A study of histological responses from Ti-6Al-7Nb alloy dental implants with and without plasma-sprayed hydroxyapatite coating in dogs

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Titanium alloys are hoped to be used much more for applications as implant materials in the medical and dental fields because of their basic properties, such as biocompatibility, corrosion resistance and specific strength compared with other metallic implant materials. Thus, the Ti-6Al-7Nb alloy that has recently been developed for biomedical use, that is, primarily developed for orthopaedic use, is to be studied in this paper, for application in dental implants. The biocompatibility test *in vivo* was carried out in dogs and the osseointegration was verified through histological analysis of the samples of the Ti-6Al-7Nb alloy with and without hydroxyapatite coating that were inserted in the alveoli. Within the controlled conditions the samples did not show any toxic effects on the cells.

1. Introduction

Metals are by far the oldest materials used in surgical procedures. Titanium and titanium alloys are relatively new materials compared with other metals, such as stainless steels and cobalt-based alloys [1]. Currently, titanium or titanium-aluminum-vanadium alloys are the metallic materials of choice for endosseous implants [2]. The Ti-6Al-7Nb alloy is of the alpha-beta $(\alpha + \beta)$ structure type, with a microstructure comparable to that of the wrought Ti-6Al-4V alloy. The chemical composition of the Ti-6Al-7Nb alloy was optimized [3].

An $\alpha + \beta$ Ti-6Al-7Nb alloy is more recently used in medical applications. The metallic implants are more commonly used in load-bearing applications for their superior fracture and fatigue resistance. Other great advantages of metallic alloys for implants are their high strength and toughness [4]. The modulus of elasticity of the typical titanium alloy, Ti-6Al-7Nb, is about half that of stainless steel. At the same time, the values of yield stress of Ti-6Al-7Nb and the stainless steel are comparable. As a result, Ti-6Al-7Nb is noticeably more resilient than stainless steel, which makes it an attractive material for stunting applications. An additional advantage of titanium alloys is their low density, which can allow the fabrication of lightweight biomaterials, for example dental implants [1].

The performance of any biomedical material is controlled by two characteristics: biofunctionality and biocompatibility [5]. Biofunctionality defines the ability of the device to perform the required function, whereas biocompatibility determines the compatibility of the material with the body [6]. Local tissue response to metal implants is closely related to the amount and toxicity of the corrosion products. Thus, minimal fibrosis is typically observed around titanium alloys, whereas fibrous layers as much as 2 mm thick are encountered with Co-Cr and, especially, steel implants. The titanium and Co-Cr alloys do not corrode in the body; when a metal implant is placed in the human body, it becomes surrounded by a layer of fibrous tissue of a thickness that is proportional to the amount and toxicity of the dissolution products and to the amount of motion between the implant and adjacent tissues. The presence of a fibrous layer prevents a firm union between the implant and the surrounding tissue, and this failure is obviously detrimental when rigid fixation of the implants is sought [1].

In the dental area, titanium and its alloys have been the materials of choice used in most surgical implant operations in recent years with an excellent biocompatibility [7]. The metallic implants to fulfil their functions should first be accepted by the body. This acceptance or biocompatibility is improved by coating the surface in contact with living tissues with Ca-phosphates, specially hydroxyapatite.

Some of the new implants utilize titanium or a titanium alloy substructure coated with a thin layer of either calcium phosphate ceramic, hydroxyapatite, or the plasma-spray technique. Tricalcium phosphate or hydroxyapatite coatings are designed to produce a bio-active

surface promoting bone growth and inducing a direct bond between the implant and the hard tissues. This phenomenon has been called biointegration [2].

Hydroxyapatite is the inorganic component of bones and teeth. It has been identified as a bioceramic with bioactive properties suitable for bone substitution and interfacing layers in surgical implants [8]. Hydroxyapatite has similar mineral constituent to bone and the high calcium/phosphorous (Ca/P) rich environment favors tissue response by accelerating and enhancing fixation to hard bone without the interaction of soft tissue. A desirable surface active characteristic promotes new bond ingrowth that is not found in the other classes of bionert materials. Hydroxyapatite is used as a substitute for bone as it promotes osteogenesis or new bone formation [9]. The application of ceramic coatings can be achieved in several ways, such as ion beam sputter deposition, electrophoretic deposition, and plasma-spraying [10–11]. Thermal spraying is an economical and effective process for coating onto metals thus, most of the efforts on coating have been directed to obtain layers of this coating. On the other hand, titanium appears to possess bioactive properties when clinically used for implantation, allowing for a very close contact between bone and metal surfaces and therefore good osseointegration.

2. Materials and methods

2.1. Materials preparation

The implants of Ti-6Al-7Nb alloy (SN056512) were obtained from IMI Titanium Limited, England/IMI-367. The specimens used in this study were 12 cylindrical-shaped screws (10 mm in length and about 3.8 mm in diameter) and these were made from machining specimens of the pure Ti-6Al-7Nb alloy and the other six specimens of the Ti-6Al-7Nb alloy with hydroxyapatite coating.

The six coupons were coated by a routine plasma-spraying technique with hydroxyapatite (HA) powder with grain size ranging from 44 to 111μm. HA powder was obtained from F. J. Brodmann® (2015M–1). Before the coating operation, to achieve well-bonded plasma-sprayed coating, surfaces were roughened by gritblasting 60-grit silica at a pressure of 80 psi (0.55 MPa) and 90° angle for 30 s. The hydroxyapatite coatings were produced by plasma-spraying using a plasma torch (SG-100-model, Miller Thermal Inc.®, USA) with pre-heated argon gas (Ar) and then hydrogen (H_2) as primary fuel gas flow and nitrogen (N_2) carrier gas flow and 70 mm stand-off distance. The spray gun was operated by hand. In order to achieve the desired uniform coating, samples were rotated while the spray gun underwent translational movement along the axis of rotation. During the coating no cooling device was used in coating/substrate, so then sample cooling was carried out only by loss of heat to environment.

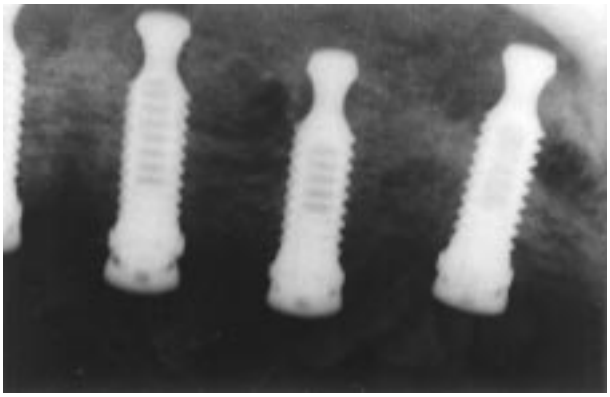

Figure 1 Representative radiograph of three screw implants after three months of implantation in dogs.

All samples were ultrasonically degreased in acetone plus ethyl alcohol solution for 10 min each. These were then air-dried and sterilized using gamma radiation, whose source was cobalt-60 (^{60}Co). The total dose of radiation per sample was 25 kGy, delivered at an average rate of 1.334 kGy/h (18 h 36 min at 50 mm distance).

2.2. Animal selection

Two male adult mongrel dogs, 24 months old, with an approximate weight of 24 kg, were used for this investigation. Animal management and care, as well as surgical procedures followed an approved protocol routinely used at the Faculty of Dentistry of Araraquara (Unesp). Periapical radiographies were carried out in both samples in order to measure both sides of the dogs' mandible bones to evaluate the quantity as well as the quality of bone and probable pathologic modifications.

2.3. Surgical procedures

The surgical procedures were carried out by firstly sedating the animals using Rompun intramuscular (i.m.) injection (1.5 ml/10 kg from Bayer of Brazil), followed by general intravenous anaesthesia (i.v.) injection of sodium thiopental (15 mg/kg for 1 g/ml dilution from Abbott Laboratories of Brazil) as needed. Routine infiltration anaesthesia of Lindocaina 2% (Cristália labs of Brazil Ltd) was administered at the surgical sites. General anesthesia with sodium thiopental was also used for short-term procedures such as suture removal and obtaining of control radiographs. All inferior premolars were removed bilaterally. After 12 weeks, the animals were anesthetized again and a full thickness flap was elevated in the alveolar ridge, and three sites were created on each side for implant placement under saline irrigation, according to a routinely used implant surgical

TABLE I Diagram of the implants put in dog mandible premolar teeth (PM), bilaterally

	Right			Left		
	1°PM	2°PM	3°PM	1°PM	2°PM	3°PM
Dog 1	Smooth	Coated	Smooth	Coated	Smooth	Coated
Dog 2	Coated	Smooth	Coated	Smooth	Coated	Smooth

protocol. The implants were inserted in the first, second and third premolar teeth alveolar sites as shown in Table I.

2.4. Histology preparation

After a healing period of 16 weeks the animals were sacrificed and the implant containing premolar alveolar parts prepared for histological interpretations. The tissue blocks containing the implants were immediately placed in 10% neutral formalin. After fixation the samples were dehydrated remaining in water for 12 h and gradually exposed to 70–99% ethanol for 24 h in each solution. After treatment with xylol for 24 and 48 h, the specimens were embedded in a metacrylate solution and after polymerization sections 80 µm thick were observed in the microscope under fluorescent light.

3. Results and discussion

Twelve weeks after implant insertion in dogs, alveolar regeneration could be detected by radiographic examination. The radiographic results suggested the presence of newly formed bone tissue around the implants or rather, a bone neoformation. The implants remained stable in the site of implantation, fitting exactly and a radiopaque lining was noted in its coronal aspect. An intimate bone to implant adaptation, suggestive of an osseointegration could be observed in the bone/implant interface. There were no observable differences among experimental conditions (Fig. 1).

A total of twelve peri-implant mandible quadrants in both animals were available for histological analysis. Formation of extra-cellular material, as collagenous fibers and bone cells, was detected. This histological

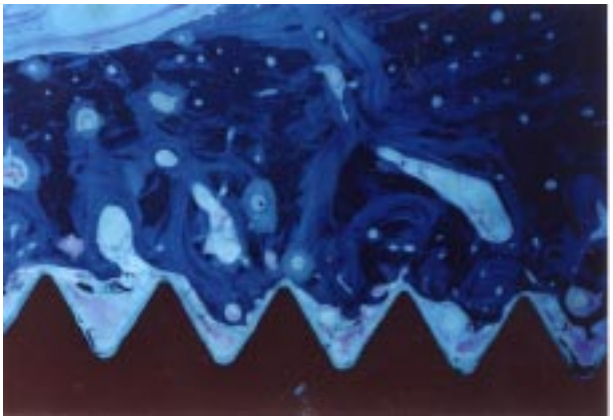

Figure 4 Optical micrograph of alveolus created for implant insertion, with ultra-violet technique. The growth and anchorage of the fibroblasts produced pre-osteoid in the implant thread (helicoidal) region of the Ti-6Al-7Nb alloy. Magnification × 126.

Figure 2 Optical micrograph of a histological sample of the Ti-6Al-7Nb alloy. The pink area represents sites of collagen, the light blue new calcification zones, and the dark blue calcified bones. The implant is black. Magnification × 126.

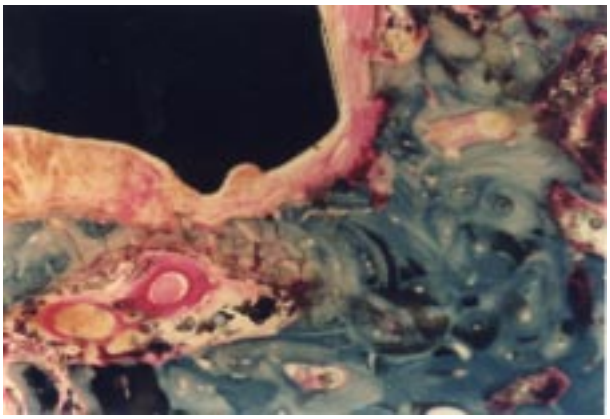

Figure 5 Optical micrograph of a histological sample of the Ti-6Al-7Nb alloy. The growth and anchorage of the fibroblasts in the implant apical region of a Ti-6Al-7Nb alloy. Magnification × 126.

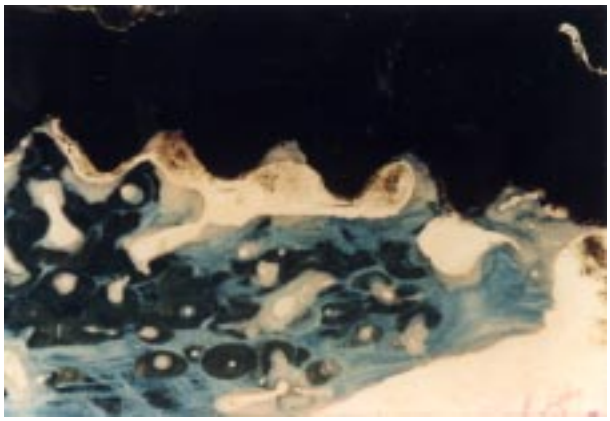

Figure 3 Optical micrograph of a histological sample of the Ti-6Al-7Nb alloy plasma-sprayed surface. Observe the bone growth in the implant direction. Magnification × 126.

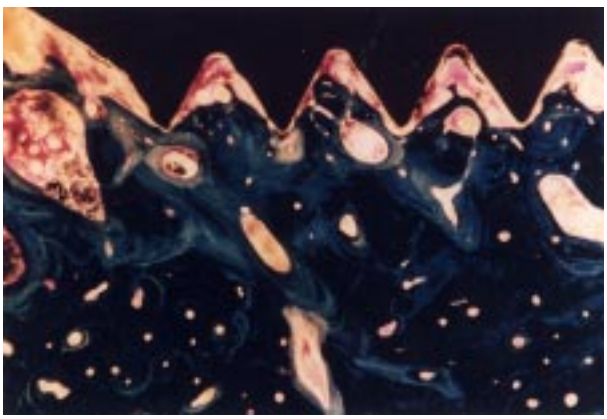

Figure 6 Optical micrograph of a histological sample of the Ti-6Al-7Nb alloy. The ossification is performed in concentric layer types. Bone reformation or remodeling. Magnification × 126.

stage was represented in different colors: collagen stained pink, initial calcification light blue, mature bone, at the time of the implant insertion, dark blue and the implant itself appeared black. It was noted that new bone invaded the area of the helicoidal part that corresponded to the bone medulla commonly filled solely by adipose and hematopoietic tissue. There was no evidence of inflammatory reaction (Fig. 2).

The material that involved the hydroxyapatite coating was completely resorbed and no presence of hydroxyapatite particles was detected in the medullary cavity or compact bone layer. The woven developed into lamellar bone and adhered to the implant surface (Fig. 3). The lamellar bone layer became thicker and connective tissue was seen in contact with the implant surface. The amount of bone-like tissue decreased, resulting in the formation of a circumferential thin layer of lamellar bone. The amount of bone tissue that circumscribed the implants increased (Figs 2 and 3).

In some regions of the implants it was verified that the bone could grow in the implant direction (Fig. 3) and this observation confirms the material biocompatibility.

The number of fibroblasts adhered to the coated implants was similar to the one observed in smooth implants. Fibroblast-like cell products were also observed disturbing the direct contact of bone tissue in the implant surface. The distribution of connective and calcified tissues seemed to be randomly scattered and the same aspect was observed from the head to the apical region as demonstrated in Figs 4 and 5.

The ossification zone (light blue) had characteristic concentric layer morphology like true osteones and through the formation of processes that develop in different periods of time (Fig. 6); the central canal of these osteone characteristic concentric rings shelters blood vessels which are very important for metabolic reasons.

Differences among the implants were found directly in the bone/implant interface zone. In this area there was a great deal of bone reformation or remodeling, and no osteogenic activity took place, and an apparently empty gap could be detected between the two structures.

The tissues reaction of the implantation in the first dog (1) was slightly superior than in the second (2). The implant Ti-6Al-7Nb alloy with HA coating in dog 1 showed a thicker lamellar bone around itself than in dog 2. This observation led us to conclude that the highest activity in new bone formation occurred in coated implants.

4. Conclusions

The results of this study are extremely encouraging for the use of Ti-6Al-7Nb alloy either with or without a hydroxyapatite coating. The biocompatibility analysis carried out by the *in vivo* test through histological observations showed that an osseointegration is reached in both implants of Ti-6Al-7Nb alloy, with and without coating. The osseointegration and anchorage seen in the bone/implant interface resulted in the acceptance of a promising stability and encapsulation of endosseous implants.

References

1. I. GOTMAN, *J. Endourology* **11** (1997) 383.
2. R. M. MEFFERT, B. LANGER and M. E. FRITZ, *J. Periodontol.* **63** (1992) 859.
3. M. F. SEMLITSCH, H. WEBER, R. M. STREICHER and R. SCHÖN, *Biomater.* **13** (1992) 781.
4. R. M. PILLIAR, *Biomater.* **12** (1991) 95.
5. D. F. WILLIAMS, ''Materials and Dental Materials'' (VCH, Germany, 1991) p. 1.
6. D. F. WILLIAMS, ''Definitions in Biomaterials'' (Elsevier, New York, 1987) p. 9.
7. F. RUPP, J. GEIS-GERSTORFER and K. E. GECKELER, *Advanced Mater.* **8** (1996) 254.
8. T. S. B. NARASARAJU and D. E. PHEBE, *J. Mater. Sci.* **31** (1996) 1.
9. K. A. KHUR and P. CHEANG, in Proceedings of the 5th National Thermal Spray Conference, June 1993 (ASM International Materials Park, Ohio) p. 347.
10. R. M. STREICHR, H. WEBER, R. SCHÓN and M. SEMLITSCH, *Biomater.* **12** (1991) 125.
11. J. D. HAMAN, A. A. BOULWARE and D. E. CRAWMER, *J. Therm. Spray Technol.* **4** (1995) 179.

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