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HISTOLOGICAL ANALYSIS OF THE OSSEOINTEGRATION OF Ti-7.5Mo DENTAL IMPLANTS AFTER SURFACE TREATMENT

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Abstract. *Metallic biomaterials are used to reinforce or to restore form and function of hard tissues. Implants and prosthesis are used to replace shoulders, knees, hips and teeth. When these materials are inserted in bone several biological reactions happen. This process can be associated to surface properties (topography, roughness and surface energy). In this work, influence of biomimetic surface treatment in osseointegration of Ti-7.5Mo dental implant was evaluated. 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. Animals were divided into two groups: untreated (control group) and treated (biomimetic surface treatment). They were sacrificed thirty days after implantation. 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, polished and mounted on glass slides. Results obtained suggest that biomimetic surface treatment was able to promote an increased of osseointegration on surface of dental implants.*

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

1. INTRODUCTION

Dental implants have been used for the replacement of teeth, and their use has been highest in the last years due to increased life expectancy of the population. The clinical success of these implants is related with osseointegration. After implantation, the implant surface interacts with water, biological fluids and, dissolved ions and the healing process initiates. According to the type of cells and their activities, two types of responses can occur: direct integration of the bone-implant without a connective tissue layer (osseointegration) or fibrous tissue capsule formation with clinical failure of the implant (Kurella et al, 2005; Le Guehennec et al., 2004). However, according (Granström, 2005) failure of osseointegration can occur due to other factors such as poor bone quality, traumatic surgical technique, overloading and also current diseases, smoking habits, ingestion of toxic drugs, osteoporosis and other causes.

Titanium and titanium alloy have become the most commonly used for this application due to their biocompatibility, excellent mechanical properties and good corrosion resistance (Gerber et al., 2005; Chanine et al., 2008; Bornstein et al., 2005). However, titanium exhibit low ductility and considerable controversy has been raised about citotoxicity of Ti-6Al-4V alloy due release vanadium and aluminum (Geetha et al., 2004). Titanium based alloys with

different compositions such as Ti-7.5Mo (Lin et al., 2005), Ti-10Mo (Ho et al, 1999; Alves Rezende et al., 2007; Alves et al., 2004), Ti-15Mo (Kumar et al., 2008), Ti-29Nb-13Ta-4.6Zr (Li et al., 2004) and Ti-13Nb-13Zr (Niemeyer et al., 2008) have been studied for biomedical applications.

However, these materials are considered bioinerts materials due when they are inserted into the human body they are cannot form a chemical bond with bone. In some of these studies, the authors have attempted to modify this bioactivity with treatments that change the material surface chemistry or roughness.

Bioactivity of an artificial material can be assessed in vitro by examining its apatite-forming ability in SBF. The formation of the apatite layer can be reproduced on the surface of bioactive materials in an acellular simulated body fluid (SBF) with ion concentrations nearly equal to those of the human blood plasma (Kokubo *et al*, 1990).

The purpose of this work was to evaluate osseointegration in implants of the experimental alloy Ti-7.5Mo after modification surface using biomimetic method with simulated body fluid condensed (5xSBF).

2. MATERIALS AND METHODS

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 homogenized under vacuum at 1100ºC for 86.4 ks to eliminate chemical segregation. The resulting samples were finally cold-worked by swaging, producing a 4 mm rod. Followed, cylindrical implants (2.5 mm diameter and 2.0 mm of height) were machined custom made for this research (Figure 1).

Figure 1 - Implant of the experimental alloy Ti-7.5Mo

Then, implants were divided into two groups with and without biomimetic surface treatment (control group), group one and two respectively. For group one, surface treatment was realized into three steps: alkaline treatment, heat treatment and soaking in SBF. First, for alkaline surface treatment, they were immersed in a 5.0M NaOH aqueous solution at 80ºC for 3 days, washed with distilled water, and dried at 40ºC for 24 h using a methodology proposed by Wei et al. for Ti-6Al-4V [22]. After alkaline treatment, implants were heat-treated at

600°C in an electric furnace under an air atmosphere, maintained at this temperature for 1 h and then naturally cooled to room temperature in the furnace.

Followed, SBFx5 solution proposed by Barrere et al. [18] was prepared by dissolving chemical reagents in the following 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) salts in 1000 ml of distilled water with vigorous stirring and constant bubbling of CO_2. Implants were placed in individual Falcon flasks containing SBF and flasks were placed on a rotatory shaker at 150 rpm for 24 hours. After soaking, samples were removed, rinsed in distilled water followed by drying at room temperature for 24 h.

Surfaces were evaluated using a scanning electron microscope (SEM, LEO 1450 VP, Zeiss, Germany).

Surgical procedure

Twelve hours before surgery, animals were fasted. Rats were anesthetized with an intramuscular injection of ketamine and xylazine (10 mg/kg). Two implants were inserted into each animal (one on the left tibia and the other on the right), which was, a total of forty-four implants.

For sterile preparation of the surgical site, the skin of rats was shaved and swabbed with povidone-iodine. Using sterile technique, a 10 mm incision was extended distally from the tibia tubercle and implants were inserted according groups under saline irrigation to avoid overheating. Then, soft tissues were replaced and sutured with a 3-0 silk suture.

After a healing time of 30 days rats were sacrificed by CO_2 asphyxiation. 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

Fig. 1 shows SEM micrographs of the implant surface Ti-7.5Mo treated with 5M NaOH solution at 80ºC for 3 days followed heat treatment at 600ºC for 1 h and soaking in SBF for 24 hours. It can be observed nanoapatite formation in all surface of the implant.

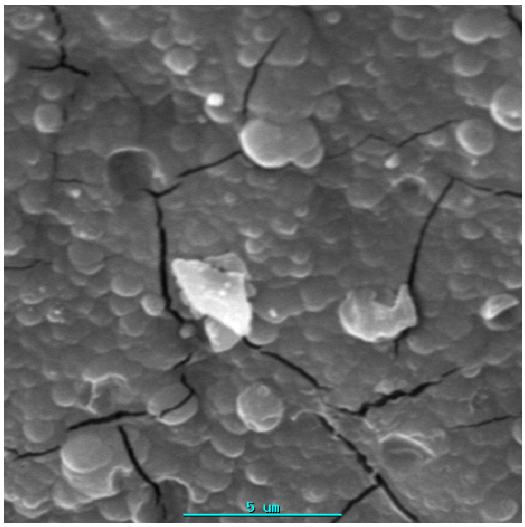

Figure 1 – Surface of the implant after biomimetic surface treatment

In histological analysis, for two groups bone-to-implant interface can be observed. For group without treatment (group two), it can be seen at 30 days postoperative new bone almost throughout the perimeter 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. Osteocytes and Havers systems are also observed. Moreover, it presents marrow spaces large and thin trabecular bone characterized in ripening stage (Figure 2).

For other group (with surface treatment) analysis of bone tissue at 30 days shows more mature bone in most of the perimeter to the implant. Thus, there are defined and small marrow spaces. Increased number of osteocytes is also found. (Figure 3).

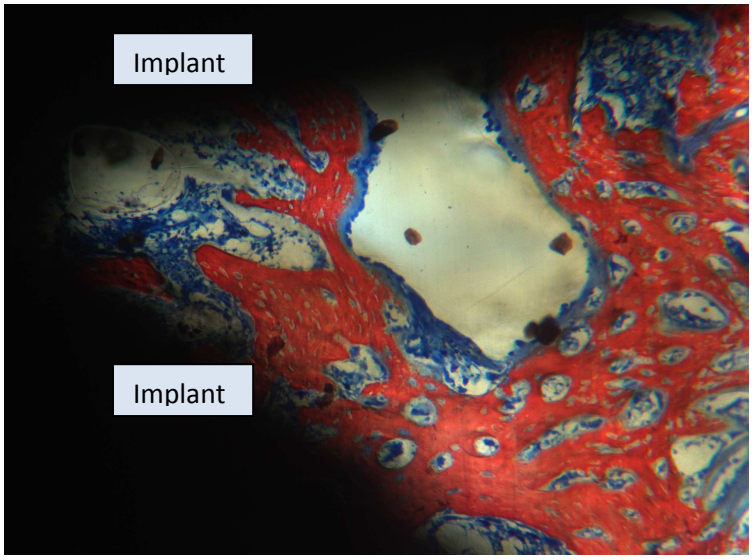

Figure 2 . Newly formed bone at the perimeter of the implant. Stenvenel's Blue and Alizarin Red

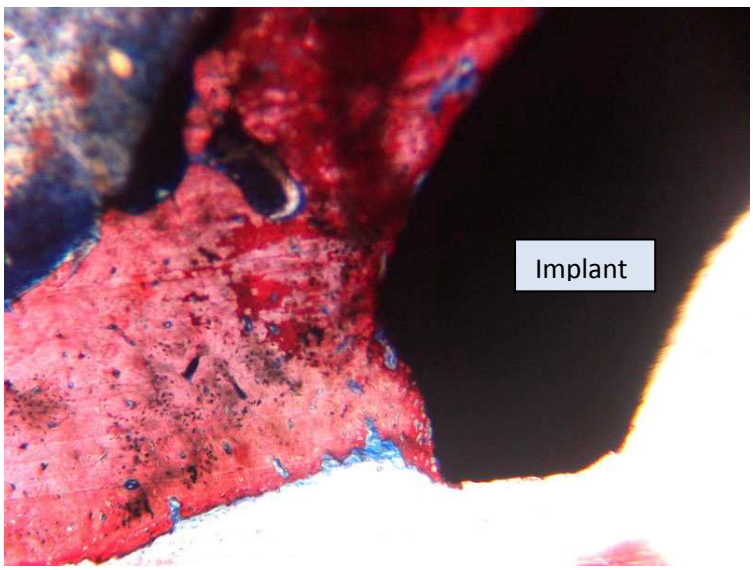

Figure 3 - Treated Group (30 days) - Mature bone at the perimeter of the implant. Stenvenel's Blue and Alizarin Red

4. DISCUSSION

With an attempt at achieving faster osseointegration to hasten the overall treatment process, the use of biomimetic agents represents a growing area of research in implant dentistry.

A calcium phosphate layer on the implant surface plays an essential role in forming the chemical bond between the implant and bone (Ban et al, 1997). At the early stage of implantation, the calcium phosphate layer needs to remain stable to be favorable for the formation of the chemical bond, which can increase the bone bonding ability of implants (Yang et al., 2010) The biomimetic technique allowed the homogeneous deposition of a carbonated apatite coating titanium implants.

When exposed to SBF, OH groups are absorbed by Ti ions in the oxide layer (Svetina et al, 2001). The TiO2 loses protons and negative TiO groups are formed. These negative sites attract Ca^{2+} ions from the body fluid that bond to the surface.30 A layer of amorphous Ca titanate is then formed and the surface becomes slightly positively charged as the layer grows due to the Ca^{2+} ions. It will then attract negatively charged P ions, which bond to the surface, and a metastable phase of CaP is formed. This layer is then crystalized into bonelike HA because it is thermodynamically more favorable for the amorphous CaP to adopt a crystalline structure in a wet environment (Forsgren et al., 2007; Svetina et al., 2001). This rough surface coated by the HA layer may act by encouraging bony ingrowth into its porous structure, providing a mechanical fixation beyond bone chemical bonding of the implant to the surrounding bone.

In this study, implants of experimental alloy Ti-7.5Mo were machined custom made. They were treated with biomimetic surface for to obtain a bioactive surface. Histological analysis revealed that implant with surface treatment and implant without treatment showed 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, the results found in this paper showed that biomimetic treatment accelerating osseous formation.

5. CONCLUSIONS

(1) osseointegration occurred for both groups, with or without surface treatment;
(2) the bone tissue in contact with group without surface treatment is still maturing while the bone tissue in contact with biomimetic coating implant exhibited characteristics of more mature bone.

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