STUDIA UBB CHEMIA, LXIV, 1, 2019 (p. 207-218)
(RECOMMENDED CITATION)
DOI:10.24193/subbchem.2019.1.17

INNOVATIVE CHEMICAL COATING PROTOCOL FOR TITANIUM ALLOY IMPLANTS

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ABSTRACT. This paper aims to investigate the "*in vivo*" behavior (over an extended period of time - six months) of hydroxyapatite and SiO_2-TiO_2 coating of Ti_6Al_7Nb alloy implants manufactured by selective laser melting. Innovative chemical implant coating by immersion technique was studied and the results were analyzed by optical and scanning electron microscopy (SEM). The results showed better osseointegration process for the coated implants and a much stabler biological behavior on the surface of the chemical treated implants.

Keywords: *chemical coating,* Ti_6Al_7Nb*, surface treatment, bioactivity, SLM, laser processing*

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INTRODUCTION

Osseointegration improvement of titanium and titanium-based alloys is one of the most challenging tasks in modern medicine. The bioinert behaviour grants titanium the first treatment of choice when bone reconstruction is wanted, having clinical application in dentistry, orthopedics and neurosurgery [1]. The drawback of commercial pure titanium, mainly the mechanical properties, seemed to be resolved in the early 2000`s by introducing alloys such as Ti_6Al_4V or Ti_6Al_7Nb with improved physical properties [2]. New technologies such as SLS (Selective Laser Sintering) or SLM (Selective Laser Melting) opened the path of creating virtually any type of custom made implant with complex shape and design [3]. This led to the search for new methods of increasing the osseointegration and improving the biocompatibility of the alloys by changing the composition and the morphology of the implant surface. Different types of coatings, such as HA (hydroxyapatite), calcium phosphate or SiO_2 with different coating techniques (immersion-coating, high-temperature sintering, high-pressure sintering, laser cladding process) have been tested with encouraging result [4]. The current paper presents a new coating protocol of HA and SiO_2-TiO_2 solutions: immersion followed by thermal treatment for Ti_6Al_7Nb implants made by SLM.

One of the most frequently encountered complications in the use of Ti implants is lack or poor osseointegration, due to fibrotic tissue formation at the implant-bone contact area [5]. The use of new surface treatment protocols may be one of the solutions to this problem and also the use of alloys that can promote osteoblast-cell growth [6].

Previous studies have analysed the osteogenic properties of Ti coatings “in vitro” and “in vivo” for periods up to 18 weeks, few of them having taken into consideration the three-dimensional characteristics of the implants [7]. Our “in vivo” study extends over a period of 24 months proving a good stability of the osseointegration in time, as well as a faster and better biological behavior for the coating protocol used.

RESULTS AND DISCUSSION

Macroscopy examination of the specimens consisting in implant and surrounding bone showed no signs of inflammation or implant displacement at 1 or 6 months, moreover the implants with SiO_2-TiO_2 coating showed bone overgrowing the neck of the implant. Optical miscroscopy showed good osseointegration of all implants, no connective tissue at 1 and 6 months post insertion, and percentages of mineralized bone vs. osteoid (unmineralized bone) surrounding the implants were calculated with the method described by Gheban D., Armencea G. et al. [8,9,10] as presented in table 1.

Table 1. Percentages of mineralized bone vs. osteoid

	1 month		**6 months**	
	Osteoid	**Mineralised bone**	**Osteoid**	**Mineralised bone**
Ti_6Al_7Nb	6.37%	93.63%	4.56%	95.44%
Ti_6Al_7Nb - SiO_2-TiO_2	1.44%	98.56%	2.57%	97.43%
Ti_6Al_7Nb - HA	0.82%	99.28%	2.70%	97.30%

Further investigation of the contact suface between the implant and the bony structure was done at 6 month by SEM. Perfect attachment of the bone to the implant site was noticed for both of the surface coatings (figure 1) and lacunar areas were present in the bone surrounding the uncoated implant (figure 2).

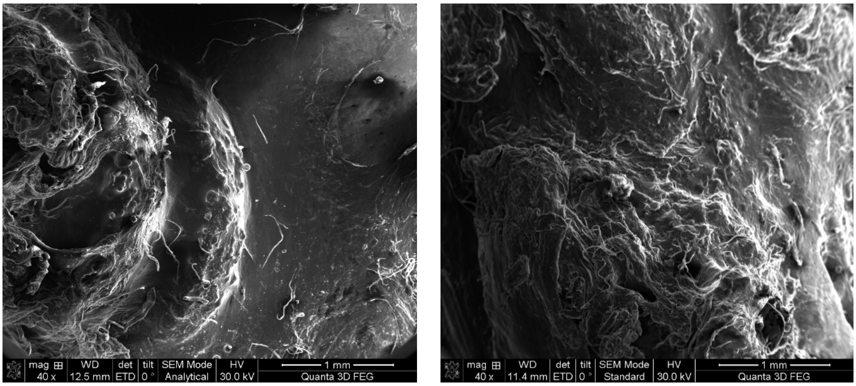


Figure 1. SEM at 40X magnification for the bone-implant area Ti_6Al_7Nb - SiO_2-TiO_2 (left) and Ti_6Al_7Nb – HA (right).

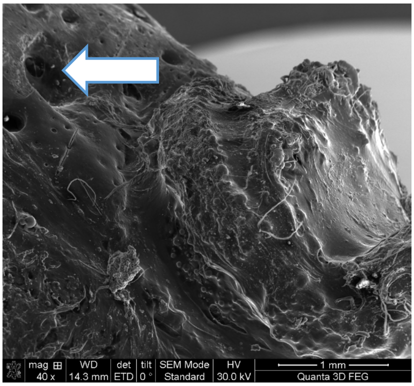


Figure 2. SEM at 40X magnification for the bone-implant area Ti_6Al_7Nb. Lacunar areas in the bone surrounding the implant (white arrow).

Greater magnification of the lacunar areas around the implant (300X) revealed round shape particles into the bone foramens that can be alloy particles detached from the implant body (figure 3), in the absence of the coating material.

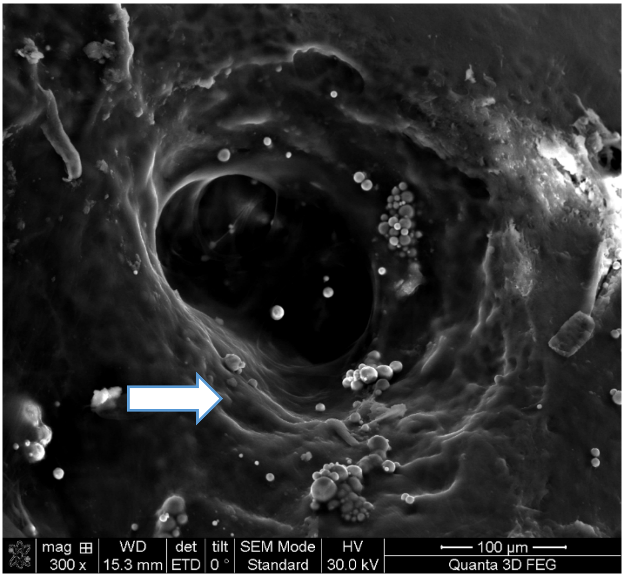


Figure 3. SEM 300X magnification of the bone foramen next to the Ti_6Al_7Nb implant showing round particles (white arrow) migrated into the lacunae

Implant osseointegration is considered to be perfect when there is no gap between the implant and the surrounding tissue, when no connective or granulative tissue exists at the implant surface [11]. None of the above were seen in our study. Better, faster and stabler osseointegration are difficult tasks to be achieved for the Ti alloys. The quality of the osseointegration (the amount of mineralized new bone formed) on the external aspect of the implant depends on the type of alloy used, on the surface morphology and coatings of the implant [12]. It is clinically proven that Ti and its alloys have a better biological behaviour than other alloplastic materials [13]. These characteristics are due to the bioinert behaviour of Ti, it alone never promoting positive effects on the recipient sites but, most notable, preventing negative biological effects from happening [14]. Osseointegration enhacement, which is an ongoing process until 6 months after insertion, can be achieved by surface treatments. Over time several types of materials such as HA, bioreactive glass, SiO_2, SiO_2-TiO_2, SiO_2-HA and different types of coating or cladding have been tried (laser cladding, dip coating-sintering technique, immersion-coating technique, HIP - hot isostatic pressing - technique, sputter) with promising results [15].

The best “in vitro” results were obtained by immersion-coating techniques, other methods having serious limitation to be considered for “in vivo” studies because of adverse effects caused by high-temperature sintering cycle on the mechanical properties of the metallic substrate materials and loss of the alloy wrought structure [16]. Combination of HA and SiO_2 proved to be ineffective in promoting cell adhesion [17]. Other authors recommend a sputter coating to be method of choice for forming a dense, adherent coating of HA onto a metal substrate [15, 16].

We have chosen to test the immersion technique for HA and SiO_2-TiO_2 coating of Ti_6Al_7Nb under specific controlled physico-chemical environment that leads to even surface distribution of the coating material. We considered the “in vivo” test for Ti_6Al_7Nb alloy because it represents a better choice for the commercial Ti_6Al_4V that proved to be cytotoxic and causing neurological problems under certain conditions [18, 19]. We studied the behavior of the implants in time, due to the dynamic mineralization and demineralization that the bone structure around the implant has, monitoring the healing process from implant insertion up to six months [20]. Other studies of similar implants proved that demineralization processes can be encountered even at 6 months after implant insertion [9], so extensive follow-up is mandatory.

Macroscopically all the implants in this study proved to be stable at 1 and 6 months, the best result having the group with SiO_2-TiO_2 coating, that showed bone overgrowth at 6 months even at the neck of the implant, so the implant bone embedding was extensive.

Optical microscopy and calculation of mineralized bone at the implant surface presented similar results for the coated implants, with higher amount of mineralization vs. the uncoated control group; result similar with other studies [20, 9]. This shows the better biological behavior of the coated implants, without any significant changes from 1 to 6 months (98.56% to 97.43% for the SiO_2-TiO_2 coating and 99.28% to 97.30% for Ti_6Al_7Nb – HA coating). However, the top mineralization surrounding the implant is present at the HA coating group at 1 month, and at 6 months both coatings have reached a stable degree of bone mineralization of about 97%, explicable by the physiological bone remodeling [21]. Even without coating, the alloy has a good percentage of mineralization, due to the fact that Nb oxides are similar to Ti oxides being stable in time, corrosion-free and thus a better chemical and biological stability than pure Ti or Ti_6Al_4V. The difference in mineralization surrounding the implant can be explained by the difference in surface morphology, coated implants having a more homogenous surface than the uncoated ones [22]. SiO_2-TiO_2 coating has the potential of electric barrier able to reduce the corrosion process, thus creating best premises for osseointegration [10,23]. Several studies have demonstrated good cell adhesion on HA or SiO_2-TiO_2 laser cladded or immersed coatings, changes in surface chemistry and surface morphology that improves bioactivity and biocompatibility of titanium endosseous implants [4]. However, the majority of these studies were done “in vitro”, and had no quantification method for the newly formed bone at the interface of the implant. Our “in vivo’ study supports by numerical percentage quantification similar studies, showing the best bone apposition on coated implants, the immersion chemical protocol for coating being one of the most facile and stable.

In our immersion chemical coating protocol, the treatment can be easily distributed on the implant surface more homogenously than other options like cladding, spraying or sputtering the substance. This even distribution of an uniform HA or SiO_2-TiO_2 solution most likely is the main factor for obtaining mineralization results close to 100% on the implant surface.

SEM was another investigation done to check the microscopic behavior of the bone–implant site; the examination was performed 6 months from implantation, time when theoretically the bond implant-bone is most stable. In the uncoated group lacunae were observed in large numbers in the bone structure next to the implant, and for the coated implants no lacunae were seen. These hollow structures can be Howship lacunae – erosions caused by osteoclasts` enzymes, caused by an intense remodeling of the bone structure next to the uncoated implants [24]. Osteoclasts remodel the bone structure, so at 6 months there still is an ongoing remodeling process at the surface of uncoated implants, on the other hand the coated implants look more stable in terms of bone remodeling. The coating procedure with HA and SiO_2-TiO_2 proved to inhibit the natural osteoclast activity, seen in the control group or in physiological bone remodeling [25]. The surface structure of coated implants suffers changes of greater corrosion resistance and also in apatite formation due to the occurrence of passive oxide layer [26]. Future studies should be done to investigate the osteoblasts and osteocytes adhesion on coating materials such as HA or TiO_2, as well as the possibility to create osteoclastic inhibition [27, 28].

In the bone lacunae of the control group round particles were observed with 300X SEM magnification. These particles could be alloy parts that can migrate in the surrounding bone during implant insertion. No signs of particle detachment were seen for the coated groups, meaning that the coating material has a stabilizing role for the Ti_6Al_7Nb alloy or at least creates a better surface environment more suitable for osseointegration.

CONCLUSIONS

The immersion technique for HA and SiO_2-TiO_2 coatings proves to be an efficient, simple and reliable process to enhance osseointegration and to biologically stabilize the surface of Ti alloy implants. This procedure offers an important alternative to the currently challenging problem of osseointegration improvement.

Future "in vivo" studies should be conducted in order to analyze the cellular behavior at the surface of these alloys.

EXPERIMENTAL SECTION

In order to study the biological behavior of the surface treatment, 30 screw type implants were manufactured by SLM (figure 4), divided into 3 groups: coating free (control group), HA and SiO_2-TiO_2 coating groups. The devices were inserted into the femur of New Zealand White Rabbits, and samples containing the implants and the surrounding bone were harvested one and six months post insertion. The samples were studied by optical and scanning electron microscopy (SEM).

Implant design

The implants were manufactured from Ti_6Al_7Nb alloy powder (ATI Allvac, Monroe NC, USA) by selective laser melting technology (Realizer SLM 250 machine, Realizer GmbH, Borchen, Germany) with a controlled porosity of 24–25%, as determined through Archimede's method ISO 2738–99 (figure 4).

Earlier published results, proved that the density and porosity of samples manufactured by SLM method using a laser power of 50 W are 3.43 g cm^{-3}, 25% total porosity and 25% open porosity, while by increasing the applied laser power above 50 W, the density of the SLM manufactured samples increases and the porosity decreases. Moreover, the samples obtained with low laser power of 50 W display mainly irregular interconnected pores with a minimum diameter of 70–100 µ and a maximum diameter of 200–400 µ [29].

Screw-type shape led to perfect primary stability of the implants (mandatory condition to promote osseointegration). The devices had 10 mm length and 3.3 mm diameter. The implants were divided into three categories: control group - Ti_6Al_7Nb with no surface treatment; Ti_6Al_7Nb with HA and Ti_6Al_7Nb with SiO_2-TiO_2 coating.

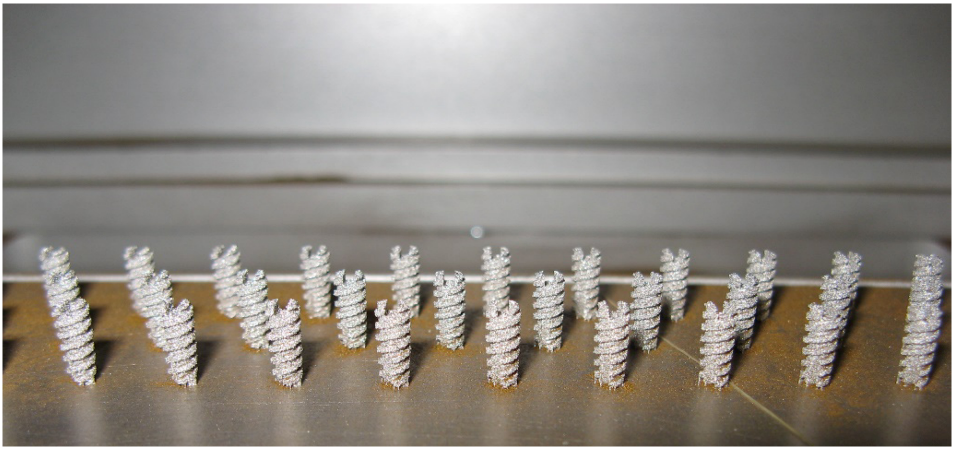
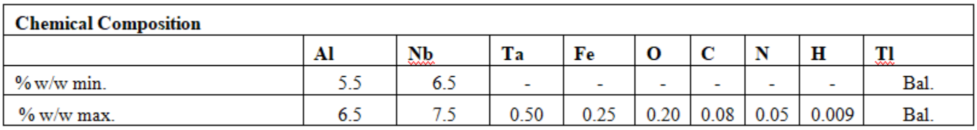


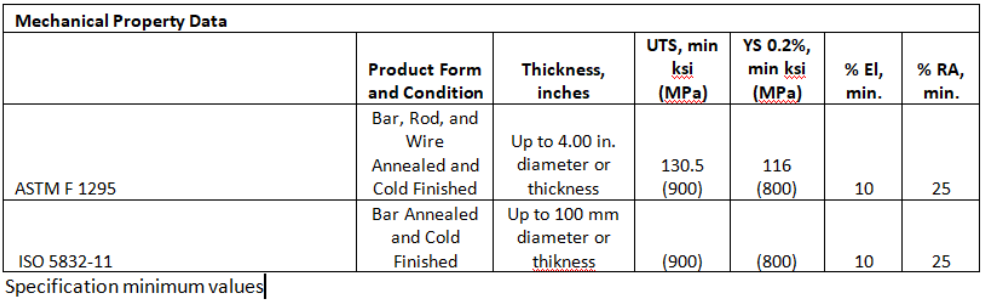
Figure 4. Final shape of Ti_6Al_7Nb implants after SLM, before the coating process

Physical properties of the alloy are: melting temperature 2,800 - 3,000°F (1,538 - 1,649°C); density 0.163 lbs/cu. in.; 4.52 gm /cc. Chemical and mechanical properties are showed in table 2 [30].

Table 2. Chemical and mechanical properties of Ti_6Al_7Nb [30].



Chemical Composition									
	Al	**Nb**	**Ta**	**Fe**	**O**	**C**	**N**	**H**	**Tl**
% w/w min.	5.5	6.5	-	-	-	-	-	-	Bal.
% w/w max.	6.5	7.5	0.50	0.25	0.20	0.08	0.05	0.009	Bal.



Mechanical Property Data						
	Product Form and Condition	**Thickness, inches**	**UTS, min ksi (MPa)**	**YS 0.2%, min ksi (MPa)**	**% El, min.**	**% RA, min.**
ASTM F 1295	Bar, Rod, and Wire Annealed and Cold Finished	Up to 4.00 in. diameter or thickness	130.5 (900)	116 (800)	10	25
ISO 5832-11	Bar Annealed and Cold Finished	Up to 100 mm diameter or thikness	(900)	(800)	10	25

Specification minimum values

Chemical coating protocol

The surface coating was done as follows:

The hydroxyapatite solution was prepared and included two types of calcium phosphate precipitates: A (pH=4.5) and B (pH=10). The precipitates were obtained by wet chemical precipitation from $Ca(NO_3)_2.4H_20$ and $(NH_4)_2HPO_4$ (Sigma Aldrich, St. Louis, Missouri, USA).

Calcium phosphate was synthesized based on a wet chemical precipitation method at room temperature using calcium nitrate tetrahydrate ($Ca(N0_3)_2\cdot 4H_20$) and diammonium hydrogen phosphate $(NH_4)_2HPO_4$). All chemicals were reagent grade (Sigma Aldrich, St. Louis, Missouri, USA) and used without further purification. Diammonium hydrogen phosphate hydrolyzed sol was added dropwise to the constantly stirred aqueous calcium nitrate solution at the molar ratio of Ca to P equal to 1.67. The mixed sol solution was then continuously stirred for about 50 minutes to obtain a white consistent sol with pH=4.5. The precipitate was taken at an aging time period of one week at room temperature. After infiltration and a suitable heat treatment, calcium phosphate turns into apatite type material.

The SiO_2-TiO_2 solution was prepared by sol-gel method from titanium isopropoxide (TIP), with the formula $Ti(OCH(CH_3)_2)_4$, and tetraethylorthosilicate (TEOS), with the formula $Si(OC_2H_5)_4$. The first stage of SiO_2-TiO_2 solution preparation included the hydrolysis of $Ti(OCH(CH_3)_2)_4$ in order to form a uniform solution. Ethanol and nitric acid were used to dilute the titanium isopropoxide to form a transparent colloid. The obtained molar ratio of $Ti(OCH(CH_3)_2)_4:C_2H_5OH:H_2O:HCl$ was 1:15:10:0.89. The second stage included TEOS hydrolysis with a molar ratio of $Si(OC_2H_5)_4:C_2H_5OH:H_2O:HCl$ = 1:7:6:25:0.28. The SiO_2-TiO_2 solution was obtained by adding the TiO_2 solution to SiO_2 solution. The final solution was left at room temperature for 30 minutes to obtain a proper homogenization.

The coating procedure was performed by immersion of the screws either into hydroxyapatite (HA) or SiO_2-TiO_2 solutions and kept in vacuum (100 mbar) for 15 minutes. After immersion the implants were placed for 30 minutes in an 100°C oven. A thermal treatment was conducted at 600°C for 30 minutes in the case of hydroxyapatite implants (figure 6 a) and at 400°C for 60 minutes for the SiO_2-TiO_2 implants (figure 6 b). The implants were sterilized using dry heat at 180°C for 2 hours.

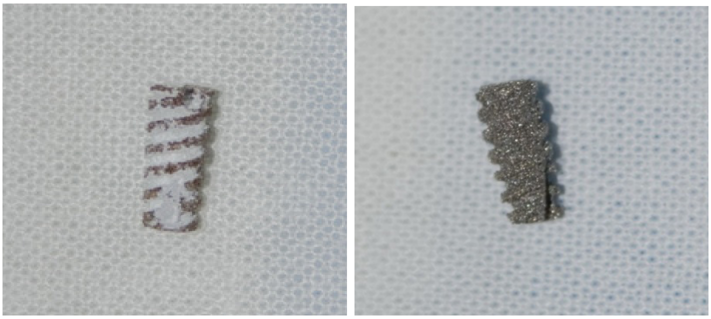


Figure 6. a) Ti_6Al_7Nb implant coated with HA; b) Ti_6Al_7Nb implant coated with SiO_2-TiO_2

Experimental design

Ten male rabbits of the New Zealand White Rabbits (Oryctolagus cuniculus) breed were used. The vivarium conditions were according to the EU Directive 63/2010. Ethics Committee approval was obtained (No. 407/03.12.2014). The control and coated implants were inserted into the femur of the specimens by low speed drilling and continuous cooling method with a torque among 25 to 30 Ncm, having perfect initial stability. Half of the specimens were sacrificed at one month and half at 6 months. The samples were prepared for optical microscopy evaluation and quantification of the quality and quantity of the bone surrounding the implants with the method described by Gheban D., Gamal M. et al., Armencea G. et al. [8, 9,31]. Scanning electron microscopy of the bone surrounding the implant was done.

ACKNOWLEDGMENTS

This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007–2013, project no. POSDRU/159/1.5/S/138776.

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