Characterization of Bone Around Titanium Implants and Bioactive Glass Particles: An Experimental Study in Rats

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Purpose: *Many situations in clinical practice require metallic implants to be combined with bone grafts and/or bone substitutes such as bioactive glass (BG). Upon implantation, silica-based BG particles are transformed into a shell containing calcium and phosphate that loses its inner silicon-rich core. The release of silicon by BG particles and its incorporation by newly formed bone tissue in the peri-implant area had not been studied to date.* ***Materials and Methods:*** *Thirty Wistar rats were used throughout. Under anesthesia, a commercially pure titanium (Ti) laminar implant was placed inside the medullary compartment of the tibia (Ti group), while in the contralateral tibia (Ti/BG group) a titanium laminar implant and melt-derived BG 45S5 particles were implanted. The animals were sacrificed 14, 30, and 60 days postimplantation. The tibiae were resected, radiographed, and embedded in methyl methacrylate resin. Sections were stained with toluidine blue and analyzed by light microscopy and energy-dispersive x-ray analysis (EDX). The presence of silicon, calcium, and phosphorus was evaluated in the BG particles and in the peri-implant bone tissue for each of the experimental times.* ***Results:*** *The histomorphometric study revealed an increase in peri-implant bone thickness in the Ti/BG group as compared to the Ti group. EDX of newly formed bone tissue showed a transient appearance of silicon at 14 and 30 days postimplantation and a rise in the calcium:phosphorus ratio in peri-implant bone tissue in the Ti/BG group.* ***Discussion:*** *The present study shows an increase in reactive medullary bone formation when BG particles are implanted around a Ti implant.* ***Conclusion:*** *The results described in the present study reveal that the release of Si by BG particles is an important issue that warrants further study.* (INT J ORAL MAXILLOFAC IMPLANTS 2002;17:644–650)

Key words: *bone, bioactive glass, dental implants, energy-dispersive x-ray analysis, silicon, titanium*

Over the last decades, different biomaterials (metal, ceramic, polymers) have been employed, alone or combined, for prosthetic rehabilitation. Within this context, titanium (Ti), in its commercially available pure grades and alloys, has become one of the most commonly used metallic implant materials for both orthopedic and oral and maxillofacial rehabilitation.[1,2]

Bone tissue reactions to Ti implants have been well documented in histologic and histomorphometric studies.[3–10] Within this context, an experimental model (laminar implant test) was developed by Cabrini and coworkers[11] to evaluate the radiographic, histologic, histomorphometric, and microchemical features, by scanning electron microscopy (SEM) and energy-dispersive x-ray analysis (EDX), of bone formed de novo around a Ti laminar implant placed in the medullary compartment of rat tibiae. This experimental model allows the characterization of the bone tissue in the different stages of peri-implant bone healing and assessment of the influence of local and systemic factors on this process.[12–19]

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Many situations in clinical practice require metallic implants to be combined with bone grafts and/or bone substitutes.[20–22] The biomaterials employed include autografts, xenografts, allografts, and alloplasts (eg, bioactive glasses [BGs]). A bioactive material was defined by Hench and Wilson as "a material that elicits a specific biological response at the interface of the material which results in the formation of a bond between the tissues and the material."[23] All of the present generation bioactive materials form a biologically active hydroxycarbonate apatite layer on their surfaces in vivo.[24,25]

The ability of BG particles to promote osseous healing has been previously demonstrated in several experimental models.[26–32] Upon implantation, silica-based BG particles are transformed into a shell containing calcium and phosphate that loses its inner silicon-rich core.[33,34] Hench was the first to propose that soluble silica from BGs plays a vital role in the stimulation of bone formation.[35]

The release of silicon (Si) by BG particles and its incorporation by newly formed bone tissue in the peri-implant area had not been studied to date. The aim of the present study was to characterize bone around Ti and BG particles implanted in marrow canals of rat tibiae by histologic, histometric, and microchemical evaluation employing the "laminar implant test." EDX was employed to assess the presence of Si in newly formed bone tissue.

MATERIALS AND METHODS

Surgical Procedure

Thirty male Wistar rats weighing on average 90 ± 5 g were employed throughout. Under anesthesia by intraperitoneal injection of 8 mg of ketamine hydrochloride (Ketalar, Parke-Davis, Morris Plains, NJ) and 1.28 mg of xylazine (Rompun, Bayer, Leverkusen, Germany) per 100 g of body weight, the skin was disinfected and shaved. A longitudinal incision of 1.5 cm was made along the frontal aspect of both tibiae. Subcutaneous tissue, muscles, and ligaments were dissected to expose the external surface of the tibiae in the area of the diaphyseal bone. An end-cutting bur (1.5 mm in diameter) was used to drill a hole that reached the bone marrow. Overheating and additional bone damage were prevented by using manual rotating impulsion.

A commercially pure Ti laminar implant (6.0×1.0×0.1 mm; Implant-Vel, Buenos Aires, Argentina) was introduced gently into the hole in each tibia and placed inside the medullary compartment, parallel to the long axis of the tibia (Ti group). In the contralateral tibia (Ti/BG group), a Ti laminar implant and melt-derived BG 45S5 particles (nominal composition by weight: 45% SiO_2, 24.5% Na_2O, 24.5% CaO, 6% P_2O_5, 90 to 710 µm; PerioGlas, US Biomaterials, Alachua, FL) were implanted. The wounds were carefully sutured.

The animals were housed in plastic cages and maintained on a 12:12 hour light:dark cycle. They were fed rat chow and water ad libitum. The guidelines of the National Institutes of Health for the care and use of laboratory animals (NIH Publication No. 85-23, Rev. 1985) were observed. The animals were sacrificed by ether overdose in groups of 10 at 14, 30, and 60 days after implant placement. The tibiae were resected, fixed in 20% formalin solution, and radiographed.

Histologic Processing

The tibiae were processed for embedding in methyl methacrylate resin.[16,36] The samples were then sectioned using a saw, and 3 slices were cut at approximately 500 µm, perpendicular to the implant. The cross sections were ground using a grinding machine and finished manually with sandpaper to obtain sections approximately 50 µm thick. One section was stained with 1% toluidine blue for histologic and histometric evaluation by light microscopy. The remaining 2 specimens were coated with a thin (20-nm) layer of silver in a vacuum evaporator for SEM and EDX.

Histomorphometry

Histomorphometric determinations were performed on sections using a light microscope (Zeiss Axioskop 2 MOT, Carl Zeiss, Jena, Germany) online with an image analysis system (Kontron KS300 v. 2, Kontron Elektronik, Munich, Germany). The thickness of bone tissue in contact with the Ti implant was evaluated.[11,16] In the Ti/BG group, a distinction was made between bone tissue related to BG particles and bone tissue unrelated to BG particles.

Microchemical Analysis

The specimens were examined in a 515 Philips scanning electron microscope (Eindhoven, The Netherlands) equipped with an EDX system (EDAX Falcon PV 8200 [3.0], Mahwah, NJ) for microchemical analysis. The presence of Si, calcium (Ca), and phosphorus (P) was evaluated in the BG particles and in the peri-implant bone tissue for each of the experimental times.

BG Particles. Five BG particles around the Ti laminar implant were selected at random. An internal area and an external area of 15×10 µm were considered for evaluation.

Fig 1 *(Left)* Lateral and *(right)* frontal radiographs. Note the presence of the laminar implant surrounded by particles of BG.

Peri-implant Bone Tissue. In the samples from the Ti group, the bone tissue around the metal implant was evaluated. In the samples from the Ti/BG group, the newly formed bone tissue around the BG particles and Ti implant was evaluated.

Statistical Analysis

The results were statistically analyzed by the Student t test. Data were reported as mean ± SD at a significance level of $P < .05$.

RESULTS

Uncomplicated healing postimplantation in all rats was observed. All implants remained in situ as determined by radiographs (Fig 1).

Microscopic Findings

Light microscopy of the histologic sections showed that a large proportion of both biomaterials was surrounded by reactive medullary bone. The rest of the surface was in contact with the bone marrow. There were no macrophages or related inflammatory cells in any of the interface regions of either of the groups.

Ti Group. Fourteen and 30 days after implantation, lamellar bone tissue was observed on most of the implant surface (bone-implant contact) (Figs 2a and 3a). Additional bone growth was observed after 60 days.

Ti/BG Group. Fourteen and 30 days after implantation, lamellar bone tissue bridges between Ti and BG particles were observed (Figs 2b and 3b). Areas of reactive bone formation, unrelated to BG particles, were detected around the implants.

Additional bone growth was observed at 60 days.

Histomorphometric Analysis

Both groups (Ti and Ti/BG) exhibited a statistically significant increase in bone tissue thickness in contact with the Ti implant as a function of time ($P < .05$). Bone tissue thickness in contact with the implant was significantly greater in the Ti/BG group than in the Ti group ($P < .05$) (Table 1).

The thickness of bone tissue in contact with the Ti implant and unrelated with BG particles, at 14 and 30 days postimplantation, did not show statistically significant differences from the Ti group at 30 and 60 days, respectively ($P > .05$).

EDX

BG Particles. The presence of Si, Ca, and P in the samples varied over the experimental period (Table 2).

Fourteen Days Postimplantation. The interior area of the particles exhibited a greater amount of Si than the external area ($P < .01$). The amount of both Ca and P was greater in the external than in the internal area ($P < .01$). Ca was more abundant than P in both areas ($P < .01$).

Thirty Days Postimplantation. Si was more abundant in the internal area than in the external area ($P < .01$). A reduction in Si content was observed for both areas as compared to 14 days postimplantation ($P < .01$). Both Ca and P were more abundant in the external area than in the internal area ($P < .01$). The levels of Ca and P were higher than at 14 days postimplantation in the internal area ($P < .01$), whereas the level of Ca fell and the level of P rose in the external area ($P < .01$).

Sixty Days Postimplantation. The amounts of Si fell below the detection level of EDX (.5 wt %). Ca was more abundant in the internal area than in the external area. Conversely, P was more abundant in the external area than in the internal area ($P < .01$). A statistically significant rise in Ca and P was observed for both areas as compared to the samples at 14 and 30 days postimplantation ($P < .01$).

Bone Tissue. Ti Group. Si was not detected at the experimental times considered. The Ca:P ratio exhibited a statistically significant increase as a function of time: 1.65 ± 0.05, 2.06 ± 0.08, and 2.35 ± 0.10 at 14, 30, and 60 days, respectively ($P < .01$).

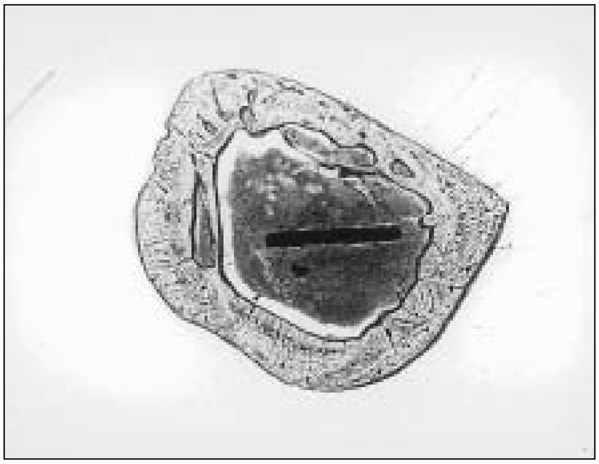


Fig 2a Ti group. Ground section. Note the presence of peri-implant bone tissue 14 days postimplantation (original magnification ×25).

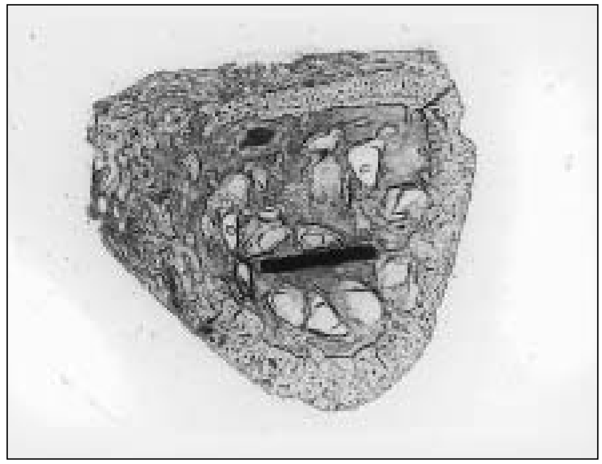


Fig 2b Ti/BG group. Ground section. Note the presence of BG particles around the Ti implant 14 days postimplantation (original magnification ×25).

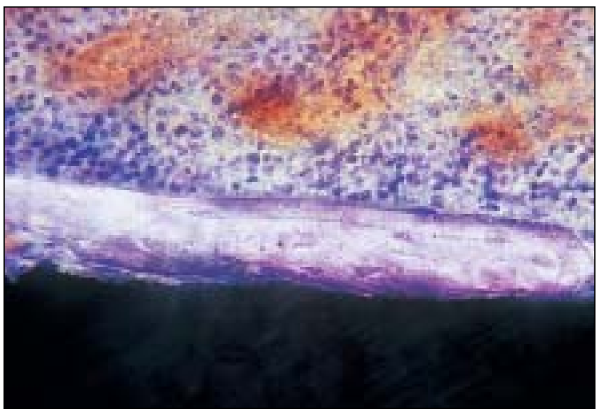


Fig 3a Ti group. Bone tissue in contact with the metal surface 30 days postimplantation (toluidine blue; original magnification ×400).

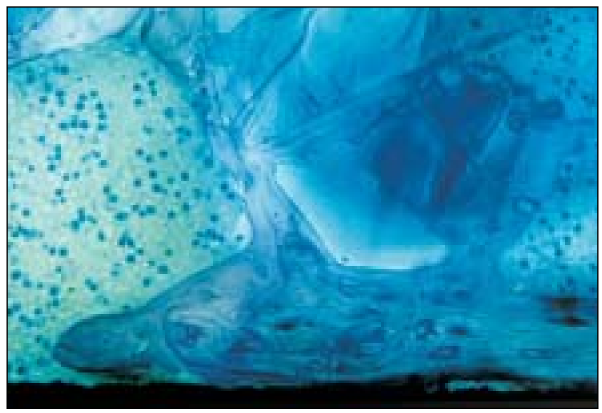


Fig 3b Newly formed bone tissue lies between the Ti implant and BG 30 days postimplantation (toluidine blue; original magnification ×400).

Table 1 Bone Tissue Thickness (µm) (Mean ± SD)

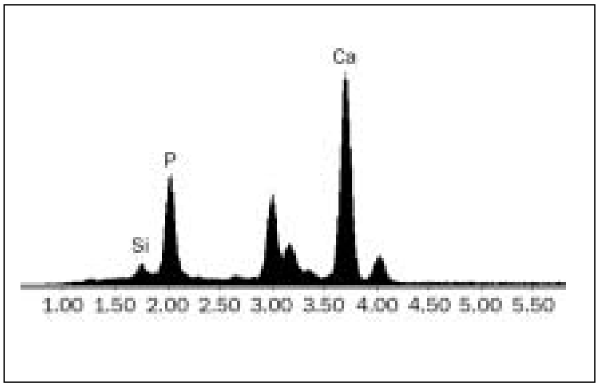
Group	Days postimplantation 14	30	60
Ti (n = 10)	22 ± 6	31 ± 6*	56 ± 12*
Ti/BG (n = 10)			
Urp	29 ± 6†	45 ± 13*‡	71 ± 6*
Rp	39 ± 6	77 ± 19*	116 ± 19*

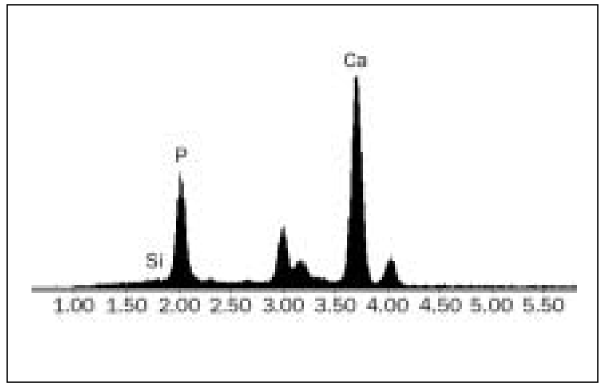
Urp = unrelated to BG particles; Rp = related to BG particles.
*$P < .05$.
†Statistically insignificant difference relative to Ti group (30 days postimplantation).
‡Statistically insignificant difference relative to Ti group (60 days postimplantation).

Table 2 Energy Dispersive X-ray Analysis (Mean ± SD)

Experimental time (days)/ Element	BG particles Inner (wt%)	BG particles Outer (wt%)	Bone tissue (wt%)
14 (n = 10)			
Si	81 ± 0.40	31 ± 0.36	4 ± 0.50
P	7 ± 0.30	21 ± 0.14	30 ± 2.00
Ca	12 ± 0.50	47 ± 0.60	66 ± 4.00
30 (n = 10)			
Si	73 ± 0.40	29 ± 0.36	1 ± 0.08
P	11 ± 0.09	25 ± 0.07	30 ± 1.00
Ca	16 ± 0.12	46 ± 0.34	68 ± 3.00
60 (n = 10)			
Si	bdl	bdl	bdl
P	30 ± 0.21	38 ± 0.28	28 ± 1.00
Ca	70 ± 0.44	62 ± 0.50	72 ± 2.00

bdl = below detection limit (.5 wt%).





Figs 4a and 4b EDX spectra corresponding to newly formed bone tissue at *(left)* 14 days, and *(right)* 30 days postimplantation.

Ti/BG Group. Si was detected at 14 and 30 days postimplantation (Figs 4a and 4b, Table 2). A statistically significant increase in the Ca:P ratio was observed as a function of time: 2.2 ± 0.05, 2.3 ± 0.06, and 2.6 ± 0.05 for 14, 30, and 60 days, respectively ($P < .01$). The Ca:P ratio was significantly higher than for the Ti group at all the experimental time-points evaluated ($P < .01$). The Ca:P ratio at 30 days postimplantation for the Ti/BG group (2.3 ± 0.06) did not differ significantly from the value seen at 60 days for the Ti group (2.35 ± 0.10) ($P > .05$).

DISCUSSION

The present study shows an increase in reactive medullary bone formation when BG particles are implanted around a Ti implant. These data are in keeping with those of Johnson and associates[37] and Turunen and coworkers.[38,39] The mechanisms involved in BG-enhanced bone repair would be associated with the chemical transformation and/or morphologic changes that occur on the surface of BG particles.[33] The microchemical findings reported herein are in keeping with previous studies.[34,40–42] Hench and Polak established that "the surface reactions release critical concentrations of soluble Si, Ca, P, and Na ions that give rise to both intracellular and extracellular responses at the interface of the glass with its cellular environment."[25] Recently, BG dissolution products have been shown to exert a genetic control over the osteoblast cell cycle, leading to differentiation and proliferation of bone cells and the expression of genes that regulate osteogenesis and production of growth factors.[25,43–46] These findings would explain the increase in osteogenesis reported herein.

Previous studies have reported on the possible distribution of Si released from BG particles. Chou and colleagues demonstrated by EDX analysis, that sol-gel Bioglass particles (US Biomaterials, Alachua, FL) implanted in proximal tibial condyles of rabbits "released a high level of silicon which then distributed to the areas of surrounding tissue."[47] Similarly, Lai and coworkers traced and quantified by flame atomic absorption spectrophotometry the Si released from BG particles implanted in the tibiae of rabbits. These authors stated that "as bioactive glass granules resorbed, there was dissolution of silica into the local bone tissue and subsequent diffusion into the bloodstream."[48] In the present study, the newly formed bone tissue showed a transient appearance of Si at 14 and 30 days postimplantation.

Si has been shown to play a role in the biomineralization process.[49] Si is required for the production of exoskeleton in unicellular organisms. However, the role of Si in vertebrate skeletogenesis is poorly understood.[49–52] The importance of Si in bone tissue mineralization was described by Carlisle, who showed, by electron microprobe studies, that Si is located in the active growth areas in the young bone of mice and rats.[53,54] These findings were then confirmed by Landis and associates, who showed, by imaging ion microscopy, that Si localization was principally extracellular.[55] In addition, Carlisle demonstrated in rats that as mineralization progresses, the concentrations of both Ca and Si rise concomitantly. When weanling rats were fed diets with a range of Si and Ca content, Si not only induced an increase in the Ca content of bone but also led to an increase in the rate of bone mineralization.[54] These findings could explain the rise in the Ca:P ratio observed in peri-implant bone tissue when BG particles were employed.

Perry and Keeling-Tucker established that "silicon may have a role to play in connective tissue synthesis and bone crystallization, but at present none of the aspects of this involvement in these processes are understood."[49,50] Franks and colleagues[56] and Lugowski and coworkers[57] suggested that the long-term local and systemic reaction to the Si in biomaterials is still unknown. Within this context, the exact effect of Si released from BG particles on tissue cells and its mode of action remain unclear.

SUMMARY

The results described in the present study and the findings previously reported in the literature reveal that the release of Si by BG particles is an important issue that warrants further study.

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