in five predetermined fields using a light microscope.

COX-2 is the rate-limiting enzyme in the conversion of arachidonic acid to prostaglandins, which affect the proliferation and differentiation of osteoblasts and regulate the differentiation and function of osteoclasts [56]. Pinto et al. [55] found that COX-2 expression was up-regulated in the BG rats on the 15th day. The authors suggested that the earlier appearance of COX-2 in the BG rats was responsible for the earlier recruitment of inflammatory cells observed in the histological analysis, resulting in more organized newly formed bone tissue.

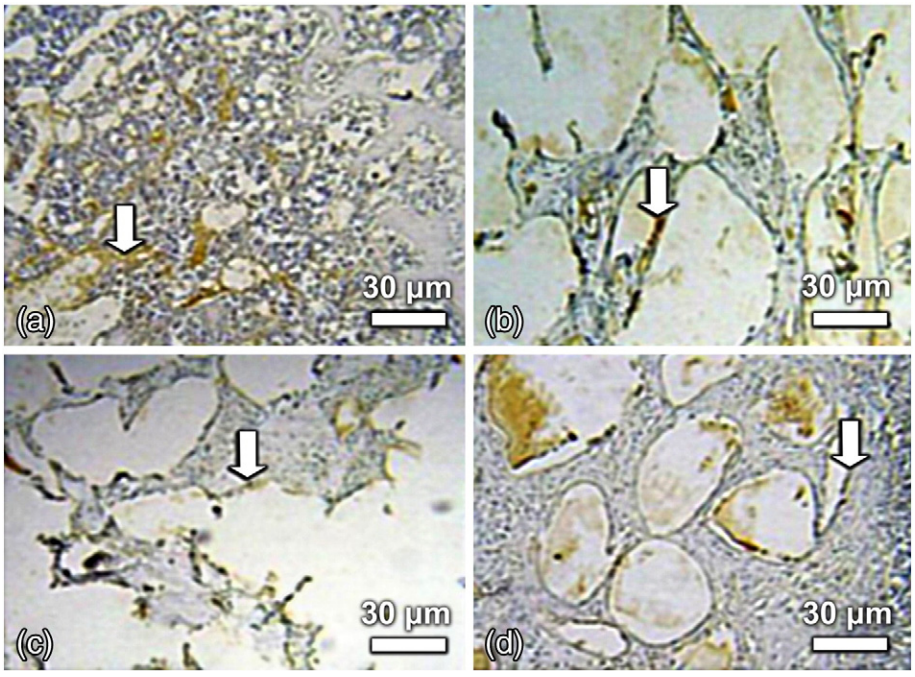

Fig. 19. Immunohistochemistry results for COX-2. (a) CG immunoexpression mainly in the medullar tissue (arrow). (b) BG, immunopositive cells detected in contact with the biomaterial (arrow); (c and d) BG60 and BG120 showing a, strong immunoexpressionin circumjacent cells or granulation tissue (arrow). Scale = 30 μm [63].

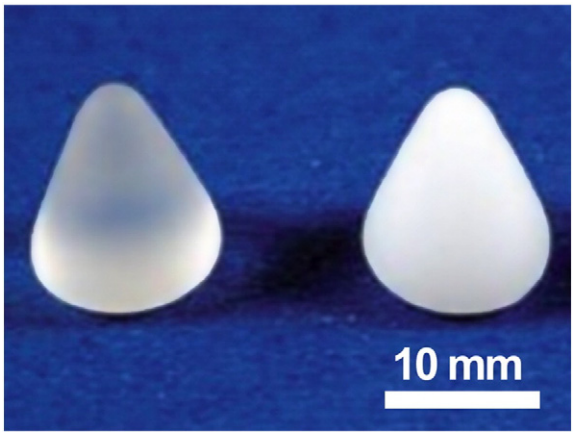

Fig. 20. Implants used in the study by Brandão. Bioglass (left side) and Biosilicate® 1P (right side) [68].

BMPs exhibit stimulatory properties for the differentiation of mesenchymal cells into osteogenic/chondrogenic lineages and increase the expression of alkaline phosphatase and osteocalcin [57,58]. The immunohistochemical analysis revealed that the BLG rats showed a late peak of BMP-9 expression 45 days after surgery in the granulation tissue still observed surrounding the biomaterial particles, corroborating the delay in bone repair presented in this group.

RANKL is a key factor for osteoclast differentiation and activation [59,60]. It has been demonstrated that cellular expression of RANKL in murine callus tissue is tightly coupled during fracture healing and is involved in the regulation of both endochondral resorption and bone remodeling [61]. The immunohistochemical analysis demonstrated slightly higher immunolabeling of RANKL around Biosilicate® particles, with or without laser irradiation, on the 15th day. These findings suggested a higher amount of macrophages/osteoclasts on the surface of the material and accelerated bone turnover, which could culminate in faster healing.

Although LLLT did not lead to an additional effect in bone regeneration, the authors concluded that the incorporation of Biosilicate® scaffolds accelerated the bone healing process.

4.1.3.2. Osteopenic rats. One the most well-known health problems regarding bone tissue is osteopenia, which represents a severe health threat to elderly people and is characterized by a lower bone density that leads to bone weakening and an increased fracture risk. This condition is considered a precursor to osteoporosis.

Researchers around the globe are trying to find alternatives to enhance bone consolidation in patients with this condition because their recovery is slower and more difficult. Acknowledging the advantages of Biosilicate® compared with other bone grafting materials, researchers have investigated its effects on osteopenic animals.

In studies incorporating LLLT, Fangel et al. [62] investigated the effects of Biosilicate® (1P) on bone fracture consolidation in osteoporotic rats. After inducing osteopenia in 40 female Wistar rats, the animals were submitted to a surgical procedure to create a 2 mm-diameter bone defect in the right tibias of the animals.

The animals were randomly divided into four groups: bone defect control group (OC); bone defect filled with Biosilicate® group (B); bone defect irradiated with laser at 60 J/cm^2 group (L60); bone defect filled with Biosilicate® and irradiated with LLLT at 60 J/cm^2 group (BL60). Immediately after the surgical procedure, the laser irradiation treatment was performed and repeated every 48 h for up to 14 days. The histopathological results showed increased deposition of new bone tissue, which leads to an improvement of the biomechanical properties of tibias treated with Biosilicate® associated to laser therapy, as shown in Fig. 17.

Another very interesting and awarded study in this area was made by Bossini et al. [63] in 2011. Forty female Wistar rats were induced with osteopenia. Eight weeks after surgery, the animals were randomly divided into four groups: bone defect control group (CG); bone defect filled with Biosilicate® (1P) group (BG); bone defect filled with Biosilicate® (1P) and irradiated with LLLT at 60 J/cm^2 group (BG60); and bone defect filled with Biosilicate® (1P) and irradiated with LLLT at 120 J/cm^2 group (BG120).

Histopathological, morphometric and immunohistochemistry analyses were performed, which indicated, by the end of 14 days, that the animals with bone defects filled with Biosilicate® and irradiated with laser at 120 J/cm^2 showed a higher amount of newly formed bone compared with the other groups. This result was mainly due to up-regulation of COX-2 expression in the bone cells and stimulation of osteoblast proliferation (Figs. 18 and 19 [63]). This study was awarded best scientific poster by the North American Association for Laser Therapy (NAALT) in 2013 [64].

4.2. Rabbit eviscerated socket

The recovery of the lost volume of enucleated or eviscerated orbital cavities resulting from eye surgery has long been of medical concern. Enucleation refers to the total removal of the ocular globe, while the term evisceration refers to the surgical removal of contents of the ocular globe (e.g., iris, lens, vitreous humor, retina and choroid), leaving the sclera (white part of the eye), eye muscles and other orbital structures intact. Among other reasons, evisceration or enucleation are necessary in cases of acute eye injury, to treat intraocular tumors, or to diminish pain in a blind eye.

Both cases require the implantation of a void-filling material to allow for the subsequent placing of an ocular prosthesis for aesthetic restoration. The first material used for this purpose was glass (in the form of spheres) in 1885 [65]. But in the last 60 years, other materials have been used, such as silicone, polymethylmethacrylate (PMMA), polyethylene (PE), polytetrafluoroethylene (PTFE) and alumina [66]. However, these materials are associated with surgical complications, such as

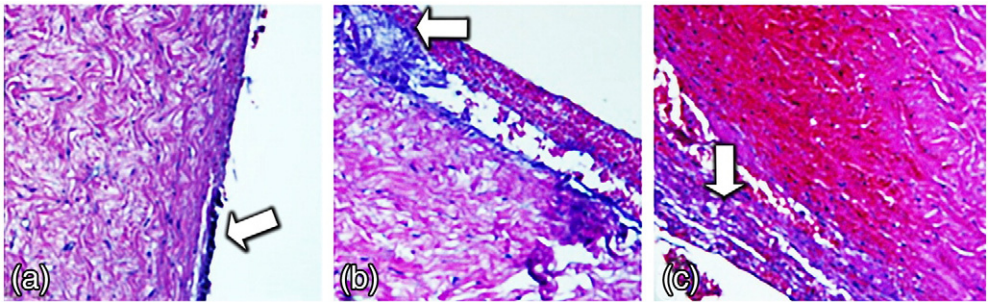

Fig. 21. Light microscopy sections showing tissue repair reaction at the outer implant interface (arrow), Stage 1 (S1). a) Bioglass; b) Biosilicate® with 1 crystalline phase; c) Biosilicate® with 2 crystalline phases. A pseudocapsule made up of fibroblasts, erythrocytes, and inflammatory cells is visible in all sections (H&E × 40) [68].

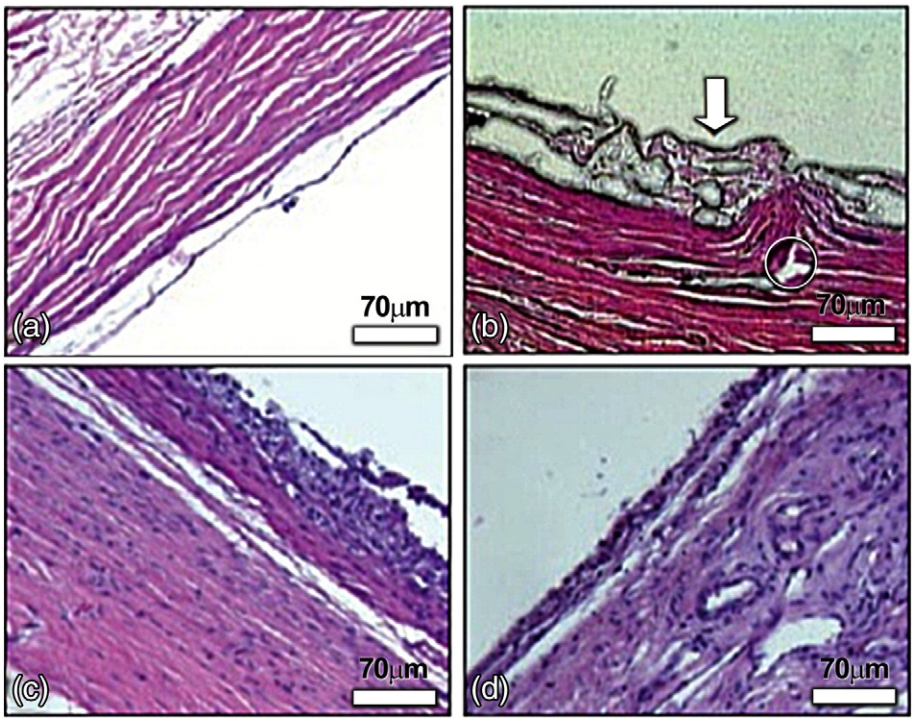

Fig. 22. Histological sections of the repaired tissues formed between the rabbit sclera and the biomaterial cones at 180 days. a) Bioglass 45S5; b) Presence of biomaterial surrounded by the host tissue; c) Biosilicate® 1P; d) Biosilicate® 2P. It is possible to observe a pseudocapsule formed by fibroblasts, the absence of edema and a small inflammatory reaction [69].

suture dehiscence, mechanical trauma, exacerbated inflammation, and frequently, implant extrusion. Low motility [66] has also been reported, which causes aesthetic problems.

Porous bioactive HA-based implants are also commercially available. However, their use involves some drawbacks, such as their brittleness, which makes it difficult to suture the extraocular muscles directly onto the implant, and their limited ability to bind to soft tissues and support fibrovascularization [66]. Recent *in vivo* tests performed on rabbits at the Botucatu Medical School, São Paulo State University (UNESP) in Brazil revealed a high percentage of extrusion of HA implants (~60%) and also the presence of small HA particles in the circulatory system [67].

Thus, the development of a material that replaces the orbital volume and is also able to bind to soft tissues is highly desirable. This would prevent implant movement and ensure the adequate motility of an ocular prosthesis.

Although the properties of the ideal orbital implant are still open to discussion, once again, bioactive glasses and glass-ceramics may potentially offer a better clinical outcome.

Because Biosilicate® is a multipurpose biomaterial, it was also considered for repair of the volume loss following enucleation and evisceration of the eye cavity. Brandão et al. [68,69] produced two interesting papers regarding the study of this application.

The first study was published in 2012 and aimed to assess the biocompatibility of Biosilicate® and Bioglass 45S5 in rabbit eviscerated sockets. Fifty-one Norfolk albino rabbits underwent evisceration of the right eye followed by implantation of cones made from Bioglass 45S5 (control group) and two types of Biosilicate® (1P and 2P) into the scleral cavity, as seen in Fig. 20 [68].

Clinical examinations, biochemical evaluations and orbital CT scans were performed at 7, 90, and 180 days post-procedure. Morphometric

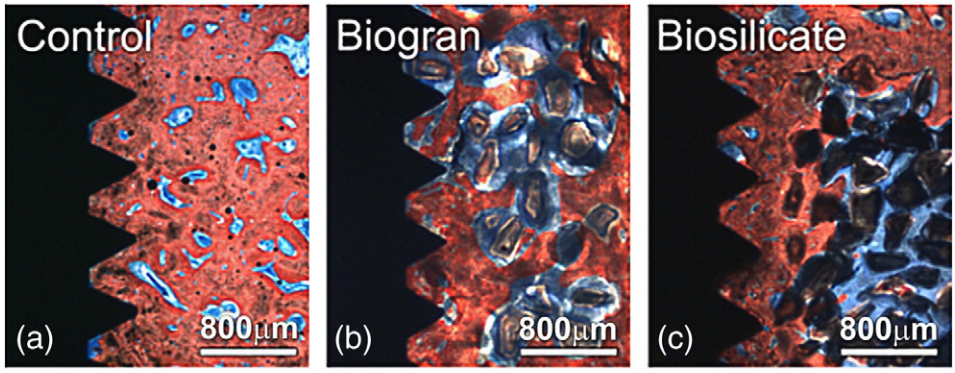

Fig. 23. Optical microscopy of ground sections of the bone-Ti implant interfacial area for the control (a), Biogran® (b), and Biosilicate® (c) groups at 8 weeks post-surgery. Low magnification image of the bone-implant interface showing densely mineralized bone tissue for the control group (a) and a large number of Biogran® (b) or Biosilicate® (c) particles embedded in both bone and bone marrow tissues. Stevenel's blue and Alizarin red staining. Scale bars indicate 800 mm (a,b,c) [70].

evaluation and scanning electron microscopy examination were also performed.

During the experimental period, no animals presented orbit infection, implant migration nor extrusion, and the morphological analysis demonstrated pseudocapsules around all of the implants (Fig. 21). The Bioglass and single-phase Biosilicate® (1P) implants induced lower inflammation and less pseudocapsule formation than the two-phase Biosilicate® (2P).

Seven days after the surgical procedure, the inflammatory reaction was most intense. As expected, the inflammatory reaction gradually diminished throughout the experiment for all groups, especially the Bioglass 45S5 group. During 180 days, no evidence of implant migration or changes in the orbital cavity were detected.

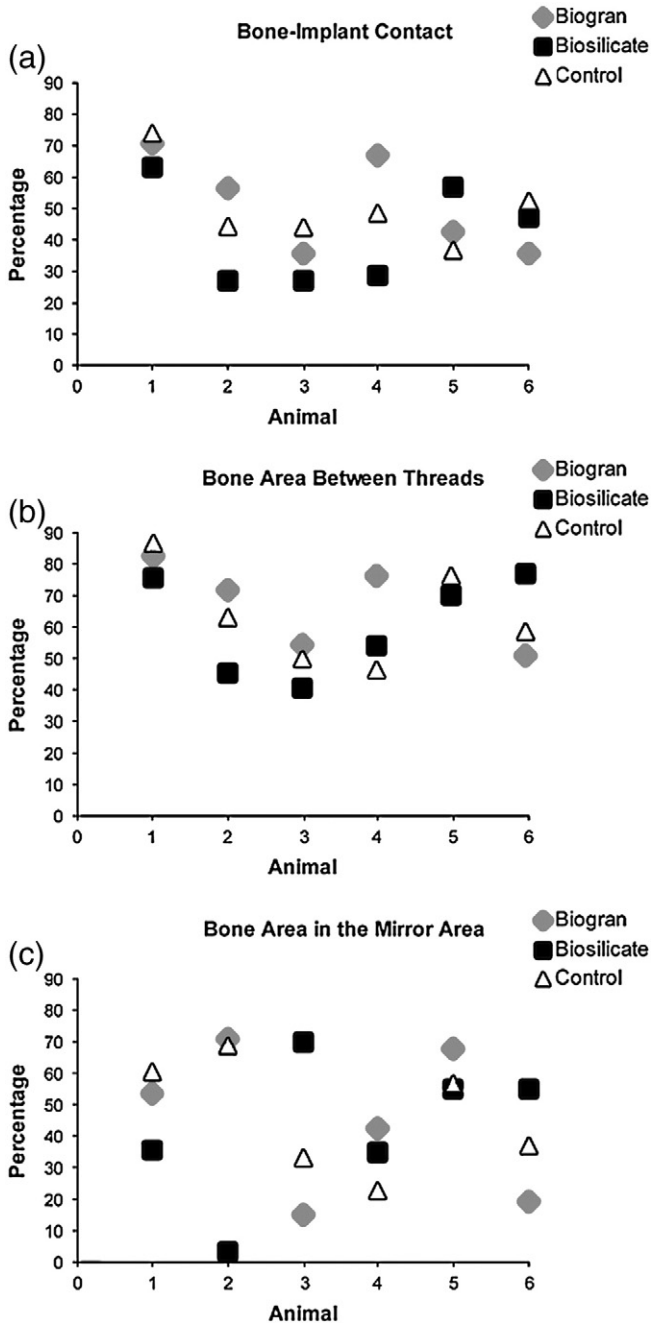

Fig. 24. Dispersion graphs of (a) bone-implant contact, (b) bone area between threads, and (c) bone area within the mirror area for Biogran®, Biosilicate®, and control groups (mean values per animal). No statistical differences were detected among the groups for such parameters [70].

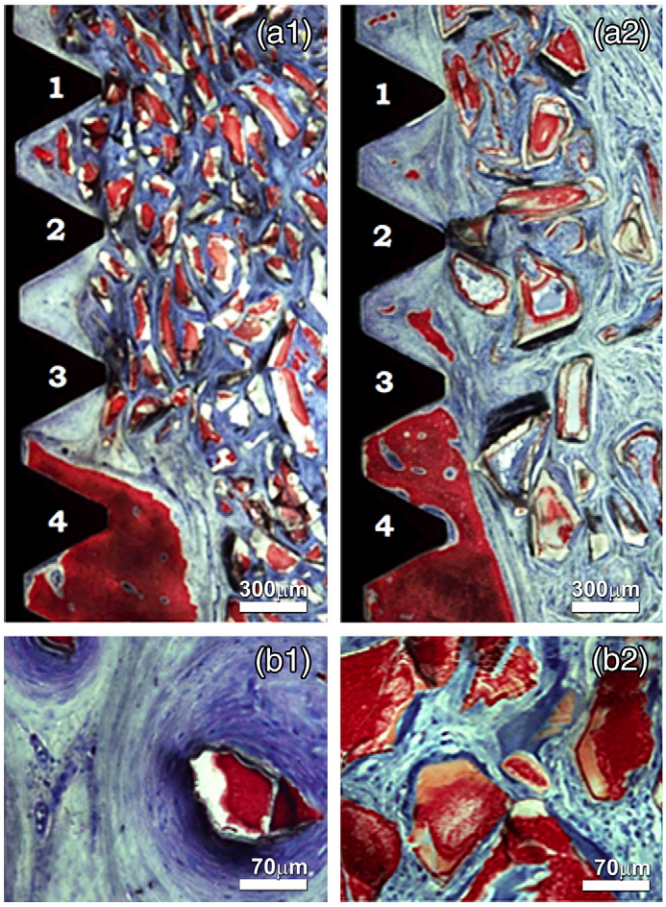

Fig. 25. Histological analysis after 18 weeks. a1) Ti implant and Bioglass (5×); a2) Bioglass particles partially degraded (20×); b1) Ti implant and Biosilicate® (5×); b2) Biosilicate® particles partially degraded with bone matrix formed around them (20×) [71].

Hence, it was inferred that Bioglass and both single- and dual-phase Biosilicate® implants could be alternative materials to manage the anophthalmic socket, the best responses being obtained with Bioglass 45S5 and single-phase Biosilicate® cone implants.

The second study evaluated the biocompatibility of Bioglass 45S5 and Biosilicate® (1P) in eviscerated cavities of 45 rabbits. The animals were sacrificed at 7, 90 and 180 days after surgery. Clinical examinations were performed daily, and biochemical examinations, histological analyses and morphometric analyses were made throughout the experiment. No cone extrusion was observed. Histologically, the pseudo capsule

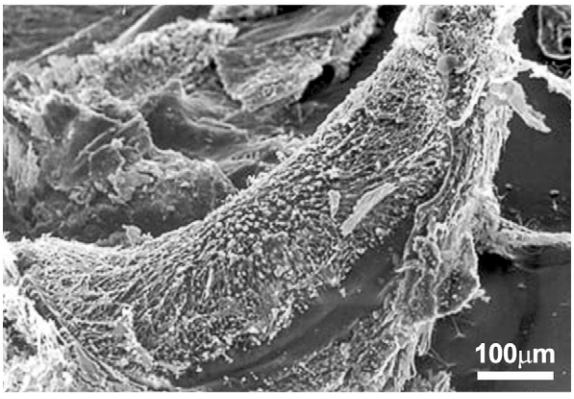

Fig. 26. Crest of the lateral canal ampulla without alteration. Biosilicate® group — 30 days [72].

formation around the cones and the inflammatory reaction was higher in the 7-day group, which progressively decreased towards 180 days [69].

Based on the findings in this study, it was concluded that Bioglass 45S5 and Biosilicate® (1P) cones show no signs of systemic or local toxicity when implanted in the cavity of eviscerated rabbits (Fig. 22).

4.3. Maintenance of alveolar ridges and osseointegration of titanium implants

The use of titanium implants has significantly increased in the past few years, especially in dentistry. Due to the need of a bone graft in some of these procedures, Roriz et al. [70] evaluated the efficacy of Biosilicate® and a bioactive glass (Biogran®) in the maintenance of the alveolar ridge and in the osseointegration of Ti implants in dental sockets.

For this *in vivo* study, the four mandibular premolars were extracted from 6 dogs, and the sockets received a graft of Biosilicate® (1P) particles, Biogran® particles or were left untreated. After the extractions, measurements of width and height on the alveolar ridge were taken. Twelve weeks later, a second surgery was performed to bilaterally insert three titanium implants. Eight weeks post-surgery, histological and histomorphometric analyses were performed, as well as the determination of the percentage of bone-implant contact (BIC), the mineralized bone area between threads (BABT), and the mineralized bone area within the mirror area (BAMA). The results indicated that the Biosilicate® and Biogran® particles preserved the alveolar ridge height without affecting its width. The analysis of variance results showed a statistic difference ($p = 0.045$) in the height of the alveolar ridge (Biogran® = Biosilicate® > control groups, Fig. 23). Regarding the clinical parameters of height, the mean values and the standard deviations found were as follows. The Biogran® group had an average alveolar ridge increase of 0.2 ± 1.3 mm, the Biosilicate® group had an average alveolar ridge increase of 0.3 ± 1.5 mm and the control group had an average alveolar ridge decrease of 1.2 ± 0.9 mm. Regarding to the width of the alveolar ridge, the Biogran® group had an average loss of 0.7 ± 1.4 mm, the Biosilicate® group had an average loss of 0.5 ± 1.4 mm, and the control group had an average loss of 1.2 ± 0.9 mm. However, no significant differences in terms of the BIC, BAMA and BABT values among the groups were detected (Fig. 24).

The authors believe that these results occurred because the experimental period chosen for this study was too long; therefore, selecting a narrower time range would positively affect the results. It was concluded that both biomaterials favored the alveolar ridge and increased its height and width. After histological and histomorphometric analyses, all of the biomaterials were determined to be safe alternatives for bone regeneration before the placement of titanium implants.

Another study in the same field was performed by Jabur et al. [71], which investigated the amount of bone formation on titanium implants in sites subjected to buccal alveolar crest defects that were filled with different bone substitutes. For this study, the mandibular premolars and first molars were extracted from 5 dogs. After 12 weeks, three implants were bilaterally placed in the sites of buccal alveolar bone defects (45 mm length, 6 mm height and 4 mm depth). Each implantation site was randomly treated with Biosilicate® (1P), Biogran®, autogenous bone or did not receive any treatment. At 18 weeks after implantation, histological and histometric (bone-implant contact — BIC, mineralized bone matrix between implant threads — BA and bone matrix within mirror area — BMA) analyses were performed.

Histological sections of the 4 experimental groups exhibited mature bone tissue in contact with implants. However, the percentage of BIC, BA and mineralized BMA in the treated or non-treated groups was not significantly different among all of the four experimental groups, indicating that the presence of the bone grafts did not interfere with bone formation or the osseointegration process. Additionally, the tissue response to Biosilicate® (1P) was similar to that of Bioglass 45S5 and autogenous bone (Fig. 25).

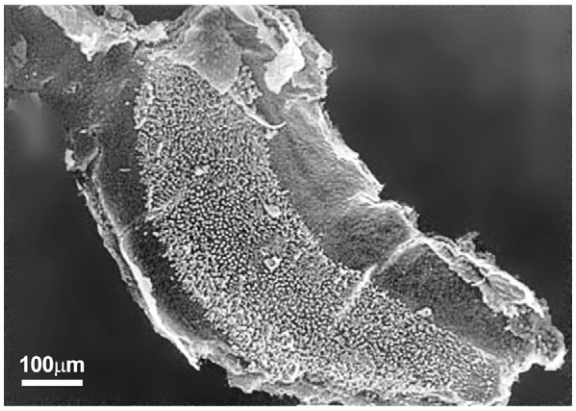

Fig. 27. Saccular macula of the Biosilicate® group at 90 days and without lesion [72].

4.4. Middle ear ossicle chain substitution

Another very satisfying clinical report used Biosilicate® for middle ear ossicle chain substitution. Ear infections can lead to the destruction of these ossicles, causing auditory transmission loss; therefore, any material that can reestablish hearing is indeed relevant. To evaluate the material compatibility, ototoxicity and vestibulotoxicity, Massuda et al. [72] tested in the first stage of the study Biosilicate® (1P) prostheses in guinea pigs.

After 30 and 90 days post-surgery, no ototoxicity or vestibulotoxicity was found in the animals whose middle ears were exposed to Biosilicate®, as can be observed in Figs. 26 and 27.

5. Clinical trials

5.1. Treatment of dentin hypersensitivity

One of the clinical studies using Biosilicate® involved testing its efficacy for cervical dentin hypersensitivity (DH) [73]. In this study, a total of 142 patients were randomized into four groups that received different desensitizing treatments, Sensodyne®, SensiKill® or Biosilicate® (1P) dispersed in a gel suspension or in a solution with distilled water. Over a period of 6 months, a total of 232 teeth were evaluated, and the study was performed using pain assessments (patients used a visual analogue scale of pain, VAS, from 1 to 10). Regarding the global diminution of pain over the course of the study, Biosilicate® mixed with distilled water (G4) displayed the greatest effect and could diminish the pain in the very first periods of the experiment, followed by SensiKill® (G2), Sensodyne® (G1) and Biosilicate® dispersed in gel (G3).

All results are shown in Fig. 28 for short periods of time (0 to 4 weeks) and longer periods of time (1 to 6 months). This study indicated that micron-sized particles of Biosilicate® could provide an immediate, effective and long-lasting treatment alternative for patients who suffer from DH. Another interesting advantage presented by Biosilicate® in this application is that, because it is a fully crystalline material, it does not present sharp cutting surfaces. Glasses, on the other hand, possess conchoidal fracture surfaces that can lead to gum irritation during brushing.

5.2. Middle ear ossicle chain substitution

After the *in vivo* tests in guinea pigs, in the second stage Massuda [74] conducted a clinical study that compared remodeled anvil ossiculoplasty and Biosilicate® (1P) (total and partial prostheses, Fig. 29) using audiometric tests three months after surgery.

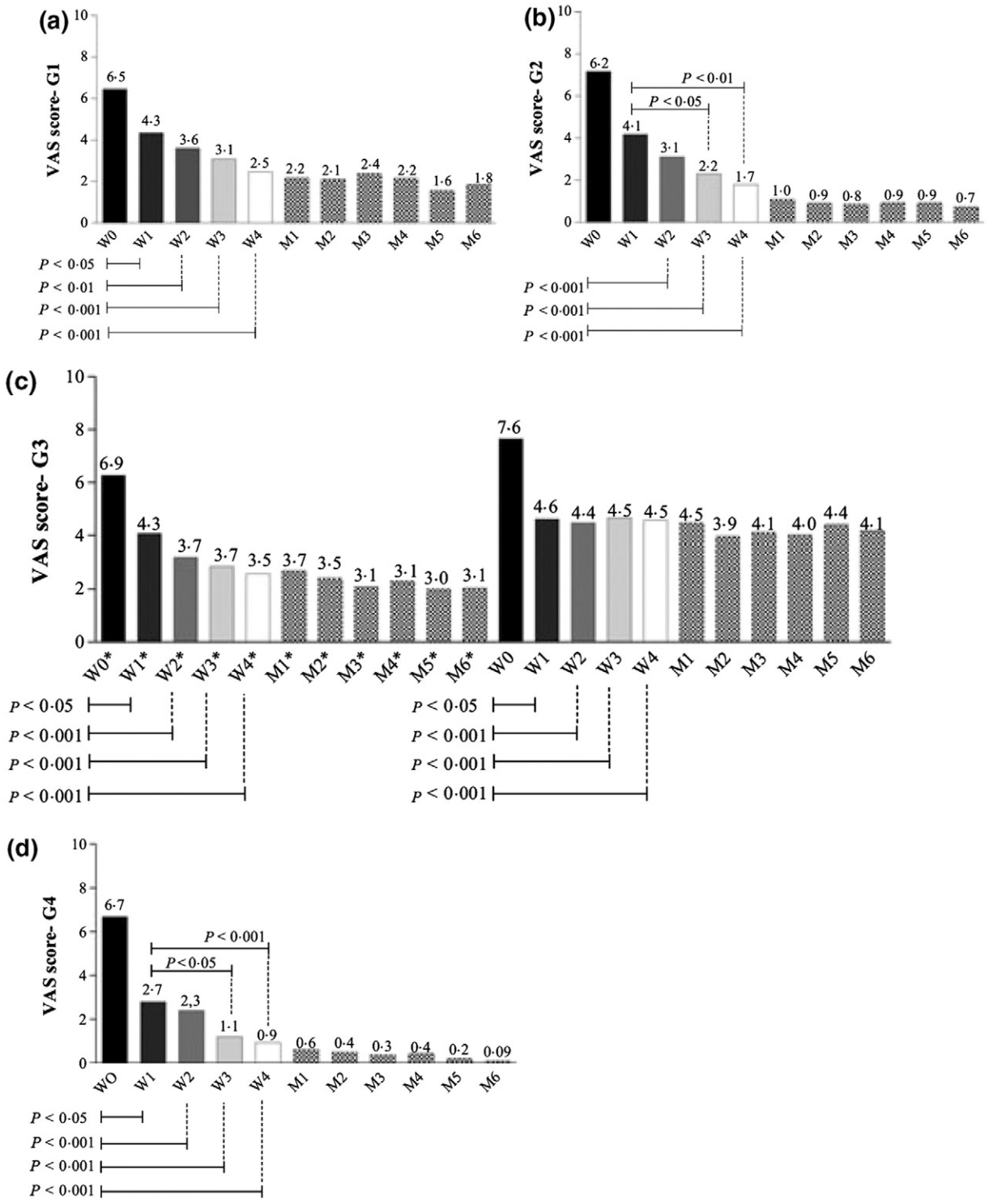

Fig. 28. Values from the pain assessment for each group. W0-4 refers to the week period of treatment and M to months. (a) The results for G1; (b) for G2; (c) for the double-blind evaluation at G3 where letter W or M plus an asterisk (*) refer to patients that used gel with Biosilicate® and the letter W or M without an asterisk refer to patients that used the same gel without Biosilicate® particles; (d) for G4. The mean values of DH pain throughout the 6 months are presented over each column. The statistical significance of the comparison of DH values in the first month of the study is presented in the graph [73].

Twenty-nine surgeries were performed: 14 with partial ossicular replacement prosthesis (PORP) with Biosilicate®, 7 with total ossicular replacement prosthesis (TORP) with Biosilicate® and 8 with bone PORP.

The results indicated that there was an improvement in the air-bone gap (≤20 dB) in all of the study groups, and all of the groups were statistically significant ($p \leq 0.001$) with 50% in PORP Biosilicate®, 29% in TORP Biosilicate® and 50% in bone PORP. This study concluded that an Otosilicate (the name given to these special shapes of monolithic Biosilicate® pieces) prosthesis is an effective substituent for ossicles, not only for its biological properties but also for its machinability, as can be observed in the Fig. 30.

This study received an award for best oral presentation in the 39th Congress of the Brazilian Association of Otolaryngology and Cervical-Facial Surgery (ABORL-CCF) in Belo Horizonte — MG in 2008 [75].

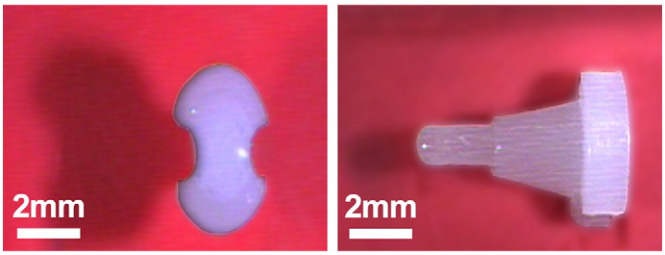

Fig. 29. Biosilicate® prosthesis (Otosilicate) [74].

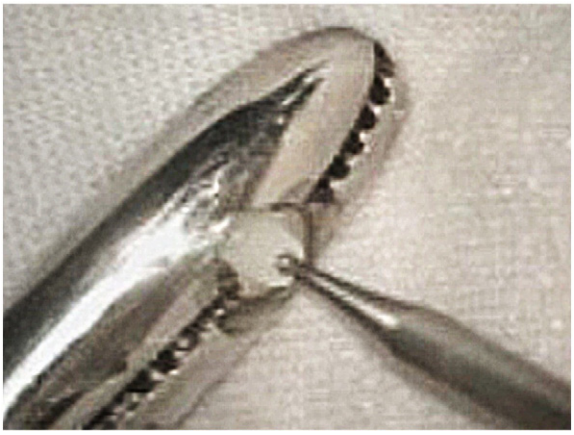

Fig. 30. Machinability of Biosilicate® prostheses during the surgical procedure [74].

5.3. Orbital implants

After successful *in vivo* tests on rabbits (Section 4.2), twelve patients who had undergone enucleation or evisceration procedures were implanted with Biosilicate® (1P) orbitals in May 2014 (see Fig. 31). They are being subjected to follow-ups after 7, 15, 30 days and thereafter at two-month intervals for a period of six months.

Table 2
A summary of the *in vitro*, *in vivo* and clinical tests performed with Biosilicate®.

Analysis		Material form
In vitro	**Acellular**	
	SBF	Monolithic discs, scaffolds and gelatin-coated scaffolds
	Cellular	
	Osteoblasts, fibroblasts, osteogenic cells	Powder, monolithic discs and scaffolds
In vivo	**Animal model**	
	Rat tibia	Solid particles, scaffolds and scaffolds + laser irradiation
	Rat calvaria	Monolithic discs
	Rabbit femur	Monolithic rod implants
	Rabbit eviscerated sockets	Orbital implants
	Dog mandibular socket	Coarse particles
	Guinea pig middle ear	Ossicle implants
Clinical	**Specialty**	
	Dentistry	Dentin hypersensitivity (fine powder)
	Ophthalmology	Orbital implants (monolithic)
	Otorhinolaryngology	Middle-ear ossicle implants (monolithic)

According to a preliminary report by the surgical team responsible for this clinical study, none of the patients showed postoperative complications or signs of inflammation, dehiscence or extrusion. Some of them have already been through the six-month follow-up period (4 patients), and some are being implanted with the external prosthesis or are in their last period of evaluation.

The laboratory tests of patients who have completed the study have revealed no changes in vital organs. These patients have also been subjected to computed tomography examinations, which showed no migration, formation of abscesses or inflammation around the implants.

A detailed study of the clinical trials will be published upon conclusion of the six-month follow-up period of the last group of patients. By then, a total of 40 patients will have received Biosilicate® orbital implants.

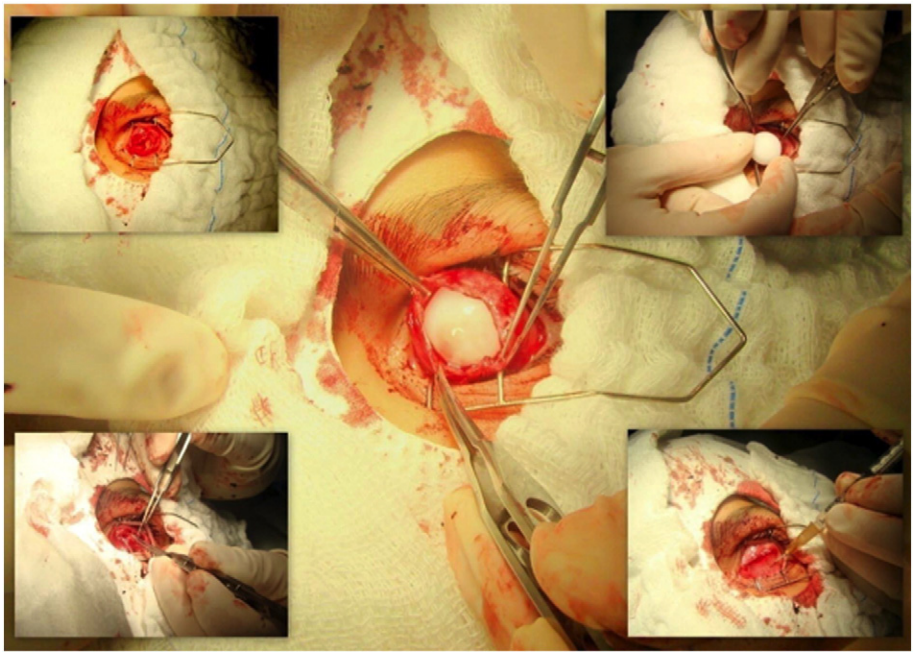

Fig. 31. Implantation of a Biosilicate® (1P) orbital implant in a patient after an evisceration procedure. Courtesy of Drs. Simone M. Brandão and Suzana Matayoshi (Botucatu Medical School, São Paulo State University, Botucatu, SP, Brazil).

Table 3
Comparison of Biosilicate® to Bioglass 45S5 observed in some *in vitro* and *in vivo* tests.

	Test	Biosilicate® results compared to 45S5
***In vitro* studies**	• Tests with SBF	HCA layer onset time: 6 h/similar to 45S5.
	• Citotoxicity and genotoxicity	Do not present cytotoxic and genotoxic potential/similar to 45S5.
	• Osteogenic cells	Similar amount of alkaline phosphatase activity; increased calcified matrix.
	• Osteogenic cells (conditioned surface)	Higher expression levels of Runx2, alkaline phosphatase (ALP) and bone sialoprotein (BSP) mRNAs than 45S5.
	• Osteogenic cells and laser irradiation	Higher osteogenic activity than 45S5.
***In vivo* studies**	• Tibial defects in rats	Higher amount of fully formed bone and higher osteogenic activity. Better biomechanical properties: increase in the maximum load at failure and stiffness than 45S5.
	• Femur defects in rabbits	Higher cortical bone formation than 45S5.
	• Eviscerated socket in rabbits	Similar results for both materials: No inflammation and little pseudocapsule formation. Also, no systemic or local toxicity was detected.
	• Osseointegration of titanium implants	Increased height of alveolar ridge and less pronounced width average loss of the alveolar ridge. Similar BIC, BAMA and BABT to 45S5.

6. Summary

After 20 years of research, Biosilicate® was evaluated in different situations and various *in vitro*, *in vivo* and clinical tests, as shown in Table 2. To evaluate the efficacy of Biosilicate®, this glass-ceramic has been compared to the gold standard Bioglass 45S5 in several experiments. In a very short form, the advantages of Biosilicate® with respect to Bioglass 45S5 are summarized in the Table 3. The analyzed properties so far indicate that Biosilicate® has similar or better properties than Bioglass 45S5. Nonetheless, several other *in vivo* and clinical studies still should be conducted in order to reach Bioglass 45S5 scientific relevance and its wide range of applications.

7. Conclusions

Biosilicate® has been evaluated in 28 theses and dissertations and in more than 30 scientific papers over the last 20 years. These studies have demonstrated its efficiency for regenerating bone tissue and treating dental hypersensitivity. Biosilicate® presents some important features for bone tissue regeneration: it is highly bioactive, osteoconductive, osteoinductive, non-cytotoxic, non-genotoxic and has antibacterial properties. In monolithic form, it is strong, tough and machinable. Its *in vitro* and *in vivo* bioactivities are comparable to that of the gold standard Bioglass 45S5. Therefore, Biosilicate® has indeed been shown to be a very versatile, multipurpose biomaterial. It can be applied in powder, monolithic or 3D forms that could be easily machined before or during surgical procedures.

Future perspectives include analyzing this biomaterial in different surgical procedures and more intensively exploring clinical trials.

Acknowledgments

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