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THE INFLUENCE OF HYDROXYAPATITE ON BONE HEALING IN TITANIUM IMPLANTS AS SHOWN BY SCANNING ELECTRON MICROSCOPY[1]

Liliane Elze Falcão Lins[2], Edmar José Borges de Santana[3], Antonio Fernando Pereira Falcão[3], Pedro Paulo Monteiro Martins[4], Tereza Raquel Vianna Calmon[3] and Viviane Almeida Sarmento[3]

[2]Department of Morphology, Medicine and Health Public College of Bahia, Salvador, BA, Brazil, [3]Department of Diagnostics and Treatment, College of Dentistry, Federal University of Bahia, Salvador, BA, Brazil, [4]College of Dentistry, Federal University of Pernambuco, Recife, PE, Brazil.

ABSTRACT

Hydroxyapatite is a biomaterial with osteoconductive properties and its use in implants may accelerate bone healing. In this work, scanning electron microscopy (SEM) was used to examine the influence of hydroxyapatite on bone healing in titanium implants in a rabbit tibial model. Two titanium implants 6 mm long and 3.17 mm in diameter were inserted into the right tibial metaphysis of each of five rabbits. The proximal implant served as the control and the distal one (experimental group) was filled with hydroxyapatite. Forty-two days after implantation, the rabbits were sacrificed and the implants analyzed. Tissue fragments with the implants were examined by SEM. Compact and trabecular bone were observed along the entire internal surface of implants filled with hydroxyapatite, but not in all of the control cases. In contrast, marrow bone retraction occurred on the internal surface of the implants in the control group but not in the experimental group ($p<0.05$). These results show that hydroxyapatite was well accepted by bone tissue in this experimental model and that there was no inflammatory reaction between the fibroblast processes and hydroxyapatite granules. In addition, hydroxyapatite accelerated the bone healing in osseointegrated implants.

Key words: Dental implantation, hydroxyapatite, rabbits, scanning electron microscopy, titanium

INTRODUCTION

Hydroxyapatite, a biomaterial that binds chemically to bone, is useful for implants because of its chemical and crystallographic similarities to the mineral constituents of bone and teeth. Nery *et al*. [7] used histological analysis to show the usefulness of bioceramic material as an alternative to bone grafting, particularly since the material was well tolerated by the tissues and caused no toxic reactions. According to Ducheyne and Groot [2], the hydroxyapatite lining porous structures stimulates calcified tissue ingrowth into the pores.

Since 1981, hydroxyapatite blocks and granules have been used in jaw reconstruction. Oguchi *et al***.** [9] used light microscopy and transmission electron microscopy (TEM) to investigate the interface between bone and chronically implanted hydroxyapatite in man. TEM showed that hydroxyapatite bound either directly to bone or to electron dense material deposited between these materials. Remagen and Prezmecky [10] studied bone augmentation with hydroxyapatite granules in 55 patients with implant. Punch biopsies were obtained from 14 days to 7 years after the implant. Increasing metaplastic formation of woven bone trabeculae was observed from the earliest time interval onwards. The hydroxyapatite was in intimate contact with the bone and was later partially included in the bone matrix. After 20 months, sufficient new bone had formed to allow implant insertion.

Wheeler *et al***.** [12] studied 36 sinus-lift graft augmentations done to place 66 implant cylinders in the posterior maxilla where vertical bone length was less than ideal. The grafts were done with porous hydroxyapatite only, or porous hydroxyapatite mixed with autogenous bone removed from the iliac crest and combined with autogenous bone removed from inside the mouth. Nineteen core biopsy specimens were taken from different grafts at time intervals ranging from 4 to 36 months. Greater bone formation was seen in specimens obtained from 19 months onwards.

Bone healing with hydroxyapatite is quicker than with titanium implants. Ichikawa *et al***.** [3] examined the three-dimensional bone response to commercially

Correspondence to: Dr. Liliane Elze Falcão Lins
Departamento de Morfologia, Escola Bahiana de Medicina e Saúde Pública (EBMSP), Rua Frei Henrique 8, Nazaré, CEP 40050-420, Salvador, BA, Brasil. Tel: (55) (71) 243-4533, E-mail: linsr@cpqgm.fiocruz.br; liliane@linsreabilitacao.com.br

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pure titanium, hydroxyapatite and a calcium-titanium mixture in the tibiae of rabbits 2, 4 and 8 weeks after implantation. Whereas the percent bone volume in the cortical bone was consistent, in the bone marrow region this percentage varied with the implant material and how long the material had been implanted. With titanium implants, the percentage increased gradually up to 8 weeks, whereas with hydroxyapatite and the calcium-titanium mixture, the increase was greatest at 4 weeks and occurred mainly close to the surface.

Hallman *et al*. [5] evaluated the compatibility of titanium implants in different grafting materials (autogenous particulate bone from the mandibular ramus, bovine hydroxyapatite with a membrane coverage, and an 80/20 mixture of bovine hydroxyapatite and autogenous bone) used for maxillary sinus augmentation procedures. Histomorphometric analysis showed no differences between the three groups, indicating that autogenous bone grafts could be substituted with 80% or 100% bovine hydroxyapatite.

Novaes *et al*. [8] compared four types of implant surfaces. Teeth were extracted from young adult male mongrel dogs and 90 days after removal, four screw-type implants with different surface treatments were inserted in the mandibular hemiarches. Each dog received two implants of each of the following surface treatments: smooth (machined), titanium plasma spray, hydroxyapatite coating, and sandblasting with soluble particles. Hydroxyapatite and sandblasting with soluble particles provided a greater bone-implant contact than the machined surface after 90 days during which the implants were maintained unloaded.

De Lavos-Valereto *et al*. [1] analyzed the biocompatibility of a Ti-6Al-7Nb alloy with and without a hydroxyapatite coating in rat cultured osteoblast-like cells. The cells were cultured on Ti-6Al-7Nb or hydroxyapatite-coated Ti-6Al-7Nb disks in Petri dishes. The presence of hydroxyapatite on the Ti-6Al-7Nb surface impaired the growth and viability of osteo-1 cells. However, this coating improved extracellular matrix formation. Thus, Ti-6Al-7Nb with or without a hydroxyapatite coating has relevant physical and biological properties as an implant material.

Although ceramics have been tested for biocompatibility, the interaction between bone and hydroxyapatite in osseointegrated implants is still unclear. In this work, we used scanning electron microscopy (SEM) to examine the influence of hydroxyapatite on bone healing in dental implants.

MATERIAL AND METHODS

In this study, the experimental model developed by Lundskog [6] was used. Five male New Zealand white rabbits 3-3.5 months old were anesthetized with an intramuscular injection of ketamine/xylazine (1:1 v/v, 20 mg/kg). The hair was removed from the surgical site and the skin was cleaned with iodinated surgical soap. Aseptic technique was used throughout the surgical procedure. An incision approximately 5 cm long was made along the medial right upper hind limb, and the mid-diaphyseal surface of the femur was surgically exposed by blunt dissection. Two cylindrical hollow titanium implants (PPMM System®) 6 mm long, with external and internal diameters of 3.17 mm and 1.3 mm, respectively, were implanted in the right tibial metaphysis of each rabbit. The proximal implant was the control implant and the distal one was filled with hydroxyapatite (Fig. 1). Two holes approximately 1 cm apart were drilled through the medial cortex using an internally irrigated 2 mm diameter PPMM bur® operated at about 1000 rpm (BLM 500 – VK Driller). Each hole was enlarged to 3 mm with an internally irrigated PPMM bur®. During all bone cutting, profuse irrigation with isotonic saline solution was maintained. Forty-two days after implantation, the rabbits were killed with an overdose of anesthetic and the implants were analyzed. Tissue fragments with the implants were removed, fixed in 4% paraformaldehyde for 2 h and then in 2.5% glutaraldehyde in 0.1 M phosphate buffer. Specimens for SEM were divided into two longitudinal, identical parts with a 0.3 mm thick diamond disc. All of the samples were cut in the Laboratory of Advanced Materials at the Federal University of Pernambuco. After post-fixation, the specimens were dehydrated in an ethanol series of increasing strength, critical point dried (Critical Point Dryer Hitachi – JEE— 4x Jeol) and coated with a gold conducting film in a vacuum device (Fine Coat Ion Sputter JFC – 1100) prior to SEM with a Jeol JSM T 200 scanning electron microscope in the Kaizo Asami Laboratory of Immunopathology, Recife, PE.

The parameters analyzed were bone formation on both the external and internal implant surface, inflammatory changes, and medular marrow retraction on the internal implant surface. The results, summarized in table 1, were analyzed statistically using the chi-square test (level of significance $\alpha = 0.05$).

The hydroxyapatite granules (Merck, Germany) were analyzed in the Laboratory of Pharmaceutical Synthesis at the Federal University of Pernambuco. The granule diameter ranged from 0.075 mm to 0.180 mm and had 100% tenor, as defined by the United States Pharmacopoeia.

RESULTS

Careful surgical technique and internal irrigation with isotonic saline solution maintained the vitality of the bone margins at the site of the implant. Clinical examination revealed no differences between the control and experimental groups after 42 days, and there was no clinical evidence of an inflammatory process.

SEM revealed less-organized, immature bone at the interface of the control implants compared to the experimental implants (Fig. 2A,B), but there were no

Table 1. Number of titanium implants for which histological parameters were analyzed.

Histological parameters	Groups		
	Control	Experimental	Total
Bone formation on external implant surface	5	5	10
Bone formation on internal implant surface*	-	5	5
Inflammatory changes	-	-	-
Bone marrow tissue retraction on internal implant surface*	5	-	5
Total	10	10	20

* Significant difference at $p<0.05$.

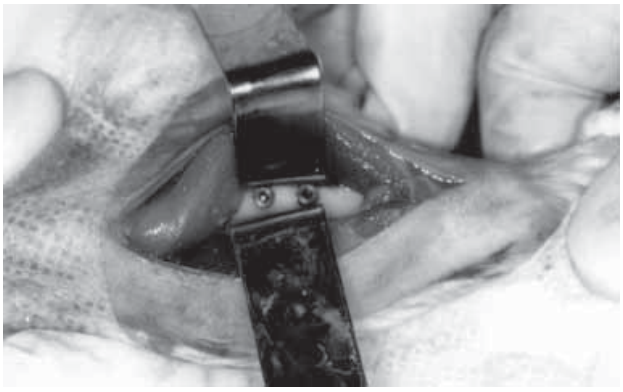

Figure 1. Clinical view of the tibial fixtures after implantation.

signs of inflammatory cells or of microorganism infiltration in either group.

Compact and trabecular bone usually did not form along the entire internal surface of the implant in the control group, but was routinely observed in implants filled with hydroxyapatite (Fig. 3). Significantly greater new bone formation occurred on the internal surface of the implant in the experimental group ($p<0.05$), whereas bone marrow retraction was observed on the internal surface of the implant in the control group ($p<0.05$). There was no significant difference in bone formation on the external surface of the implants in the two groups.

Although the control cylinders were filled with a cellular, fibrous, vascular tissue compatible with bone marrow, the cylinders in the experimental group showed compact bone formation on the apical internal surface, with well-organized concentric lamellae but no inflammatory reaction at the bone-implant interface (Fig. 4A). The collagen fibers of the bone adhered tightly to the titanium surface and showed no inflammatory changes (Fig. 4B).

Examination of the central region of the implants in the experimental group revealed trabecular bone formation surrounded by marrow tissue, but again there were no signs of a toxic reaction in the cells or

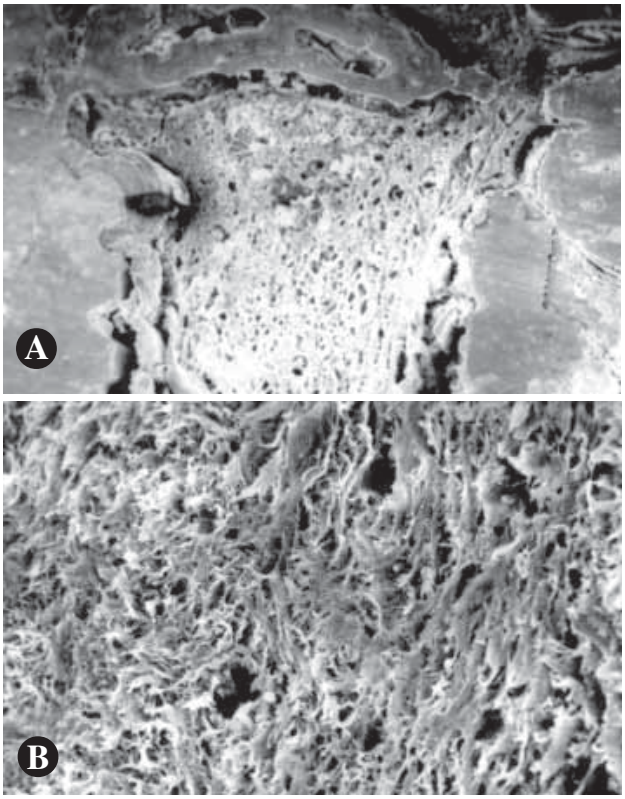

Figure 2. **A**. Scanning electron micrograph of fibrous and vascular tissue formation within a control fixture. **B**. Magnification of (A) showing vascular and fibrous tissue without inflammation. Bar = 500 µm (A); 100 µm (B).

the surrounding ground substance. The hydroxyapatite was well accepted by bone tissue. The upper internal region of the implants in the experimental group showed proliferating fibroblasts on granules of hydroxyapatite, as well as vascular growth. There was no inflammatory reaction between the fibroblasts and the hydroxyapatite granules (Fig. 5).

DISCUSSION

The rabbit tibial model for implants was developed by Lundskog [6] and the quality of this bone

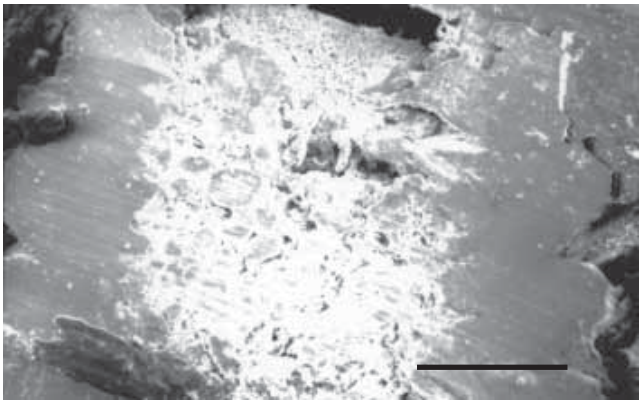

Figure 3. Scanning electron micrograph of the experimental group showing compact, trabecular bone formation along the internal surface of the fixture. Bar = 1000 µm.

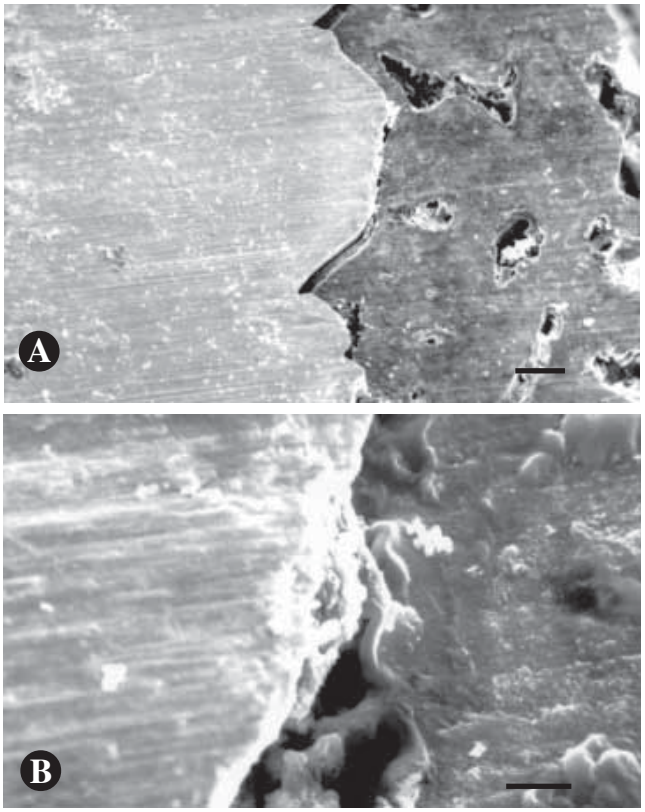

Figure 4. **A**- Scanning electron micrograph of the experimental group showing the implant-bone interface with compact bone formation and well-organized concentric lamellae. **B**- Magnification of (A) showing bone and collagen fibers tightly adhered to the titanium surface and the absence of an inflammatory reaction. Bar = 50 µm (A); 5 µm (B).

was classified by Jaffin and Berman [4] as type II, which is the best type of bone for implants, hence the choice of this model for our experiments. The intervals chosen for analysis were based on work by Roberts *et al*. [11] who established the time course of interface development for endosseous implants in human and rabbit cortical bone.

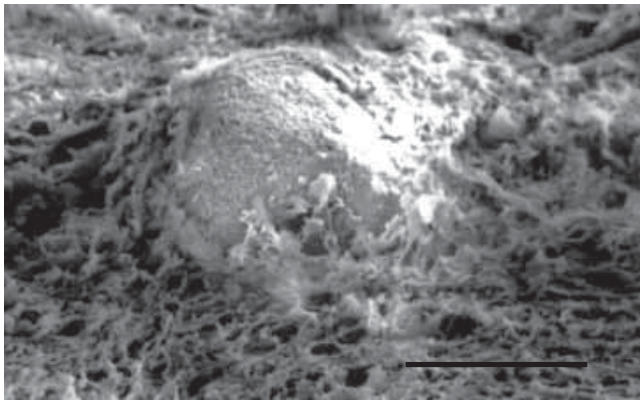

Figure 5. Scanning electron micrograph of fibroblast filaments and hydroxyapatite granules in the experimental group. Note the lack of an inflammatory reaction. Bar = 100 µm.

SEM showed that the responses to the implants in the control and experimental groups were similar, particularly with regard to the lack of an inflammatory reaction. Collagen fibrils from bone adhered to the implant surface. This close relationship formed the morphological basis for the good mechanical stability of titanium implants. The ability of fibroblasts and osteoblasts to adhere closely to the titanium implants resulted in a biological seal with no signs of microorganism or inflammatory cell infiltration.

As shown here, hydroxyapatite granules ranging from 0.075 mm to 0.180 mm in diameter were well tolerated by bone and marrow tissue for up to 42 days after implantation. This biocompatibility of hydroxyapatite agrees with the findings of Nery *et al*. [7] who showed that the material was well tolerated by tissues and caused no toxic reactions.

Bone healing in the experimental group was accelerated compared to the control group. The osteoconductive action of hydroxyapatite was seen in all implants in the experimental group, as shown by the presence of fibrovascular tissue, osteoblastic activity and new bone formation. No bone tissue formation was seen in implants of control group and there were more vessels than connective fibers. These results agree with those of Oguchi *et al*. [9] and Remagen and Prezmecky [10] who also observed a direct contact between bone and hydroxyapatite but no inflammatory reaction.

In conclusion, the hydroxyapatite granules accelerated bone healing in rabbits, with the advantage of not causing adverse tissue reaction.

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