Advances in Science and Technology Vol. 76 (2010) pp 259-263
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doi:10.4028/www.scientific.net/AST.76.259

Osseointegration of Titanium Alloy Macroporous Implants Obtained by PM with Addition of Gelatin

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Keywords: Osseointegration, Macroporous, Implants, Titanium Alloy, Gelatin.

Abstract. Studies of titanium and its alloys commonly used as biomaterials aim to improve bone-implant interface related problems, which may determine the quality, bone repairing time and therefore the implant clinical success. The goal of this study was to evaluate, in rats, osseointegration of macroporous implants produced by powder metallurgy (PM) method with controlled addition of gelatin. As control group, samples of commercially pure titanium (cpTi) and Ti-13Nb-13Zr alloy obtained by the PM process were used. To obtaining the porous samples, at most 15% in weight of gelatin was added to metallic powders, the samples were thermally treated in vacuum furnace, and sintered at 1150ºC. The osseointegration evaluation was performed in Wistar rats, males, for a 28 days period. The morphological analyses, optical microscopy and scanning electron microscopy (SEM), evaluated qualitatively the osseointegration. The PM process modified by addition of gelatin provides with success the obtaining of porous metallic implants. Pore size obtained by this technique allowed the necessary nourishing to cell survival, proving that pores and channels form a high interconnectable network represented by the osseointegration and osteoconduction feature of the porous alloy.

Introduction

Titanium and its alloys are the most used metallic biomaterials for applications in orthopedics and dentistry due to its excellent mechanical and biological properties [1]. However, there are technical problems associated with use of titanium as implant material. The high rigidity when compared to the surrounding bone, can lead to problems of stress shielding and subsequent dislocation of the implant [2]. Thus, the difference in modulus between bone (10 - 30 GPa) and dense metallic biomaterials (100 GPa for the cpTi and 230 GPa for the alloy Co-Cr) has been identified as the major reason of implant failure [3]. To confront these problems, materials have been developed with open porosity for bone regeneration, osteoconductive [2,4]. The advantage of using materials with a porous structure is the ability to allow a biological anchorage of bone grown inside the pores [4]. Moreover, the value of elastic modulus can be adjusted to be in balance with the trabecular bone, preventing bone resorption at the implant interface [2].

Investigations of porous materials for biomedical applications date from 1970 involving materials and the concept of osseointegration. Although ceramics have excellent corrosion resistance, the porous ceramic structures can not be used under conditions subject to loads due to their inherent fragility. Also the porous polymer systems can not withstand mechanical stress. This lead researchers to focus on porous metals, including titanium, due their higher fracture toughness and fatigue properties required for applications under load [2-4]

The porous matrix has to be designed to satisfy certain requirements in order to mimic the architecture of natural bone. Besides the biocompatibility of the chosen material, the porous structure should have high porosity and be interconnected to allow sufficient space for cell migration, anchorage and proliferation of new bone tissue, vascularization and transport of body

fluids [1]. Since the vascularization does not occur in pores of diameter less than 100 μm appropriate pore size to the reorganization and bone vascularization reported is in the range of 100-500 μm [4,5].

Aiming to maintain the characteristic of strength and change the condition of porosity, was added an organic agent (gelatin) during processing to form open pores and interconnected channels in the Ti-13Nb-13Zr alloy. Biological characterization was evaluated by the osseointegration of implants in rats, *in vivo* tests.

Materials and Methods

Conformation of samples

Were added 15 wt% gelatin powder (CAAL®) to commercially pure titanium (cpTi) particles before the sintering process. From the powder of the Ti-13Nb-13Zr alloy, were added 5, 10 and 15 wt% gelatin, homogenized and dissolved in boiling water until high viscosity. The formed paste was cooled in butter paper and put on kiln at 30° C for 24 hours. After drying process, the paper was removed from the material and it was broken into metal pestle and mortar coated with stainless steel and after this stage was selected by mesh sieve of 20.

The powders in its various concentrations were submitted to the cold isostatic pressing in silicone mold, with pressure of 20,000 psi. Before sintering, the samples were heat treated in a vacuum furnace (10^{-2} mBar) at 300 °C during 90 minutes, for the decomposition of gelatin and removal of carbon from it. Samples in crucible of alumina (Al_2O_3) and quartz tube were sintered to 1150 ° C for 14 hours, and 10^{-5} mBar.

They were obtained in a cylindrical (5mmx2mm) desired size ("near net shape") without the need of the step of machining. The implant properly identified and packaged, were sterilized with a dose of 25 kGy through gamma radiation (Co60, Gammacell model 220 of the Nuclear and Energy Research Institute/IPEN-USP). The cpTi by presenting known characteristics of biocompatibility was used as a control group in an experiment.

"in vivo"Test

Animals, anesthesia and medication

Eighteen animals *Rattus norvergicus*, male, Wistar, with approximate age of 10 weeks, were designated as animal model in this study, being allocated 3 animals per group. The animals were allocated in the vivarium IPEN, in heated room with constant temperature (21°C), free of stress, under lighting at intervals of 12 hours (light / dark) controlled by timer, in separated cages, treated with diet-based of dry rations and water *Ad libitum*. The animal experiments followed the ethical principles of the Brazilian College of Animal Experiments (BCOAE).

Before the surgery, the anesthesia was performed with Ketamina (75mg/kg) + Xylazine (10mg/kg) administered via intraperitoneal. After the surgery a single dose of morphine (10mg/kg) was applied with the aim of promoting analgesia, reducing the stress of post-surgery pain and improving the welfare of animals. To avoid complications caused by infectious bacterial contamination during the event surgery, was given a single dose, via intramuscular of Pentabiótico® (0.2ml/animal) a combination of five broad spectrum antimicrobial.

Surgical Procedure

The region of the femur of the animals had the hair removed and anti-sepsis with chlorhexidine gluconate to 2% and povidone-iodine. After with scalpel (No. 5) fitted with blade 15, the skin was incised exposing the muscle fascias. The exposure of the femur was obtained after muscle separation of *vastus lateralis* and *biceps femoris* to the periosteum, which was moved in order to provide access to the area and implant site.

The bed of insertion of the implants was prepared with the help of drills coupled with a counter-angle with reduction of 16:1, driven by an electric motor with a speed of 1,000 rpm and 30 N/cm of throttle, under constant irrigation (isotonic solution sodium chloride 0.9%). With the help of a gripper the implant was placed on the right mouse femur. After the insertion of materials, a simple interrupted stitch was done in the muscles to held them together and the skin was sutured with a simple continuous stitch both sutures used mononylon (3/8 CR - 3.0 cm). After the procedure of suture, the asepsis was performed once again.

Preparation of samples

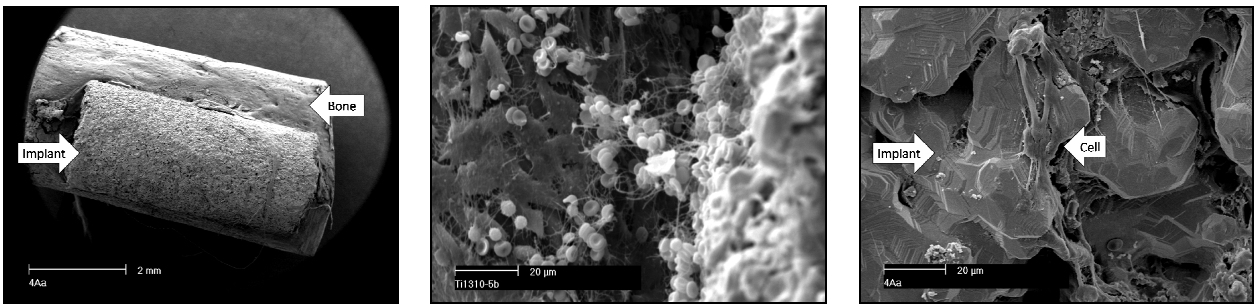
The mouse, being a small animal, has a metabolic process six times faster than that of humans. This can be evidenced also in the mechanism of bone tissue repair. Therefore in this work, we adopted the time of 4 weeks for evaluation of osseointegration. The euthanasia of animals was conducted in CO_2 chamber. The soft tissues adjacent to the area were dissected, a sample of 4 mm in the long axis of the femur (involving the central area of initial injury, 2 mm in diameter) was removed with the aid of a cutting disk.

For histology, soon after the removal of samples, they were immersed in neutral solution of formalin 10% for 30 days. The procedure for inclusion of the samples followed the protocol of Resin Technovit ® 9100 NEU until the stage of producing the slides. The histomorphology was performed from the analysis of slides stained with Hematoxylin-Eosin (HE) and Masson Trichrome (MT).

To analyze the morphology and bone integration in the bone-implant interface, one sample of each experimental group was taken for evaluation by scanning electron microscopy (SEM). After the removal procedure of bone-implant sample, they were immersed for 24 hours in a solution of Karnovsky. Then the samples were dried until they are free of moisture, an important factor to avoid electrostatic charging during observation in SEM. For the analysis took a thin coating of gold to improve the quality of the captured images.

Results and Discussion

In vivo test results, samples were analyzed by SEM related aspects to the anchoring of the implant tissue (Fig.1A). The repair process immediately after the surgical installation of a porous implant is an inflammatory process response by increasing vascularity and cellular activity in the region. The clot formed and fibrin network that fills the spaces inside the pores (Fig.1B), is replaced by osteoprogenitor mesenchymal cells that promote the formation of a disorganized and immature trabecular bone at this early stage. The second stage of the process of "bone ingrowth" refers to the modeling and remodeling induced by stress in mature trabecular bone. The anchoring cells from the bone tissue were observed in the microstructure of titanium (Fig.1C).

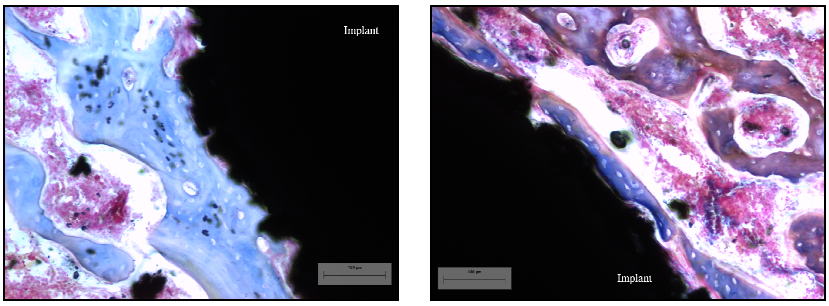


A B C

Figure 1. A - Sample Alloy + 5%: SEM implanted sample. B – Formation of fibrin-clot after surgery. C - Sample Alloy + 5%: SEM surface of the implant and bone tissue.

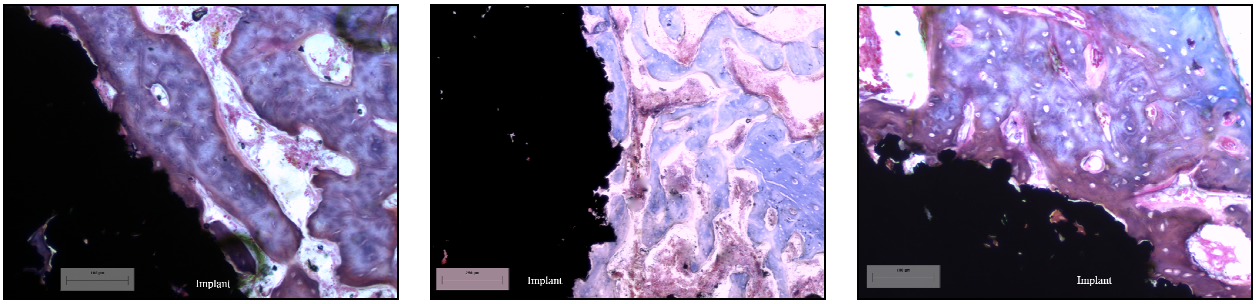
The morphological structure of the histological sections was performed by optical microscopy, which proceeded by Masson Trichrome staining (TM). Besides showing very well the nucleus and cytoplasm, this technique helps to differentiate the old bone tissue (seen in dark blue), neo-formed bone tissue (seen in light blue) and non-mineralized bone tissue (seen in red), could be identified in micrographs of the different stages of maturation

After 4 weeks for recovery of animals, the dense implants (cpTi and Alloy samples) showed neo-formed bone predominantly by light blue, around the implant surface (Fig.2). The porous samples showed, however, a non mineralized bone tissue surrounding the area represented by the red color (Fig.3). This difference may be related to the formation of bone tissue within the channels and pores of the implant, because of the greater difficulty of nutrients and cells penetration, the tissue maturation around the implant may take longer.



A B

Figure 2. Micrograph of a histological sample TM: A - cpTi e B – Alloy.



A B C

Figure 3. Micrograph of a histological sample TM: A - Alloy + 5 %, B – Alloy + 10 % e C – Alloy + 15 %.

It was possible to observe the maturation of bone tissue within the pores and interconnected channels of porous implants. For bright field microscopy was evident the presence of osteocytes inside the pores, showing osseointegration and osteoconductive porous alloy (Fig.4).

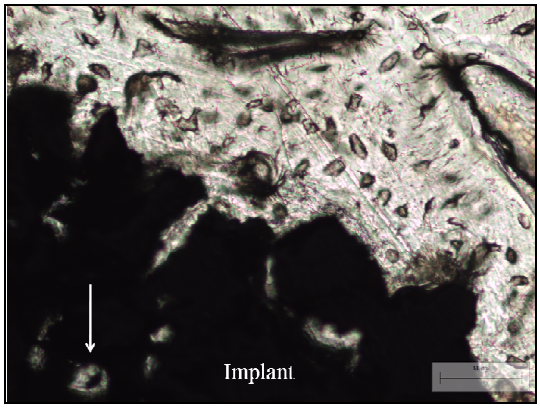


Figure 4. In bright field micrograph of a histological staining without the presence of osteocytes within the pores of the implant.

Conclusion

The PM process modified by addition of gelatin, provides with success the obtaining of porous metallic implants. Pore size obtained by this technique allowed the necessary nourishing to cell survival, proving to pores and channels, form a high interconnectable network showing thus the osseointegration and osteoconduction feature of the porous alloy.

Acknowledgements

We would like to thank the Brazilian institutions, FAPESP and CNPq for financial support.

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