Efficacy of a bioactive glass–ceramic (Biosilicate®) in the maintenance of alveolar ridges and in osseointegration of titanium implants

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Key words: biomaterials, bone repair, dental sockets, osseointegration, titanium implants

Abstract
Objectives: The aims of this research were to evaluate the efficacy of a bioactive glass–ceramic (Biosilicate®) and a bioactive glass (Biogran®) placed in dental sockets in the maintenance of alveolar ridge and in the osseointegration of Ti implants.

Material and methods: Six dogs had their low premolars extracted and the sockets were implanted with Biosilicate®, Biogran® particles, or left untreated. After the extractions, measurements of width and height on the alveolar ridge were taken. After 12 weeks a new surgery was performed to take the final ridge measurements and to insert bilaterally three Ti implants in biomaterial-implanted and control sites. Eight weeks post-Ti implant placement block biopsies were processed for histological and histomorphometric analysis. The percentages of bone–implant contact (BIC), of mineralized bone area between threads (BABT), and of mineralized bone area within the mirror area (BAMA) were determined.

Results: The presence of Biosilicate® or Biogran® particles preserved alveolar ridge height without affecting its width. No significant differences in terms of BIC, BAMA, and BABT values were detected among Biosilicate®, Biogran®, and the non-implanted group.

Conclusions: The results of the present study indicate that filling of sockets with either Biosilicate® or Biogran® particles preserves alveolar bone ridge height and allows osseointegration of Ti implants.

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Titanium [Ti] implant placement has been widely used to restore the function and aesthetics of edentulous areas post-tooth extraction and alveolar ridge maintenance procedures. To preserve bone height and width and, therefore, long-term Ti implant osseointegration, several biomaterials have been suggested to partially fill dental sockets and/or bone defects (Nevins et al. 1998; Carmagnola et al. 2000; Norton & Wilson 2002; Stvrtecky et al. 2003). Guided bone regeneration associated or not with several types of grafts and/or biomaterials – such as autogenous bone, freeze-dried bone allograft, hydroxyapatite, anorganic bovine bone, and bioactive glasses – has been used before Ti implant placement to achieve such goal, or to increase alveolar bone ridge dimensions (Kostopoulos & Karring 1994; LeKovic et al. 1997; Fugazotto 1998; Watzinger et al. 2000; Novaes & Souza 2001; Pinho et al. 2006). Despite the good results that can be obtained with such strategy, some drawbacks may affect the outcome, including the ability to adequately apply the technique (the operator’s ability with barrier handling and fixation), a large period of barrier maintenance, risk of membrane exposure requiring its removal before the necessary period to allow bone formation, and the high cost of membranes (Fritz et al. 1996).
Among all those alternatives used before Ti implant placement in post-extraction sockets, bioactive glass particles seem to represent a safe choice when bone repair is needed, not only because of its well-known stimulatory effects on bone cell functions, but also because it may prevent the loss of alveolar ridge contour and allow osseointegration of Ti implants to occur [Norton & Wilson 2002; Svrtecky et al. 2003]. Bioactive glasses are synthetic materials, therefore eliminating the possibility of disease transmission and favoring their availability. It is well known that the great success of bioactive glass is mainly due to its high bioactivity, which favors an apatite layer formation on the particle surfaces that attracts undifferentiated and osteogenic cells to the implanted site ultimately promoting bone formation [Hench 1980; Schepers et al. 1993; Wheeler et al. 1998]. The commercial bioactive glass particulate material Biogran™, using Bioglass® 45S5 particle size within the 300–355 μm range, has been considered ideal for such clinical application [Wheeler et al. 1998].

Recently, some members of our research group developed a fully crystalline glass-ceramic in the NaO-CaO-SiO2-P2O5 system [Biosilicate®, Pl 0300644-1] (Zanotto et al. 2004). In contrast to what was expected about the decrease of the bioactivity level following the increase of the material’s crystallinity, in vitro experiments demonstrated that Biosilicate® is highly bioactive and supports enhanced bone-like matrix formation compared with its parent glass and to Bioglass® 45S5 in an osteogenic cell culture system [Moura et al. 2007]. Therefore, it would be relevant to verify whether Biosilicate® could promote in vivo bone repair when the use of synthetic biomaterials is an indicated procedure. The present study aimed to evaluate clinically the ridge maintenance in a dog mandible, and to analyse histologically and histomorphometrically bone repair adjacent to and in contact with a conventional Ti implant placed in dental sockets that were previously filled with Biosilicate® or Biogran™.

Materials and methods

Bioactive glass–ceramic [Biosilicate®, Vitrovita, São Carlos, SP, Brazil] and bioactive glass [Biogran®, Orthovita Inc., Malvern, PA, USA] were used in this study. To prevent any particle size effect, Biosilicate® was milled to the same size range of Biogran® (300–355 μm).

Six mongrel dogs weighing around 18 kg each were used in this study under a research protocol approved by the Ethical Committee for Animal Study of the University of São Paulo. The animals presented general good health, with intact teeth and absence of periodontal disease or oral injuries of any nature. Animals were anesthetized through intramuscular injection of 2% Rompun 20 mg/kg (Bayer, Porto Alegre, RS, Brazil) at the dosage of 0.05 ml/kg, and, after sedation, an intravenous injection of thiopental [Cristália, Itapira, SP, Brazil] 1 ml/kg (20 mg thiopental diluted in 50 ml saline) was applied. Then, local anesthesia was made by infiltrating 2% mepivacaine plus adrenaline 1:100,000 in both quadrants of the mandible. Local antisepsis was made with 2% chlorhexidine. Incisions were made in the crevice region of the first, second, third, and fourth premolars. Minute buccal and lingual full thickness flaps were elevated to disclose the marginal alveolar bone. The second, third, and fourth premolars were sectioned with high-speed diamond burs to avoid tooth fracture [Fig. 1a]. Teeth were carefully removed using elevators and forceps. Some of the remaining alveolar bone septa were removed with burs under abundant sterile saline solution in order to produce similar sized dental sockets and the sockets were debrided. After that, titanium screws (1.5 mm in width and 3 mm in length) were attached on the buccal side (onto center) of alveolar ridge to serve as reference points to trans-surgical measurements [Lekovic et al. 1997; Camargo et al. 2000]. At this time, alveolar ridge width (buccal/lingual distance) and height (distance from reference screw point to alveolar crest) were measured by one calibrated periodontist using a compass on the reference points. The measurements were transferred to a probe, registered in millimeters and taken as initial values [Fig. 1b and c].

Middle sockets were always related to the control group, whereas the mesially and distally located ones were randomly selected with a coin to be filled with either bioactive glass–ceramic [Biosilicate® group] or bioactive glass [Biogran® group]. Such experimental design was chosen aiming to avoid mixing of biomaterials particles. Flaps were coronally positioned covering the edentulous ridge and were sutured using 3.0 silk interrupted sutures, avoiding excessive tension on suture line [Fig. 1d–f]. The dogs received intramuscularly injections of 20,000 UI of penicillin and erythromycin (0.1 g/kg) starting on the day before the surgery and then every 4 days during 2 weeks. Following surgery, the animals were fed only water-softened dog food. In addition, the dogs were sedated for thorough ultrasound prophylaxis every 2 weeks. The suture was removed after 10 days.

At 12 weeks post-extraction, Ti implants were placed. Briefly, under the same protocol of anesthesia described above, the animals were submitted to a new surgery, in which horizontal incisions were done over edentulous ridge and full-thickness mucoperiosteal flaps were raised. At that time, alveolar ridge width and height measurements were carried out as described above and taken as final values. Using buccal screws as reference, bone perforations with specific burs [Fig. 1g and h] were performed before Titamax II (Neodent, Curiúta, PR, Brazil) implants placement [9 mm in length and 4.5 mm thick]. All implant placement procedures were carried out according to the manufacturer’s instructions [Fig. 1i]. Flaps were sutured using silk interrupted sutures and removed after 10 days. Follow up of the healing process was performed on a weekly basis. Eight weeks post-Ti implant placement, the dogs were anesthetized as described above and block biopsies were removed [Fig. 1j]. The animals were then euthanized with a lethal dose of pentobarbital. Alveolar ridge maintenance, height and width, were calculated from the difference between final and initial values of the clinical measurements.

Histological processing

Block biopsies were ground-sectioned for light microscopy analysis, as previously described (Maniatopoulos et al. 1986). Briefly, immediately after harvesting, the block biopsies were immersed in 10% formalin buffered with 0.1 M sodium cacodylate, pH 7.3, for 48 h and transferred to a solution of 70% ethanol and left for 72 h. Bone segments were then dehydrated in graded concentrations of ethanol and embedded in resin (LR White Hard Grade, London, UK). Following polymerization, resin blocks were sectioned with an annular
blade using Microslice 2 precision saw (Ultra Tec Manufacturing Inc., Santa Ana, CA, USA) to produce one longitudinal mesiodistal section per implant. Each section was polished and mounted on glass slides, and the resulting 40-μm-thick mounted sections were further ground and polished to a thickness of 20 μm. Sections were stained with Stevenel’s blue and Alizarin red S for histological (Maniatopoulos et al. 1986) and histomorphometric analysis.

Histological and histomorphometric analysis

Light microscopy of ground sections were used for the histological description of tissues adjacent to or in direct contact with implant surfaces and for the histomorphometric analysis. Such analysis was relative to the medial third of implants, which involved three consecutive areas between four threads (Tavares et al. 2007). The measurements were determined from the second thread downward toward the fifth one both mesially and distally. For each implant, the mean values of mesial and distal measurements were used. In order to evaluate, respectively, the amount of bone at the bone–implant interface and between threads, bone-to-implant contact (BIC) and mineralized bone area between threads (BABT) were determined. In addition, mineralized bone area within mirror areas (BAMA) was also determined to evaluate parent bone content outside the threaded area. The mirror area was defined as a symmetric area to the trapezoid between two threads, sharing the larger base of the trapezoid (Rosa et al. 2006; Tavares et al. 2007).

For the measurements, images were digitally recorded using a Leica DM LB light microscope (Leica, Bensheim, Germany), with N Plan (× 2.5/0.07, × 10/0.25, × 20/0.40) and HCX PL Fluotar (× 40/0.75) objectives, outfitted with a Leica DC 300F digital camera. The acquired digital images were analysed by a single trained and calibrated examiner (κ ranging from 0.8 to 0.9) blind to treatments for BIC measurements using the Leica QWin software (Leica Microsystems, Nussloch, Germany), whereas BABT and BAMA were determined using ImageJ software, version 1.34s (NIH, Bethesda, MD, USA). Some images were processed with Adobe Photoshop software (Adobe Systems) for preparing Figs 2 and 3 (see “Results”).

Statistical analysis

The data are presented as mean ± standard deviation. The statistical unit for the analysis was the animal (n = 6 to each group) for each parameter evaluated. The data were submitted to one-way ANOVA for dependent samples (the animal as the dependent factor). The level of significance was set at 0.05.

Results

Clinical findings

In general, the healing process following tooth extraction and implant placement was uneventful. At 12-week re-entry surgeries, no particles of any biomaterials were clinically observed and the socket areas appeared to be filled with a hard tissue. Thirty-four out of 36 implants used in the present study were retrieved for the morphologic and histomorphometric analysis; one implant of each biomaterial group was
lost. No primary stability was achieved for five implants at the time of the placement procedure – two of the Biogran® group and three of the Biosilicate® one. From these, one implant of each group was associated with implanted sites that exhibited dehiscences during the initial healing phases and was not used for the analyses. The other three implants became stable overtime and were considered for the measurements.

**Clinical measurements**

The mean ± standard deviation values for the difference between initial and final alveolar ridge height measurements were 0.2 ± 1.1 mm for the Biogran® group, 0.3 ± 1.1 mm for the Biosilicate® group, and −1.2 ± 0.7 mm for the control group, with a statistical difference between control and test groups (P = 0.012). The mean standard deviation values for the difference between initial and final width measurements were −0.7 ± 1.1 mm for the Biogran® group, −0.5 ± 1.3 mm for the Biosilicate® group, and −1.2 ± 0.7 mm for the control group (non-significant; P = 0.437). Tables 1 and 2 summarize the clinical measurements.

**Histological findings**

In all experimental groups bone healing resulted in the formation of lamellar bone trabeculae adjacent to or in direct contact with the implant surface (Figs 2 and 3a–e,h,i). Biosilicate® and Biogran® particles exhibited mostly diffuse borders and were entirely or partially surrounded by a calcified bone matrix (Fig. 3b,c,e–i) or by a

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**Fig. 2.** Light microscopy of a Ti implant placed in the dental socket previously filled with biomaterial particles [Biosilicate®], at 8 weeks post-Ti implant placement. Biomaterial particles were distributed in both mesial and distal parts, although, as expected, not uniformly in the tissues adjacent to the implant surface. Ground section. Stevenel’s blue and Alizarin red S. Scale bar = 1 mm.

**Fig. 3.** Light microscopy of ground sections of the bone–Ti implant interfacial area for control (a,d), Biogran® (b,h,i), and Biosilicate® (c,e–g) groups at 8 weeks post-Ti implant placement. (a–c) Low magnification of bone–implant interface showing a dense 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. (d) The dense lamellar bone in the control group exhibited small vascular canals. (e) Biosilicate® particles were either surrounded by bone matrix (arrow) or by bone marrow tissue. (f) Individually, Biosilicate® particles exhibited bone apposition associated with both internal [*] and external surfaces, which showed signs of erosion. (g) In areas with Biosilicate® particles, bone marrow was characterized by a fibrous/dense connective tissue (also observed for Biogran® group). (h) Bone–implant interface of the Biogran® group showing particles partially surrounded by bone trabeculae. (i) A Biogran® particle in direct contact with Ti surface, a finding that was only rarely observed. Stevenel’s blue and Alizarin red S. Scale bars indicate 800 μm (a,b,c), 200 μm (d,e,g,h,i), and 100 μm (f).
Table 1. Initial and final heights, and variation in the height of the alveolar ridge in millimeters (mean ± SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Measurements (mm)</th>
<th>Initial height</th>
<th>Final height</th>
<th>Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biogran*</td>
<td>4.7 ± 1.5</td>
<td>4.9 ± 2</td>
<td>0.2 ± 1</td>
<td></td>
</tr>
<tr>
<td>Biosilicate*</td>
<td>4.7 ± 1.2</td>
<td>5 ± 2.1</td>
<td>0.3 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.2 ± 0.8</td>
<td>3.1 ± 1.2</td>
<td>-1.2 ± 0.7</td>
<td></td>
</tr>
</tbody>
</table>

*Significant (P = 0.012).

Table 2. Initial and final widths, and variation in the width of the alveolar ridge in millimeters (mean ± SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Measurements (mm)</th>
<th>Initial width</th>
<th>Final width</th>
<th>Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biogran*</td>
<td>6.9 ± 0.8</td>
<td>6.2 ± 1.3</td>
<td>-0.7 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>Biosilicate*</td>
<td>7 ± 1.2</td>
<td>6.6 ± 1.6</td>
<td>-0.5 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7 ± 0.7</td>
<td>5.7 ± 1.3</td>
<td>-1.2 ± 0.7</td>
<td></td>
</tr>
</tbody>
</table>

Non-significant (P = 0.437).

Histomorphometric analysis

Although no statistically significant differences were noticed between the groups in terms of BIC (P = 0.348), BABT (P = 0.492), and BAMA (P = 0.339), the Biogran group exhibited a tendency toward higher values for BIC and BABT, followed by the control and Biosilicate groups (Fig. 4). The BIC values were: Biogran group, 51.3 ± 15.4% [ranging from 35.7% to 70.5%]; Biosilicate group, 41.6 ± 16.1% [ranging from 26.9% to 62.9%]; control group, 49.8 ± 12.7% [ranging from 36.7% to 73.6%]. The BABT values were: Biogran group, 67.6 ± 12.7% [ranging from 50.3% to 82.1%]; Biosilicate group, 59.0 ± 16% [ranging from 39.9% to 76.5%]; control group, 63 ± 15.7% [ranging from 45.8% to 86.6%]. The BAMA values were: Biogran group, 44.9 ± 23.7% [ranging from 15.1% to 70.8%]; Biosilicate group, 42.3 ± 23.3% [ranging from 3.3% to 70%]; control group, 46.5 ± 18% [ranging from 23% to 68.9%].

Discussion

The results of the present study demonstrated that filling of dental sockets with bioactive glass or Biosilicate particles, in addition to preserve the height of the alveolar ridge (compared with the non-implanted sites), allow bone formation adjacent to Ti implants at 8 weeks despite the physical presence of some particle remnants. Indeed, no significant differences in terms of BIC, BAMA, and BABT values were observed among Biosilicate, Biogran, and the control group [with no biomaterials]. A tendency toward higher values of BIC, BAMA, and BABT was noticed for Biogran compared with Biosilicate.

The strategy to use bioactive glass particulate materials in alveolar bone ridge defects before, or concurrently to Ti implant placement has been based on the capacity of this class of biomaterials to prevent bone loss or even to promote an increase in height and width of alveolar ridge. In the present study, bone loss in height of about 1.2 mm for the control group was within the levels that usually take place after tooth extraction. This has been attributed to the predominance of resorptive activities in the context of bone remodeling [Lekovic et al. 1997, 1998; Lasella et al. 2003]. Concerning the results of bone gain in height in test sockets, both bioactive materials were equally effective, a finding that could be due to a tendency to over fill [coronally] the sockets in order to limit the unevenness of the lingual and buccal crestal ridge heights. This finding was also observed by Simon et al. [2000], who used in human maxillary anterior area extra filling with biomaterial aiming to maintain ridge dimensions before implant rehabilitation. No significant differences were observed for the width measurements, although a tendency for Biogran and Biosilicate groups to exhibit reduced bone loss compared with the control group was noticed.

It has been demonstrated high success rates of endosseous implant therapy when Ti implants are placed in bone sites filled with bioactive glasses, irrespective to the time of biomaterials filling, whether before, during, or after Ti implant placement [Johnson et al. 1997; Schepers et al. 1998; Hall et al. 1999; Tadjoeedin et al. 2000, 2002; From et al. 2002; Norton & Wilson 2002, Stvrtecky et al. 2003]. In the present study, the strategy of filling sockets with bioactive glass or Biosilicate particles 12 weeks before Ti implants placement resulted in the lost of only two implants during the 8-week period out of five that exhibited no primary stability. In these cases, the lost of the implants took place in sockets that developed dehiscences during the initial healing phases. The possibility that the presence of biomaterial particles affected primary stability should also be taken into consideration.

One of the key parameters for evaluating the tissue response to biomaterials implant procedures is particle dimension. Johnson et al. [1997] observed higher percentage of bone formation in direct contact with Ti implants placed in rabbit femora using the bioactive glass Perioglas, which exhibits particles ranging from 90 to 710μm. Although Perioglas and the bioactive materials used in the present study belong to the same quaternary system [P2O5–Na2O–CaO–SiO2], Biogran and Biosilicate have been provided as particles with dimensions in a narrowed range [300–555 μm]. Some slight differences between the size distributions and shape of the Biogran and Biosilicate particles may occur, because the fracture profile between glass and crystal-line materials differs, which may generate particles with different characteristics, such as shape, roundness, and surface roughness/topography.

Despite variations in particle dimensions, Hall et al. [1999] demonstrated no
relevant differences between Perioglas® and Biogran® as to bone formation in areas of peri-implant defects. Further studies should take into account variations in the range of Biosilicate® particles aiming to address the controversial subject of bioactive glass-based materials as regards to the correlation between particle dimensions, dissolution rate, and the ability of the particles to affect bone healing and remodeling processes.

Controversial results have also been observed in the literature concerning the quantity of bone formation around Ti implants when bioactive glasses are used as bone biomaterials. The results for the Biogran® and Biosilicate® groups in the present study were similar to the control group, a finding that is supported by the work of Hall et al. (1999), which showed no statistical differences between Biogran® and non-implanted site groups in terms of mean percentages of BIC. Similar findings were observed by Carmagnola et al. (2008), which showed no advantages to the osseointegration of Ti SLA implants with the filling of a self-contained, type 4 bone rabbit tibiae defects with either deproteinized bovine bone matrix [Bio-Oss®], nanocrystalline biocompatible synthetic hydroxyapatite [Ostim-Paste®], or bioactive glass particles [Perioglas®] before implant placement. However, using Ti implants of the IMZ system (Friatec AG, Mannhein, Germany), Schepers et al. (1998) observed higher mean values of BIC for the Biogran® group compared with the control one (50.8% and 33.4%, respectively). At least part of such divergent results could be attributed to the use of different Ti implant systems, with varying shapes, surface chemistry, and topography (Stentz et al. 1997; Abrahamsson et al. 2001; Novaes et al. 2004), in addition to the use of different animal models and experimental and clinical conditions (Carmagnola et al. 2008). Despite the beneficial clinical outcomes as to alveolar ridge height in comparison with the non-grafted sites, the strategy to use biomaterials in bone defects aiming to improve osseointegration needs to be re-evaluated and further improved.

It is worth noting that only rarely were a few glass particles observed in intimate contact with Ti implant surface; indeed, only three out of 23 implants exhibited such histological finding. The present results are in accordance with a previous study (Schepers et al. 1998), which showed no particles in direct contact with Ti implant surface. That could be explained by the occurrence of higher rates of bone remodeling in such areas (Schepers et al. 1998; Gorustovich et al. 2002; Nishida et al. 2006), a fact that is confirmed by the results showing a tendency for higher values of BAMA compared with BABT. In addition, the possibility that fracture of biomaterial particles during the Ti implant placement procedure could accelerate the dissolution rates of Biogran® and Biosilicate® cannot be ruled out. Consistent with the occurrence of rare particles in intimate contact to the Ti implant surface are the observed differences in the dynamics of particles replacement by bone matrix; the closer to the Ti surface, the more advanced stages of bone formation on internal and external particle surfaces (Nishida et al. 2006).

In conclusion, the results of the present study indicate that filling sockets with...
Biogran® or Biosilicate® particles preserve alveolar bone ridge height and does not significantly affect bone quantity adjacent to Ti implants at 8 weeks, despite the presence of the particles in the surrounding bone and bone marrow tissues. Further studies should comparatively evaluate the effect of Biosilicate® or Biogran® particles in larger bone defects, as a strategy to support osseointegration of Ti implants when the use of synthetic materials is indicated as a clinically relevant procedure.

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References


