

EFFECTS OF 45S5 BIOACTIVE GLASS PARTICLES ON TITANIUM PERI-IMPLANT BONE HEALING. A HISTOMORPHOMETRIC STUDY IN RATS.

[A. Gorustovich](#)¹, & [M.B. Guglielmotti](#)¹

¹ [Department of Oral Pathology](#), School of Dentistry, University of Buenos Aires, Buenos Aires, Argentina

INTRODUCTION: Many situations in orthopedic and oral and maxillofacial clinical practice require metallic implants to be combined with bone substitutes eg, bioactive glasses (BGs). The ability of BG particles to promote osseous healing has been demonstrated in several experimental models. In a previous study, we performed histologic and microchemical determinations by scanning electron microscopy (SEM) and energy-dispersive x-ray analysis (EDX) of newly formed bone tissue surrounding titanium (Ti) implants and bioactive glass particles used as bone filling material[1]. The aim of the present study was to perform a histomorphometric evaluation of bone healing around titanium implant placed simultaneously with bioactive glass particles in hematopoietic bone marrow of rat tibia.

METHODS: Thirty male Wistar rats weighing 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, shaved, and 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 reaching the bone marrow. Overheating and additional bone damage were prevented by using manual rotating impulsion. A commercially pure titanium laminar implant (5x1x0.1mm; 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)[2]. In the contralateral tibia (Ti/BG group) a titanium laminar implant and 15 mg of melt-derived BG 45S5 particles (nominal composition by weight: 45% SiO₂, 24.5% Na₂O, 24.5% CaO, 6% P₂O₅; 90-710 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 N° 85-23, Rev.1985) were observed.

The animals were sacrificed by ether overdose in groups of 10 at 14, 30 and 60 days after implantation. The tibiae were resected, fixed in 20% formalin solution, and radiographed.

Histologic Processing: The tibiae were processed for embedding in methyl-methacrylate resin. The samples were then sectioned using a saw, and three 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 about 35 μm thick. The sections were stained with 1% toluidine blue for histologic and histometric evaluation by light microscopy (Zeiss Axioskop 2 MOT, Carl Zeiss, Jena, Germany).

Histomorphometry: Histomorphometric determinations based on standard stereologic methods were performed with an image analyzing system (Kontron KS300 v.2, Kontron Elektronik, Munich, Germany). Tracings of the projection of the sections were made at x160 magnification. The peri-implant bone area and the percentage of bone-to-metal contact were evaluated.

The results were statistically analyzed by Student's *t* test. Data were reported as mean \pm SD at a significance level of $P < .05$.

RESULTS: Uncomplicated healing post-implantation in all rats was determined by radiographs .

Histologic Findings: Light microscopy of the histologic sections showed that observed. All implants remained *in situ* as a large proportion of both biomaterials was surrounded by newly formed bone. The rest of the surface was in contact with the bone marrow.

There was no occurrence of macrophages or related inflammatory cells in any of the interface regions of either of the groups.

Ti group: 14 and 30 days after implantation, lamellar bone tissue was observed on most of the implant surface (bone-to-metal contact). Additional bone growth was observed after 60 days.

Ti/BG group: 14 and 30 after implantation, lamellar bone tissue bridges between Ti and BG particles were observed (*Figure 1*).

Areas of bone formation, unrelated with BG particles, were detected around titanium implants. Additional bone growth was observed at 60 days.

Histomorphometric Analysis

Both groups (Ti and Ti/BG) exhibited a statistically significant increase in peri-implant bone area as a function of time ($P < .05$). Compared to Group Ti, Group Ti/BG showed a statistically significant ($P < .05$) increase in peri-implant bone area at all the experimental times (*Table 1*). No statistically significant differences were found between groups when comparing percentage of bone-to-metal contact (*Table 2*).

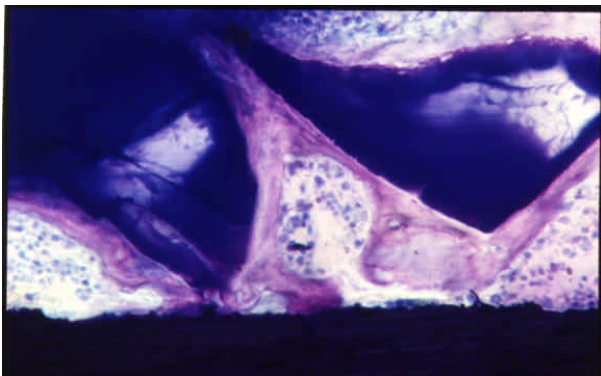


Fig. 1: Newly formed bone tissue lies between the Ti implant and bioactive glass particles 14 days postimplantation (toluidine blue; original magnification x400).

Table 1. Peri-implant bone area (mm²)

	Days postimplantation		
	14 (n:10)	30 (n:10)	60 (n:10)
Ti	1168±114 *	1444±109 *	2554±84 *
Ti/BG	1600±102	1793±103	2698±75

Mean ± SD * $P < .05$ mm² of projection

Table 2. Bone-to-metal-contact (%)

	Days postimplantation		
	14 (n:10)	30 (n:10)	60 (n:10)
Ti	31±4 *	38±3 *	39±3 *
Ti/BG	35±3	41±2	42±4

Mean ± SD * $P > .05$

DISCUSSION & CONCLUSIONS: The results of the present study evidence that simultaneous implantation of 45S5 BG particles around a titanium implant caused an increase in osteogenic activity in the peri-implant micro-environment.

In a previous study using this experimental model a transient appearance of Si at 14 and 30 days postimplantation and a rise in the Ca:P ratio in peri-implant bone tissue when BG particles were employed were observed[1].

Bioactive glass dissolution ions (Si, Ca, P, and Na) plays an important role in the formation of a bone-like apatite on its surface *in vitro* and *in vivo*[3], and 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[4]. These findings would explain the increase in osteogenesis reported herein.

REFERENCES: ¹A.Gorustovich, M. Rosenbusch, and M.B. Guglielmotti (2002) *Int J Oral Maxillofac Implants* **17**: 644-650. ²M.B.Guglielmotti, S.Renou, and R.L. Cabrini (1999) *Int J Oral Maxillofac Implants* **14**:565-570. ³T.Kokubo et al (2000) What kinds of materials exhibit bone-bonding? in *Bone Engineering* (ed J.E. Davies) em squared Inc, pp 190-194. ⁴L.L.Hench and J.M. Polak (2002) *Science* **295**: 1014-1017.

ACKNOWLEDGEMENTS: This study was supported by Grant UBA O014 of the University of Buenos Aires.