BIOLOGICAL RESPONSE TO A BIOACTIVE GLASS COATED IMPLANT: EXPERIMENTAL DATA.

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Abstract

A biomaterial constituted by a metallic titanium alloy (TiAl6V4) coated with a bioglass layer (Na_2O (7-24%), K_2O (2-8%), CaO (9-20%), Al_2O_3 (0.1-2%), MgO (0.1-2%), SiO_2 (46-53%) and P_2O_5 (4-8%) was implanted in the cancellous bone of adult rabbit knee, in orthotopic situation, under conditions of mechanical stability.

The animals were sacrificed after 30 (Group I), 90 (Group II), and 180 (Group III) days postoperatively. The bone pieces, containing the samples under investigation, were studied using radiographs, optical microscopy, scanning electron microscopy and electron probe micro-analysis.

Radiologically, osteointegration of the implant surface was observed in all cases at the 180^{th} day after the surgery. A new bone formation was observed in the animals of Group I and the specimens observed after 90 days of the surgery (Group II) presented

a higher amount of new bone tissue. At the 180th day, the bioglass coating of the metallic titanium alloy was not detected and a total contact between the metallic surface and the new bone tissue was observed. The incorporation of the bioglass layer into the bone matrix occurred without interposition of non mineralized tissues and without the presence of inflammatory cells.

These results strongly suggest that the studied ceramic coating material of the metallic implant is bioactive, biocompatible, bioresorbable, and possess osteoconductive properties.

Introduction

Orthopaedic surgeons and basic scientists have translated advances of biomaterials science into novel management options for their patients. Implanted biomedical prosthetic devices are intended to perform safely, reliably, and effectively in the human body for prolonged periods of time. The development of cementless implants emerged as a natural evolution of the joint arthroplasty concept, allowing a direct confrontation of an artificial structure with an active biological environment in permanent remodeling, aiming at a precocious osteointegration and a successful joint replacement.

Biomaterials are widely used in the composition of joint substitution prosthesis. Pure titanium implants are currently employed in ortopaedic surgery, as this metal offers the best available surface to interface and anchorage with the bone. Nevertheless, 10 years retrospective studies revealed osteolytic areas and implant failure when porous metallic surfaces were used. This type of problem has not yet been detected with hydroxyapatite coated hip prosthesis (1,2). Nevertheless, the main problem with these implants concerns the osteolytic mechanisms caused by the presence of wear particles.

Bioglass has been known for almost 20 years and it has been proven its excellent osteointegration behaviour. However, despite their good biocompatibility, only very few prosthesis with this ceramic material are implanted in orthopaedic surgery. A major drawback is their poor mechanical strength associated with a high production cost. On the other hand, bioglass formulations, in form of granules, fibers and blocks, are brittle and may form particular debris, contributing to the release of inflammatory cytokines (3).

However, a single process was developed by which powdered granular bioglass is synthesized and applied by plasma spraying, opening the possibility of ortopaedic application as a coating implant, in the aim of a better bone anchorage. Exposing a metal substrate to the plasma allows the production of coatings, which are obtained with adhesion strengths over 30 MPa (4). The advantages of the plasma-spray technique include a high depositional velocity, a reduced alteration of the metallic substrate and a minimum dimensional tolerance.

Certain glasses and glass-ceramics materials belong to the biologically active group of glasses, that, when placed in contact with cellular tissues, demonstrate good biocompatibility both *in vivo* and *in vitro* and an absence of inflammatory and toxic processes (5). Furthermore, in the presence of precursory osteogenetic conditions, they also demonstrate an osteoconductive predisposition, which tends to favor a particularly good biological bond at the interface between the glass and the bone tissues. Aditionally, certain bioactive glasses are able to connect to bone through a chemical bond (6). However, the bioactivity of a glass is compositional dependente (region A according to the ternary diagram of Hench (5)), and the rate, the strength and stability of the bond vary not only with the composition but also with the microstructure of the bioactive material.

Since the epoch-making development and investigations of Bioglass® by Hench many publications and developments of comparable materials (7,8,9,10,11) have been reported. Nevertheless, works describing the clinical results of bioglass coated prosthesis are rarely found in orthopaedic literature (12). On the contrary, numerous experimental studies *in vivo* are currently under investigation (13,14,15,16).

In order to evaluate the biological behavior of a biomaterial constituted by a metallic titanium alloy (TiAI6V4) coated with a bioglass layer, an experimental in vivo study was performed using rabbits. The implants were placed in orthotopic situation, in the cancellous bone of the rabbit knee, under conditions of mechanical stability.

Experimental procedure

A metallic titanium alloy (TiAl6V4) similar to those used for a human orthopaedic prosthesis with a cylindrical form (10 mm x 3mm) was submitted to a superficial treatment, following the spraying process. The bioglass coating, with a constant thickness of about 80 μm, was reasonably homogeneous with the presence of microcavities (Fig. 1). The implants were subsequently sterilised by gamma radiation.

The bioglass (Biovetro®, Cgbdp Group), with an amorphous structure, had the following composition: Na_2O (7-24%), K_2O (2-8%), CaO (9-20%), Al_2O_3 (0.1-2%), MgO (0.1-2%), SiO_2 (46-53%) and P_2O_5 (4-8%).

Fifteen Californian rabbits, males 9 months old (body weight 3.600±0.020 Kg), were used in this study. 30 implants were impacted ("press-fit" technique) at the medial condyle of the femur, two in each rabbit, through a medial parapattelar approach. The animals were anesthetised by an i.m. injection of xylazine hydrochloride (Rompum® 2%), 0.5ml/kg, and ketamine hydrochloride (Ketalar®) 0.37ml/Kg, under aseptic conditions. After the surgery the animals were allowed unrestricted movement in their

cages and were maintained according to the Portuguese law for animals (Portaria 1005/92, 23/10 /1992). The rabbits were sacrificed with an overdose of ketamine hydrochloride at 30 (Group I), 90 (Group II), and 180 (Group III) days postoperatively. Each group corresponded to ten animals. The femora were resected just above the condyles, using a manual saw, and the soft tissue was removed. Preliminary, 8 implants were introduced in 4 animals in order to optimise the surgical technique and these rabbits were sacrificed at intervals of 7 and 15 days, after the implantation.

The bone pieces, containing the samples under investigation, were fixed in buffered formaldehyde 4% and radiographed (Odel, Sirius 1000, 40-50 KV, 8-10 mAs). After this preliminary observation, undecalcified, methyl methacrylate embedded specimens were prepared. Sections with 30 μm thick were taken perpendicular to the longest implant axis (using a cutting-grinding technique) and then stained with both the van Gieson and the Toluidine Blue methods for histological examination. The histological sections were observed using optical microscope with polarized light, scanning electron microscopy and electron probe micro-analysis (EPMA). For the two latest techniques the samples were coated with a 300 nm thick gold layer deposited by sputtering. The analysis performed by EPMA consisted of chemical elemental distribution for calcium and silicium obtained with an accelerating voltage of 20 keV and a beam current of 100 nA.

Results and Discussion

Radiologically, no lucent lines were detected in the bone-implant interface in the three experimental groups. At the 180^{th} day after the surgery, osteointegration of the implant surface was observed in all cases (Fig. 2).

The preliminary studies enabled to detect, as early as the 15^{th} postoperative day, by microscopic observation of the implant, the formation of new bone on the implant

surface as a diffuse interface without cleavage zones and without the interposition of fibrous tissue (Fig. 3). These findings are characteristics of an osteocoalescence process and are more evident at 30^{th} and 90^{th} days. These observations indicate that there is a strong chemical bond between the bioglass material and bone living tissue, revealing a good bioactivity of the bioglass. On the other hand, neither the presence of fibrous encapsulation nor the infiltration of inflammatory cells was detected in any of the implants.

In Group I the new bone formation directly contacting the bioglass layer or the metal alloy was observed on the same analyzed specimen (Fig.4). In the animals of Group II, and as expected, more new bone tissue was detected when compared to that present after 30 days. At the 180^{th} day after implantation, non bioglass areas were observed and a total contact between the metallic surface and the new bone tissue was found (Fig.5). Moreover, neither a corrosion process, located on the surface of the metallic titanium alloy, nor the presence of delaminating on the bioglass layer were observed.

The elemental X-ray distributions maps obtained by EPMA, for silicium and calcium, confirmed the observations of light microscopy. In fact, a decrease of Si (corresponding to bioglass layer) accompanied by an increase of Ca content (corresponding to new bone formation) was found at the implant interface (Fig.6).

As mentioned before, in this work the implants were introduced under mechanical stability, in a well-vascularized bone environment, although there was no direct loading of the implant. The lack of direct loading may negatively affect new bone formation. Nevertheless, the microcopic studies showed an increase of new bone formation with increasing implantation time which can be due to the following factors.

The physiologic mechanical stress may have affected the incorporation of the bioglass layer because a new bone formation, originated from the host, was observed

on the implant surface, initially with characteristics of immaturity (woven bone), followed by physiological process of bone remodeling and bone maturation.

On the other hand, the roughness of the implant surface as well as its chemical composition favorably influenced the bone reaction, leading to an early bone ingrowth and mechanical fixation as been shown by other authors (2). In fact, at 15^{th} postoperative day, a process of new host bone formation on the bioglass layer in a firm union was observed, without interposition of fibrous tissue, suggesting a chemical bonding between the implant surface and the new formed bone. Furthermore, neither the interposition of fibrous tissue nor the presence of inflammatory cells was detected.

After 180 days, and as Figure 6 illustrates, the metallic titanium alloy contacted directly with the bone tissue, on most of its extension, through a contact osteogenesis process, without interposition of a non mineralized tissues. The bioglass layer was almost completely reabsorbed and gradually replaced by new host living bone, indicating the incorporation of the implant surface without the interposition of connective tissue. These observations demonstrate that the implant surface is bioresorbable.

The *in vivo* studies carried out in this work have shown that throughout the experimental time a gradual and controlled reabsorption of the bioglass layer and a new bone ingrowth in direct contact with the metallic titanium alloy. According to the literature, this is the result of the reaction between the surface of the bioactive glass with the host tissue, on the implantation site, leading to the formation of a gel with an ion composition similar to that of the ossification front formed during natural bone remodeling allows its recognition, by osteoblasts, as a substrate for the deposition of bone matrix (11,19).

In conclusion, the above results demonstrate that the studied ceramic coating

material is bioactive, biocompatible, bioresorbable and possesses osteoconductive properties. Such characteristics open new perspectives for clinical and experimental studies. Furthermore the use of a bioglass layer as a carrier of bone growth factors can accelerate the osteointegration of an implant with an identical composition.

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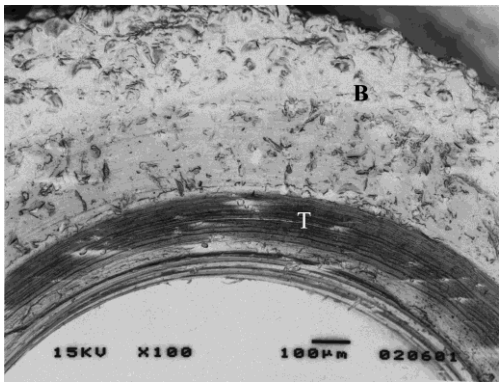

a) b)

Fig.1 – SEM micrographs of the implant: a) transverse section of the metallic titanium alloy (T) coated with a bioglass layer (B) (50x); b) surface view of the bioglass coating (150x).

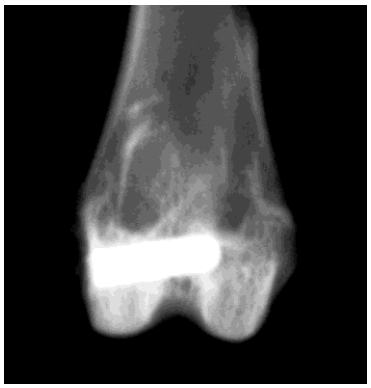

Fig. 2 – Radiograph image of an implant after 180 days of insertion, showing osteointegration of the implant surface, without lucent lines in the bone-implant interface.

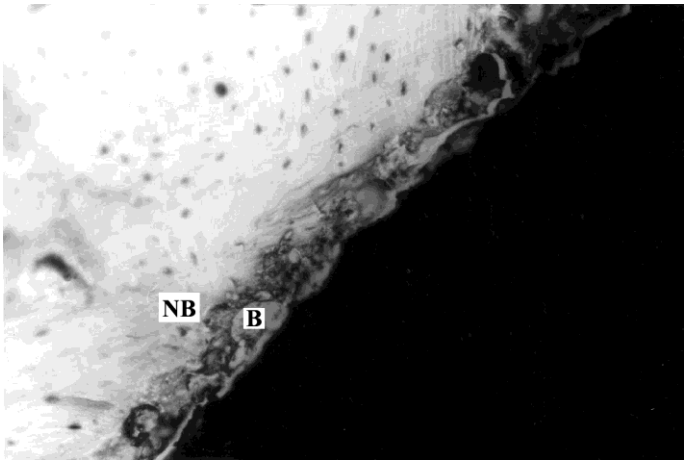

Fig. 3 – Light micrograph of an implant at 15th day. A processof new host bone formation (NB) on the bioglass layer (B) in a firm union was observed, without interposition of fibrous tissue. (Undecalcified section stained with Toluidine Blue, original magnification x40)

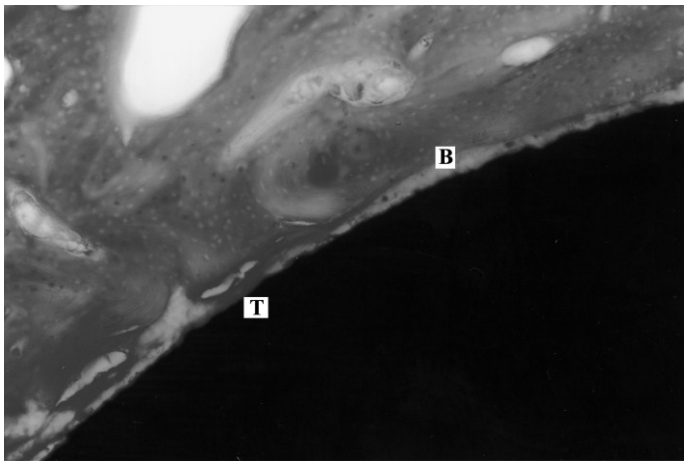

Fig. 4 - Light micrograph of an implant at 30th day. Note the new bone was in some areas contacting directly with the bioglass layer (B) and in others contacting with the metal alloy (T). (Undecalcified section stained with van Gierson, original magnification x200)

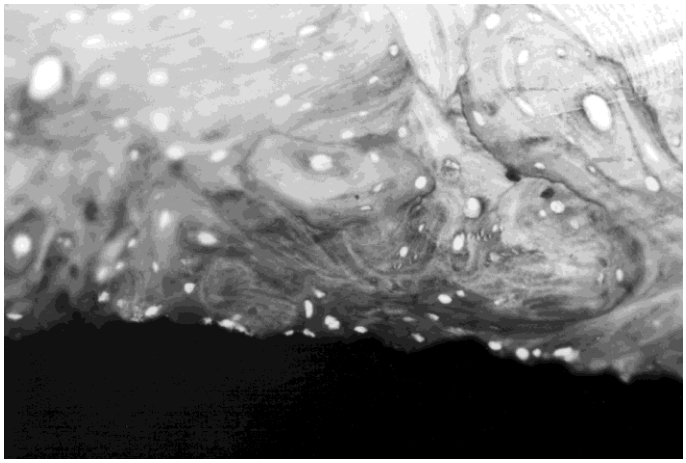

Fig. 5 - Light micrograph of an implant at 180th day, showing a direct contact between the new host bone tissue and the metallic titanium alloy. Neither a bioglass layer nor a connective tissue was observed (Undecalcified section stained with Toluidine Blue, original magnification x200)

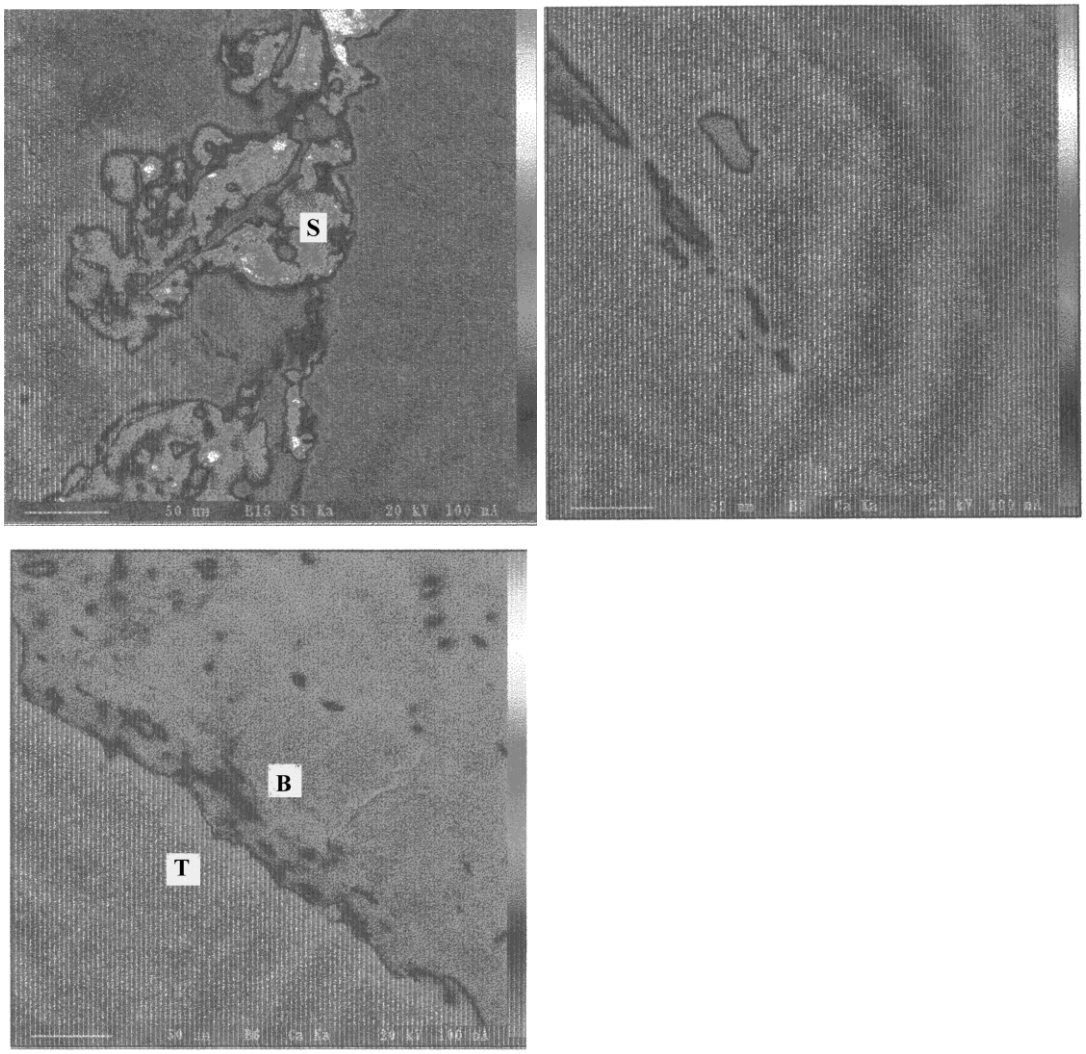

Fig. 6 – a) Elemental X-ray distribution map obtained by EPMA of an implant at 15th day after the surgery showing areas with high Si content (S) at bone/implant interface b) At 90^{th} day a low Si content and a high Ca content were found. c) At 180^{th} day a Calcium based structure (bone tissue (B)) was detected, in direct contact with the metallic titanium alloy (T).