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BONE RESPONSE TO CALCIUM PHOSPHATE-COATED AND ALENDRONATE SODIUM IMMOBILIZED TITANIUM IMPLANTS

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Abstract: *Many materials with different surfaces have been developed for dental and orthopedics implants. Among the various materials for implants, titanium and bioactive such as calcium phosphates and hydroxyapatite, are widely used clinically. Alendronate sodium is a potent inhibitor of bone resorption used successfully in the treatment osteoporosis. Studies indicate that alendronate sodium increases the rate of early bone formation around dental implants. The purpose of this study was to characterize and evaluate the binding capacity of alendronate sodium implant surface of Ti-7.5Mo treated by biomimetic method and analyze the response of bone to the surface of these implants. Ingots were obtained from titanium and molybdenum by using an arc-melting furnace. They were submitted to heat treatment at 1100ºC for one hour, cooled in water, cold worked by swaging. Then, screw-shaped implants (2.0 mm diameter by 2.5 mm length) were manufactured and they were implanted in the rat's femur. The bone-implant interface was evaluated histological and after insertion of implants into the femur of rats. These results suggest that calcium phosphate coating with alendronate sodium are able to promote the osteogenesis on surfaces of dental implants.*

Keywords: *Dental implants, titanium alloys, alendronate sodium, histological analysis*

1. INTRODUCTION

Bisphosphonates are potent inhibitors of bone resorption and are known to be osteoclastic inhibitors that bind strongly to hydroxyapatite through their high affinity for calcium, in both synthetic hydroxyapatite and natural hydroxyapatite within the bone (Zenios et al., 2004).
In dentistry, reports indicate that bisphosphonates increase the rate of early bone formation around dental implants by the local application of alendronate to calcium phosphate coated and machined titanium implants after implantation (Miyaji et al., 2005). There is also evidence suggesting that some bisphosphonates may prevent osteocyte and osteoblast apoptosis (Peter et al., 2005). Other effects include the induction of osteoblast proliferation, the inhibition of inflammatory cells and improvement of screw fixation (Bobyn et al., 2005).

Yoshinari et al, 2001 showed that the activity of the osteoblastic cells cultured on titanium plates immobilized with bisphosphonates increased, indicating that bisphosphonate-immobilization has no toxic effect on osteoblastic cells, and that it provides a favorable microenvironment with osteogenic ability. Therefore, this method is expected to promote osteogenesis at the bone tissue around the implants through the local action of the bisphosphonates in vivo.

In a murine study, the local delivery of ibandronate resulted of external fixation with the use of stainless-steel screws and histological analysis performed five weeks postoperatively

showed inhibition of bone resorption at the bone-screw interface, after systemic administration of alendronate sodium (Zenios et al., 2004). Furthermore, in a canine study, enhancement of bone growth into porous metal implants was found following a single systemic dose of zoledronic acid (Bobyn et al., 2005).

In a recent study, implants coated with hydroxyapatite and impregnated with zoledronate showed better fixation in osteoporotic murine bone than that seen in the control group (Peter et al., 2006).

The purpose of the present study was to evaluate the bone response to titanium implants coated with thin calcium phosphates and followed by immobilization of bisphosphonates in vivo.

2. MATERIALS AND METHODS

2.1 Preparation of specimens

The Ti-7.5Mo alloy was produced from sheets of commercially pure titanium (99.9%) and molybdenum (99.9%). Samples were first melted in an arc furnace under an argon atmosphere. The ingots were then homogenised under a vacuum at 1100ºC for 86.4 ks to eliminate chemical segregation. Then, screw-shaped implants (2.0 mm diameter by 2.5 mm length) were manufactured.

Screw-shaped implants were immersed in a 5.0-M NaOH aqueous solution at 80ºC for three days, washed with distilled water and dried at 40ºC for 24 h using a methodology proposed by Wei et al. (2002). After alkaline treatment, samples were heat-treated at 600°C in an electric furnace under an air atmosphere, maintained at this temperature for 1 h and then allowed to cool to room temperature in the furnace.

Samples were divided in two groups according SBF immersion: Group I (SBFx5) and Group II (SBFx5 and alendronate sodium).

The SBFx5 solution proposed by Barrère et al. (2002) was prepared by dissolving the chemical reagents NaCl (40 g), $MgCl_2.6H_2O$ (1.52 g), $CaCl_2.2H_2O$ (1.84 g), $Na_2HPO_4.2H_2O$ (0.89 g) and $NaHCO_3$ (1.76 g) in 1000 ml of distilled water with vigorous stirring and constant bubbling of CO_2. Samples of Group I placed in individual falcon flasks containing SBFx5 and samples of Group II containing SBFx5 with 0,4 µg alendronate sodium. Flasks were placed on a rotatory shaker at 150 rpm for 24 hours.

2.2 *In vivo* implant

Six female 6-month-old Wistar rats were used for this experiment. Surgical procedures were conducted under general anesthesia using intraperitoneal injection of sodium thiopental associated with subcutaneous injection of morphine sulfate. Bilateral implantations were performed at the distal end of the femurs. Group I implantations was in right femur and Group II was in left. They were sacrificed thirty days after implantation.

2.3 Histological analysis

For histological analysis implants with surrounding tissue were removed and immersed in formaldehyde. Samples were embedded in polymethyl methacrylate and after polymerization cut with a saw and polished to thickness of 180 µm and mounted on glass slides. Samples were stained with Stevenel´s blue and Alizarin red stains for light microscopy.

3. RESULTS AND DISCUSSION

In histological analysis for Group I (SBFx5) can be seen side by side, vital bone maturing and mature bone tissue close to the bottom of the implant. Regions of fibrous tissue interposition are not found. The newly formed bone has features of normality with marrow spaces filled with blood vessels. Are also observed osteocytes and Havers systems. On the other hand, mature bone tissue exhibits trabecular bone set, small marrow spaces and large numbers of osteocytes (Figure 1).

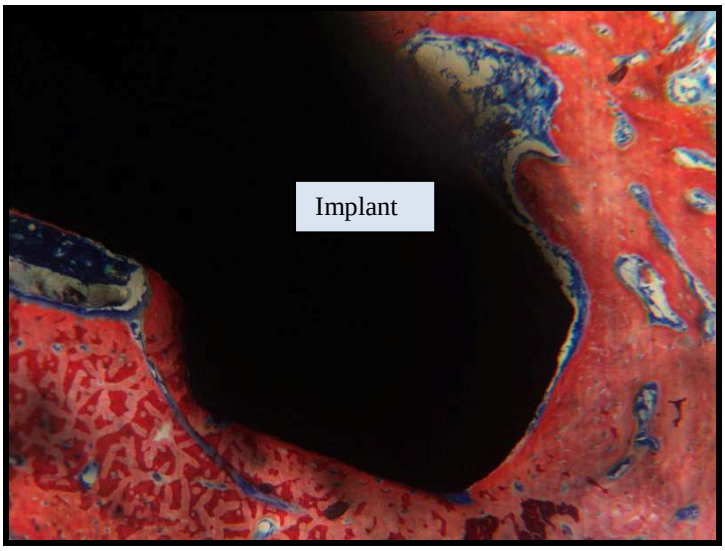

Figure 1 - Bone tissue along with newly formed bone near the bottom of the implant. Stenvenel's Blue and Alizarin Red. 160X.

For Group II (SBFx5 and alendronate sodium), there were no regions of interposition of fibrous tissue. When compared to the control group, the analysis of bone tissue at 30 days shows a more mature tissue in most of the perimeter to the implant. Thus, there are defined and small marrow spaces. Increased number of osteocytes is also found (Figure 2).

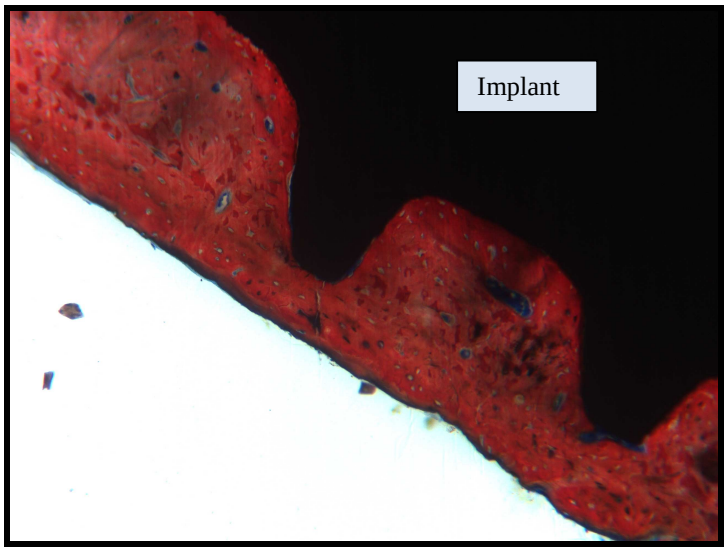

Figure 2. Mature bone at the perimeter of the implant. Stenvenel's Blue and Alizarin Red. 160X.

4. DISCUSSION

The success of dental implant treatment depends on the healing of both hard and soft tissues. While osseointegration provides initial success, the biological seal of the peri-implant soft tissue is crucial for maintaining the long term success of implants.

Calcium phosphates implants are well known for good osteoconductivity as well as for direct binding to bone tissue in vivo (Cooper et al., 1998). Several hypotheses explain the effect and final biological mechanism of the calcium phosphates coatings. Calcium phosphates implants acts as a nucleation site and exhibits crystallographic properties in process of the newly developed structure. The calcium ion dissolves from the calcium phosphates surface, resulting in the deposition of a mineralized layer and stimulates the bone cells to continue extracellular matrix synthesi and calcification (Hayakawa et al., 2000).

Bisphosphonates (BPs), such as alendronate, risedronate, ibandronate, and clodronate, are an important group of drugs used for the treatment of metabolic and oncologic pathologies involving the skeletal system. The two main categories of BPs are non–nitrogen-containing and nitrogen-containing BPs (Green, 2004). Non–nitrogen-containing BPs are metabolized rapidly, whereas nitrogen-containing BPs are much more potent and are not metabolized (Frith et al, 1997; Tenebaum et al., 2002). The mode of action of BPs depends on the chemical structure of the drugs (two phosphate groups attached to a central carbon atom that forms a three-dimensional structure); however, additional mechanisms of action exist. This molecular construct enables the molecule to attach to bone, disrupt osteoclastic function, and induce apoptosis (Fleich, 1998; Reszka & Rodan, 2003; Otomo-Corgel, 2007). BPs are commonly used in the treatment of various osteometabolic diseases including osteoporosis, Paget's disease, multiple myeloma, tumors that metastasize to the bone, and malignant hypercalcemia because of their properties of inhibiting bone resorption by osteoclasts (Javed &Almas, 2010).

Alendronate sodium is very potent inhibitors of bone resorption and the mechanism of this phenomenon is likely to be at the cellular level, especially as it applies to the activity of osteoclasts. Also potent inhibitors of osteoclastic bone resorption, alendronate sodium have a direct effect on osteoblasts (Schmidt et al., 1996).

Gandolfi et al., (1999) demonstrated that good differentiation and osteoblastic activity occur in cells in contact with bisphosphonates. Giuliani et al., (1998) suggested that bisphosphonates might have, in vivo, a potentially relevant influence on cells of the osteoblastic lineage, distinct from their inhibitory action on osteoclasts.

Histological analysis revealed that, both in the Group I as in the Group II no interposition of fibrous tissue at the perimeter of the implant, which confirms the concept of osseointegration when observed under light microscopy (Brånemark et al., 1985, Zarb & Albrektsson 1991). Moreover, both groups showed bone tissue observed features of normality: blood vessels, osteocytes and Haversian systems. This shows that the bone was found in the interface characteristics of vitality, as well as interfaces observed by Carvalho et al. (1994, 1997). However, in the group treated bone tissue is characteristic of more mature, corroborating Chen et al (2010). For these authors alendronate (ALO) and calcitonin (CT), as commonly used antiosteoporosis drugs in current clinical practice, have been experimentally confirmed to produce the effectiveness of promoting osseointegration at the interface between prosthesis and host bone and enhancing the long-term stability of the prosthesis (Chen et al, 2010).

5. CONCLUSIONS

In this in vivo study, the greatest degree of bone contact was found around the alendronate sodium-immobilized implants. The results are according by Cochran et al., (1998) explanations of the mechanism for a greater degree of bone contact is that a more favorable osteophilic property such as a higher rate of bone cell attachment or proliferation, could be responsible for this mechanism and that the higher bone mineral density in the bone tissues adjacent to the implants could be responsible for the inhibition of osteoclastic bone resorption or direct promotion of osteoblasts.

However, these results suggest that calcium phosphate coating with alendronate sodium are able to promote the osteogenesis on surfaces of dental implants

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