In vivo behavior of surface modified Ti$_6$Al$_7$Nb alloys used in selective laser melting for custom-made implants. A preliminary study

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Abstract

The objectives of this study were to test the biocompatibility and to evaluate the osseointegration of Titanium–Aluminum–Niobium (Ti$_6$Al$_7$Nb) alloy used in the manufacturing of personalized implants with selective laser melting (SLM) technology and to compare the growth viability of osteoblastic-like cells on different Ti$_6$Al$_7$Nb alloy samples (plain, coated with hydroxyapatite or SiO$_2$–TiO$_2$) implanted into the cranial bone of Wistar rats. In terms of biocompatibility, the cone-beam computer-tomography head scans taken at the moment of sacrifice of each group (one, two and three months) showed no implant displacement, no osteolysis and no liquid collection around the implants. At one month, around all types of implants new bone formation was noticed, although around the plain Ti$_6$Al$_7$Nb implant a large amount of powder debris was present. Still, no inflammatory reaction was seen. At two months, the distance between the implants and the calvarial bone margins diminished. A thin layer of fibrous tissue was noticed around the Ti$_6$Al$_7$Nb implant coated with hydroxyapatite but no bone contact was achieved. In the group sacrificed at three months there was still no bone contact, but noticeable were the SiO$_2$–TiO$_2$. In the group sacrificed at three months SiO$_2$–TiO$_2$ particles detached from the implant and completely integrated in the tissue were noticeable. All results suggested that the Ti$_6$Al$_7$Nb alloy with or without infiltration is well biologically tolerated.

Keywords: Ti$_6$Al$_7$Nb, additive technology, selective laser melting, custom-made implants, osseointegration.

Introduction

Due to their excellent biocompatibility, Titanium and its alloys are on the top of the list of fundamental biomaterials used for dental, orthopedics, neurological and cardiovascular implantation [1, 2]. The use of Titanium as a biomedical material had a big impact in the medical field since 1950’s. Titanium alloys, such as Titanium–Aluminum–Vanadium (Ti$_6$Al$_4$V) and Titanium–Aluminum–Niobium (Ti$_6$Al$_7$Nb) have been created to improve the mechanical properties of commercial pure Titanium (cpTi) [3–5]. However, the cytotoxicity and the adverse reaction of the Vanadium against the tissue have limited its clinical use [5]. This led to the use of Ti$_6$Al$_7$Nb as one of first choice materials in the reconstruction of cranio-facial bone defects.

Over the past decade, the interest in chemical modifications of implant surfaces has widely grown in order to improve the implant-bone interface [6, 7]. For this, Ti$_6$Al$_7$Nb implants, fabricated by selective laser melting technology, can be immersed in hydroxyapatite (HA) and SiO$_2$–TiO$_2$ solution. Selective laser melting (SLM) allows the generation of complex three-dimensional metal parts by selectively melting successive layers of metal powder on top of each other, using the thermal energy supplied by a focused and computer-controlled laser beam [8]. Generally, bioactive materials such as bioglass or HA can bond directly to living tissue via an apatite layer [9]. This is the reason the physico-chemical or surface properties of an implant can be modified to improve the osteogenic capacity of synthetic materials.

Among other in vivo evaluation tests, osseointegration is used to measure the biocompatibility of intraosseous implants [10].

The objectives of the present study were to test the biocompatibility of the Titanium alloy used in the manufacturing of personalized implants with SLM technology, to compare the growth viability of osteoblastic-like cells on different Ti$_6$Al$_7$Nb alloy samples (plain, coated...
with HA and coated with SiO₂–TiO₂) and to evaluate the osseointegration behavior of the alloys implanted into the cranial bone of Wistar rats.

**Materials and Methods**

Commercially available Ti₆Al₇Nb alloy (ATI Ti₆Al₇Nb®, ATI Allvac, Monroe NC, USA) was used to fabricate the test samples by selective laser melting technology, using a Realizer SLM 250 machine (Realizer GmbH, Borchen, Germany). The samples were produced in the form of discs with 5 mm diameter and 1 mm thickness with a controlled porosity of 24–25%, determined through Archimede’s method ISO 2738–99. They were divided into three groups. One group was left uncoated. One group was coated with hydroxyapatite (HA) and last one was coated with SiO₂–TiO₂ (Figure 1).

![Figure 1 – Hydroxyapatite-coated Ti₆Al₇Nb implants.](image)

The coating procedure was done by immersing the discs into a hydroxyapatite and SiO₂–TiO₂ solution. They were kept in preliminary void for 15 minutes. After that, they were dried in a special oven at 100°C for 30 minutes. The thermal treatment was performed at 600°C for 30 minutes for the implants infiltrated with HA and at 400°C for 60 minutes for the implants immersed in SiO₂–TiO₂.

**Experimental design**

Thirty-six male Wistar rats were included in the study, divided into six groups, each of them having six animals. All rats were the same age (two months old) and approximately the same weight, kept in standard conditions of temperature, humidity, day/night cycle and they all had the same access to food and water, *ad libitum*, throughout the experiment. The vivarium conditions were according to The European Committee for Animal Protection No. 86/606, 24 November 1986. Body weight and clinical signs were recorded regularly.

The rats were anesthetized with a Xylazine/Ketamine cocktail using a dosage of 8 mg Xylazine and 80 mg Ketamine per kg of body weight. Disinfection of the cephalic extremity was done with a polyvidone iodine solution, after preliminary hair cut. An antero-posterior incision of the skin and periosteum has been done, from the inter-orbital level to the superior cervical level, exposing the bone surface. With a continuous cooling system, the bony defects were created, using diamond burs, having the same diameter as the implants. The implants were placed into the fronto-parietal and inter-orbital bony defects (Figure 2), each animal having two samples implanted into the calvaria (uncoated–coated with HA and uncoated–coated with SiO₂–TiO₂).

![Figure 2 – Non-coated and coated implants with HA or SiO₂–TiO₂ placed into the calvarial defects of Wistar rats.](image)

Layer-to-layer suture was performed at the end of the procedure.

The rats were sacrificed at one, two, and three months post-implantation with a diethyl-ether overdose after a preliminary cone-beam computer-tomography (CBCT) scanning of the head. The biological samples were taken from the bone, periosteum and dura-mater and immersed in 10% formalin solution until their processing time.

**Histological examination**

The bony specimens containing the implant samples were labeled, washed and stained with Villanueva Osteochrome bone stain for 72 hours (manufacturer’s instructions).

The dehydration process used different concentrations of ethanol (70%, 80%, 95% and absolute ethanol).

For the infiltration process, the specimens were kept in Technovit 7200® (Heraeus–Kulzer GmbH, Wehrheim, Germany) with different concentrations of ethanol and absolute Technovit 7200®.

The embedding process was performed using Technovit 7200 VLC® (Heraeus–Kulzer GmbH, Wehrheim, Germany). The thick sections of 250–350 μm were cut with a saw band (EXAKT 300CL cutting System®, Exakt GmbH, Norderstedt, Germany), right through the sagittal sinus of the parietal bone.

To obtain thin sections of the histological samples, EXAKT 400 CS® and EXAKT AW 110® grinding system (Exakt GmbH, Norderstedt, Germany) were used, with different textures of polishing paper discs until 50-μm thickness of specimen was reached. A digital caliper for thickness measurement was used.

The histological slices were examined by optical microscopy. A Zeiss Axiosvert® D1 microscope (Carl Zeiss Microscopy GmbH, Germany) was used, the images being captured with an AxioCam MRC® photo camera (Carl Zeiss Microscopy GmbH, Germany). The software dedicated for this procedure was Axiovision® Rel 4.6 (Carl Zeiss Microscopy GmbH, Germany). The study was approved by the Ethical Commission of the “Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania (No. 391/01.09.2011).
Results

At the moment of sacrifice, each group of animals (at one, two and three months) was scanned with cone-beam computer-tomography (CBCT), at the level of the head. The images obtained pointed out that, in each group scanned, the implants were not displaced from the implantation area. On the other hand, images showed no zones of osteolysis around the implants and no images of liquid collection over the dura-mater or under the periosteum or skin (Figure 3).

Figure 3 – CBCT head scan of Wistar rats, showing no osteolysis or liquid collection around the implants.

At one month, around the untreated Ti$_6$Al$_7$Nb implant new bone formation could be noticed, starting from the bone margins towards the implant, but a fibrous tissue formed at the implant surface, surrounding it (Figure 4A).

Also noticeable was the presence of Ti$_6$Al$_7$Nb powder debris in the fibrous tissue surrounding the implant, without any inflammatory reaction or hemorrhage.

In case of Ti$_6$Al$_7$Nb implant coated with HA, the amount of Ti$_6$Al$_7$Nb powder debris seen was smaller (Figure 4B). A slight hemorrhage close to the implant surface and also a defect of healing of the fibrous tissue could be seen at the same time.

A small amount of new immature bone tissue was noticed starting from the margin of the bone defect but not in contact with the implant.

In case of Ti$_6$Al$_7$Nb implant coated with SiO$_2$–TiO$_2$, detachment of SiO$_2$–TiO$_2$ particles was observed, which were integrated in the newly formed osseous tissue, producing no inflammatory reaction, but osteoconduction. The bone-implant distance was smaller (Figure 4C).

Figure 4 – Villanueva Osteochrome bone stain – one month: (A) Ti$_6$Al$_7$Nb implant; (B) Ti$_6$Al$_7$Nb implant coated with hydroxyapatite; (C) Ti$_6$Al$_7$Nb implant coated with SiO$_2$–TiO$_2$. Notice new bone formation and the amount of powder debris around each implant.

At two months, around the Ti$_6$Al$_7$Nb implant, the bone-implant distance diminished, but there was still no contact (Figure 5A). Fibrous tissue was still present around the implant. The powder debris was also present but no inflammatory reaction could be noticed. There was no hemorrhage between the implant and the bone.

In case of Ti$_6$Al$_7$Nb implant coated with HA a uniformity of the fibrous tissue at the implant surface could be noticed. At this time interval the layer of fibrous tissue was thin but no bone contact to the implant was present (Figure 5B).

A little progressive growth of newly formed bone from the calvarial margins toward the center of the bone defect was noticed in case of Ti$_6$Al$_7$Nb implant coated with SiO$_2$–TiO$_2$. But, the newly formed bone was not integrated within the implant surface. There was still
fibrous tissue present in between, without adequate osseointegration (Figure 5C). There was no inflammation but hemorrhage at the interface between bone and implant.

In the group with Ti6Al7Nb implant, sacrificed at three months, histological evaluation indicated continuous bone growth. At the level of the fibrous tissue present around the implant some defects were noticed (Figure 6A). No reject inflammatory reaction was seen in this group.

In case of Ti6Al7Nb implant coated with HA, new bone formation could also be noticed, connected to the implant via a small layer of fibrous tissue. There was no inflammatory reaction accompanying this evolution (Figure 6B).

The Ti6Al7Nb implant coated with SiO2–TiO2 showed osseointegration defects. SiO2–TiO2 particles outside the implant surface, completely integrated in the tissue, with no inflammatory reaction were also noticeable. New bone formation was present even at three months after implantation (Figure 6C).

Figure 5 – Villanueva Osteochrome bone stain – two months: (A) Ti6Al7Nb implant; (B) Ti6Al7Nb implant coated with hydroxyapatite; (C) Ti6Al7Nb implant coated with SiO2–TiO2. Notice the diminished distance between implant and bone and the uniformity of the fibrous tissue.

Figure 6 – Villanueva Osteochrome bone stain – three months: (A) Ti6Al7Nb implant; (B) Ti6Al7Nb implant coated with hydroxyapatite.
Endosseous titanium implants are key factors to achieve rods and nails, spinal devices, screws, wires, etc., for joint replacement systems, fracture fixation plates, alloy is widely used in the medical device industry, primarily Ti6Al7Nb as a material for the custom-made fabrication of the implant.

Improve the bone cells adhesion and the osseointegration of the implant with different coating materials can enhance bone-implant integration [16]. Thus, treating the post-surgical and long-term mechanical stability and reliability of the implant. SLM uses CAD data in order to generate specimens into a layer-by-layer fashion [11–13]. Through this technique, implants with unlimited geometric shapes and an irregular structure can be manufactured. This is very important in mimicking the human bone and in obtaining personalized implants for visceral- and neurocranium bone defects reconstruction.

Different titanium alloys can be used with SLM to produce custom-made structures. ATI Titanium 6Al–7Nb Alloy (UNS R56700) was developed in 1977 by a team of researchers at Gebruder Sulzer in Winterthur, Switzerland [14]. The objective was to create a titanium alloy for medical and surgical device applications with properties nearly identical to Ti6Al4V, substituting Niobium for Vanadium as the beta-stabilizing element [15]. Ti6Al-Nb alloy is widely used in the medical device industry, primarily for joint replacement systems, fracture fixation plates, rods and nails, spinal devices, screws, wires, etc. [14].

The shape, surface composition and morphology of endosseous titanium implants are key factors to achieve post-surgical and long-term mechanical stability and enhance bone-implant integration [16]. Thus, treating the surface of the implants with different coating materials can improve the bone cells adhesion and the osseointegration of the implant.

In this study, in order to test the behavior of the Ti6Al-Nb as a material for the custom-made fabrication by SLM, the surface treatment of implants was done with hydroxyapatite and SiO2–TiO2. Hydroxyapatite is the inorganic component of bones and teeth. It has been identified as a bioerodent with bioactive properties for bone substitution and interfacing layers in surgical implants [10]. SiO2–TiO2 implant coatings are known for their ability to form calcium phosphate (CaP) on their surface [17, 18]. This is the reason why they are taken into consideration as an alternative for bioactive ceramic-based coatings. Also, it was demonstrated that by releasing silica in bone environment, bone-forming cells are activated leading to enhanced bone growth [17, 19, 20].

The findings of this study prove that the titanium alloy materials, by themselves or impregnated with HA or SiO2–TiO2, are osteoconductive materials. The surface topography and chemistry seem to be the two key factors [21]. One month after implantation Ti6Al-Nb, Ti6Al-Nb–HA, and Ti6Al-Nb–SiO2–TiO2 showed bone formation at the edge of the implant, all with formation defects (wider defect for the third material), but none of them had inflammatory reactions, even the free particles of SiO2–TiO2 and HA showed integration in the new formed bone. Areva S et al. reported in their study the high osteoblast activity, even bone formation after 14 days of culture, except for the SiO2–TiO2 coating. Even so, gradual release of SiO2 from the coating extends the proliferation and differentiation of the osteoblast [22]. Ti6Al-Nb powder debris were present in the fibrous tissue probably because of the friction between the implant and host bone during insertion [7]. For the alloy with HA coating, powder debris seen was smaller. Thus, HA may act like a barrier against the detachment of Ti debris [7].

At two months the defects in osseointegration were larger for Ti6Al-Nb coated with SiO2–TiO2, persistent for Ti6Al-Nb and absent for the HA coating. For the three months period, only the alloy with SiO2–TiO2 coating still shows areas of integration defects and possibly osteolysis. In the SiO2–TiO2 coated implants, there were SiO2–TiO2 particles that detached from the implant’s surface and were totally included in the newly formed bone tissue, without an inflammatory foreign body reaction.

The integration of the Ti6Al-Nb and Ti6Al-Nb coated with HA, at three months, clearly shows the higher capacity of osteoinduction for these two alloys, the HA having better histology results. Still, all of the materials showed defects of osseointegration of various degrees. The defects could have been caused by the inadequate drilling process when placing the implants. The implants and the drill having exactly the same dimensions could have led to poor primary stability, which can be the cause of the integration defects. It is also important to consider the roughness of the implants. In the present study, we found no significant association between the surface roughness and the cell attachment. These findings are similar to those of Rosa AL and Beloti MM in Ti6AlV alloy study, who reported that primary human and rat bone marrow cell attachment were not affected when cells were cultured on different surface roughness of cpTi and Ti6AlV alloy [23].

Studying the surface properties and cell response of Ti6Al-Nb alloy, Spriano S et al., in their multi-step chemical and thermal processes, concluded that a good cell spreading and no cytotoxicity can be underlined. They also observed a good chemical interaction of the surfaces with the culture medium [24].

Discussion

Conventional implant fabrication technique by casting and cutting process has the disadvantages of restrictions in geometric shaping of the implants. In contrast, additive manufacturing techniques have the advantage of tailoring the structure’s architecture owing to their layer-wise building and their digital link with a computer-aided design (CAD) model. Selective laser melting (SLM) is an additive manufacturing technology using a laser beam as the energy source for fusing the titanium alloy powder into different structures. As all additive-manufacturing techniques, SLM uses CAD data in order to generate specimens into a layer-by-layer fashion. Through this technique, implants with unlimited geometric shapes and an irregular structure can be manufactured. This is very important in mimicking the human bone and in obtaining personalized implants for viscero- and neurocranium bone defects reconstruction.

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Studying the surface properties and cell response of Ti6Al-Nb alloy, Spriano S et al., in their multi-step chemical and thermal processes, concluded that a good cell spreading and no cytotoxicity can be underlined. They also observed a good chemical interaction of the surfaces with the culture medium.
Future studies, with implants that could have slightly larger dimensions than the drill, are to be taken into consideration, providing better local conditions for primary stability. Following the current protocol, the osteoinductive character of the three materials studied was proven, even the SiO$_2$–TiO$_2$ powder being integrated in the newly developed bone. Moreover, these particles of SiO$_2$–TiO$_2$ implants and Ti 6Al7Nb coated with HA showed better stability. Following the current protocol, the osteoinductive consideration, providing better local conditions for primary insertion is required in order to achieve bone formation at the margins of the defect. Further in vivo studies using these kinds of surface treatments of Ti$_6$Al$_7$Nb alloy are needed to confirm the current findings.

**Conclusions**

The current animal study points out that the Ti$_6$Al$_7$Nb alloy shaped into form by selective laser melting additive technology is well biologically tolerated, without producing any adverse reactions. The samples used in this study, based on the Ti$_6$Al$_7$Nb alloy were integrated into fibrous tissue but no inflammatory reaction was seen. Ti$_6$Al$_7$Nb implants and Ti$_6$Al$_7$Nb coated with HA showed better results, which could suggest a possible enhancement of osteoconductive properties by hydroxyapatite infiltration. For clinical purposes of custom-made implantation, rigid insertion is required in order to achieve bone formation at the margins of the defect. Further in vivo studies using these kinds of surface treatments of Ti$_6$Al$_7$Nb alloy are needed to confirm the current findings.

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