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Titanium alloys with hydroxyapatite or SiO_2+TiO_2 coatings used in bone reconstruction

G. ARMENCEA[a], C. BERCE[b], H. ROTARU[a], S. BRAN[a], V. STEFAN[c], D. LEORDEAN[d], C.-A. JULA[a], D. GHEBAN[c], M. LAZAR[a], G. BACIUT[a], M. BACIUT[a], R. S. CAMPIAN[a]
[a]*Faculty of Dental Medicine, University of Medicine and Pharmacy "Iuliu-Hatieganu", Cluj-Napoca*
[b]*Laboratory Animal Facility, University of Medicine and Pharmacy "Iuliu-Hatieganu", Cluj-Napoca*
[c]*Faculty of Medicine, University of Medicine and Pharmacy "Iuliu-Hatieganu", Cluj-Napoca*
[d]*Faculty of Mechanics, Technical University, Cluj-Napoca*

The osseointegration of Ti_6Al_7Nb implants obtained by selective laser melting, both uncoated and coated with SiO_2+TiO_2 or hydroxyapatite, has been analysed. The mineralization of uncoated implants, although relatively high after one month, decreases with time showing a minimum after three months, but recovering the starting value after 6 months. A high degree of mineralized bone for coated samples has been shown after one month, then little increasing with time. The best osseointegration was evidenced for implants coated with hydroxyapatite. The reasons for the observed behavior are discussed.

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1. Introduction

Biomaterials are widely used in the reconstruction of the craniofacial skeleton or in oral implantology. The implant consolidation after the osseointegration has been obtained by a structural and functional connection between the bone and implant surface [1]. The ideal biomaterial should be biocompatible with the surrounding tissues, stable over time and able to maintain their volume and form. An implant is considered to be osseointegrated when bone develops directly on the implant surface, without any fibrotic tissue grown at the interface [2]. The most common factor responsible for good osseointegration is the bone to implant contact [3,4].

The titanium alloys are the most used materials for implants manufacturing. The bioinert nature of titanium and some of their alloys confers good biocompatible properties and do not induce negative reactions as inflammation. The surface morphology of the implants influences the bone metabolism. A thin oxide layer covers the surface of pure titanium, at atmospheric conditions. More extensive oxide grown occurs on titanium implants subject to biological tissues [5]. It is expected that the actual interface of titanium implants to the living tissue is a hydrated titanium peroxy matrix [1]. In order to improve the osseointegration of titanium-based implants, a rough surface must to be created. An adequate porosity, similar to that of the bone, can be obtained by selective laser melting [6,7]. The rough surface increases the bone cells attachment and in addition the bone mineralization.

The implant surface roughness can be changed by coating with different materials. In addition, the coating materials influence also the osseointegration, as result of higher cellular activity [8,9]. A high value of mineralized bone was observed when titanium-based implants were coated with SiO_2 [10,11] or hydroxyapatite [12]. The titanium-based alloys covered with hydroxyapatite, after 18 weeks of implantation seems to be well tolerated by the bone [8]. Addition of growth factors did not improve the mineralization process [11].

The previous studies analysed the osseointegration for a period of time up to 18 weeks after implantation. In the present paper we extended the previous studies, by analysing the evolution of the bone mineralization on implants, for a higher period of time, by using both uncoated Ti_6Al_7Nb as well as coated implants with SiO_2+TiO_2 and hydroxyapatite (HA).

2. Materials and methods

The Ti_6Al_7Nb alloy **(**ATI Allvac, Monroe NC, USA) was used to create the sample implants, 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. In order to have a good bone contact, the implants were of screw-type shape, having 10 mm length and 3.3 mm diameter. The osseointegration has been studied by using both uncoated as well as implants coated with SiO_2+TiO_2 or hydroxyapatite (HA). The coating procedure was made by implant immersion into hydroxyapatite or SiO_2+TiO_2 solution, during 15 min. The implants were then dried at 100^0C, for 30 minutes and thermally treated. The thermal treatment was done at 600^0C for 30 min. for the implants infiltrated with hydroxyapatite and at 400^0C for 60 min. for those immersed in SiO_2+TiO_2 solution [14].

The above mentioned samples were implanted in the femur of 3 groups of rabbits, each including 6 individuals. The study was approved by the Ethical Committee of the “Iuliu Haţieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania. The femur approach was done through the muscle bodies without tempering the muscle fibers. A periosteal scraper was used to fully expose the antero-lateral part of the femur. Two cylindrical orifices were created at the proximal area of each femur under continuous cooling with saline solution, at 800 rot/min and 30 Ncm torque, by using cylindrical 10 mm long burs. In the test femur, two coated implants (with SiO_2+TiO_2 or hydroxyapatite) were introduced, while in the right femur the uncoated implant has been inserted. The implants were placed with a 30 Ncm torque having perfect initial stability. Suture in layers was performed after the implantation procedure.

After one, three and six months the rabbits were sacrificed. The samples containing the implants and the surrounding bone were immersed in 10% formalin for two days and then histological examined. Micro-CT was performed for each specimen in order to evaluate the position of the implant and the bone apposition between the threads of the implants. The rabbits had no post-op complications. No implant displacement or osteolysis around the implant threads were shown. In all cases no inflammatory reactions or fibrous tissue were noticed.

The samples were decalcified in azotic acid for 3 days, dehydrated using ethanol, immersed in xylene and then embedded in parrafin. The histological slices of 4 µm thickness were obtained with a Leica microtome cutting system. Before the histological staining, the slices have been removed of the paraffin (immersed in xylene and then in ethanol) and then hydrated in distilled water. Tricrom Masson staining was then performed, in order to have a high contrast between the mineralized bone (intense blue) and osteoid (intense red). The histological slices were then examined with a Leica microscope.

Cleaning of the artefacts (bone marrow, muscle fiber etc.) was then performed - Fig. 1. Adobe Photoshop software technique, as already described [15,16], was used for the image analysis. Panoramic image of the slice was used by merging images done at 50x magnification.

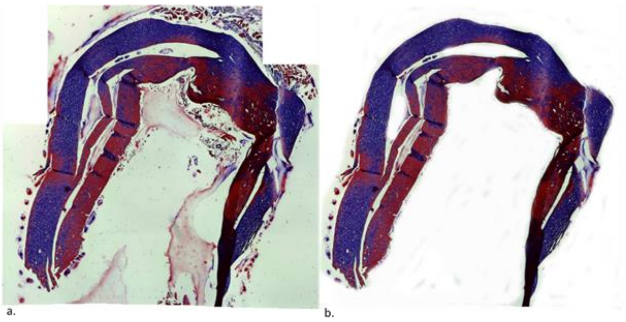

Fig. 1. Panoramic image for Ti_6Al_7Nb sample after 3 months implantation, done at 50x magnification showing mineralized bone (blue) and osteoid (red), before (a) and after (b) artefact cleaning.

The “histogram” function of the programme offers a quantification of pixels for each colour. Once the total number of pixels for each colour is known, the exact percentage for each colour can be determined, representing the percentage of bone and osteoid in the slice. Statistical analysis was performed with MedCalc Statistical Software version 15.2.1 (MedCalc Software bvba, Ostende, Belgium). Data were tested for normality of distribution using the Kolmogorov-Smirnov test. Differences between groups were calculated with one-way ANOVA test and with Tukey post-hoc test.

3. Results

The most challenging tasks, in bone reconstruction, are the osseointegration period of the implants and enhancement of the implant integration. The percentage of mineral bone formed around the three types of implants as function of time are given in Fig. 2.

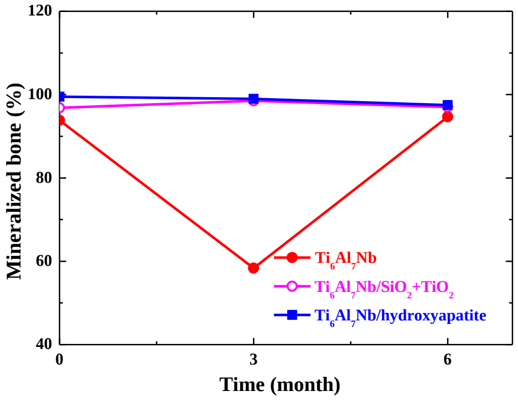

Fig. 2. The mineralized bone content as function of time for the three types of implants.

After one month, the percentage of mineralized bone is 93.83% for Ti_6Al_7Nb implant, and little higher for the coated ones, of 96.83% for those coated with SiO_2+TiO_2 and 99.50% for the hydroxyapatite coating. The histological examination evidenced, that for the uncoated Ti_6Al_7Nb, there was a continuous demineralization process from one up to three months after implantation. Then, the degree of mineralization increased, being after 6 months near the same as evidenced after one month. Thus, the uncoated Ti_6Al_7Nb implant involved a stabilization period of six months. There is a possibility of complication onset at three months, even implant displacement in the presence of stress. This fact suggests that the high porosity (24-25%) induced by laser melting method for uncoated implants is not sufficient to induce a relatively rapid mineralization.

Both the coated implants behave similarly concerning the mineralization of bone as function of time, better results being obtained when the Ti_6Al_7Nb has been coated with hydroxyapatite. As previously reported [13], there is a fast reaction of bone towards the coated implants suggesting a higher cellular activity. The above data shows that the coating material is important for osseointegration. When coating with SiO_2+TiO_2, the osteoblast adhesion is improved. When coating with hydroxyapatite there is lower ability to induce cell adhesion and proliferation, but an increased capacity to induce early mineralization. Thus, depending on local conditions of bone lesions, one of these types of implants can be used, as already suggested [11]. In the present study the implant with hydroxyapatite seems to be more efficient in enhancing the mineralization process.

Statistical analysis of the implants were also made, these showing a significant statistic correlation as described by *p* values. In case of the uncoated implants the measurements differed in time by $p<0.001$, in Ti_6Al_7Nb-SiO_2+TiO_2 by $p=0.001$ (<3 months) and 0.003 (3 to 6 months), while for Ti_6Al_7Nb-HA, values $p= 0.319$ between 1 and 3 months and $p<0.001$ for higher period of time were evidenced.

4. Discussion

Selective laser melting of titanium alloy powder is a manufacturing technology for obtaining samples with irregular shape. This technology is useful to create custom made implants used in bone defects reconstruction. One of the most challenging tasks in bone reconstruction is shortening the osseointegration period of implants and enhancing bone-implant integration, things that will provide the post-op implant mechanostability. In order to improve the osseointegration of Ti_6Al_7Nb alloy, the SiO_2+TiO_2 and hydroxyapatite as coating materials were used. The present data suggest that at least six months should be taken into consideration for a better osseointegration assessment of uncoated implants. Thus, the osseointegration must be studied for longer time, than in previously performed studies [13, 16], in order to obtain reliable data.

The implants coated with SiO_2+TiO_2 or hydroxyapatite have the potential of electrical barrier able to reduce the corrosion process. As a result, the mineralized bone content at the implant site shows a good time stability as evidenced after one, three or six months, namely: 97.00% - 98,50% - 96,83% for Ti_6Al_7Nb - SiO_2+TiO_2 and 97.50% - 99.00% - 99,50% for Ti_6Al_7Nb -HA. The osseointegration occurs as a continuous process without any kind of relapse. Implants coated with HA show better osseointegration properties than those with SiO_2+TiO_2, possibly because of a favourable electrical barrier, thus diminishing the surface corrosion process, as evidenced at 3 months. After 6 months, the two types of coated implants showed no statistical difference in terms of mineralized bone content surrounding the implant.

Although the implants with both SiO_2+TiO_2 or HA show near the same degree of mineralized bone, their use can be selected according to the types of bone lesions [11]. The use of coated implants is useful whenever local or general conditions could temper with the normal osseointegration process. The HA-coated dental implants may be valuable treatment modalities when placing implant in type IV bone, in fresh extraction sites, in grafted maxillary and/or nasal sinuses, or when using shorter implants [17].

5. Conclusions

Both the surface morphology and the nature of the coating material determine the bone metabolism. The roughness of implant surface influences the osteoblasts proliferation and fasten the osseointegration. When coating the implants, the energy surface is enhanced in particular when using hydroxyapatite. As a result, the cellular and proteic adhesion is improved and thus the osseointegration. As already suggested [3], the electrical barrier present in HA coated implants induce an early mineralization that supports their osseointegration.

All the above data suggest that bone defect reconstructions for patients with altered health status can be made by using titanium-based alloys coated with SiO_2+TiO_2 or hydroxyapatite.

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References

[1] R. Branemark, P. I. Branemark, B. Rydevik, R. R. Myers, J. Rehab. Res. Dev. **38**, 175 (2001).

[2] M. Ramazanoglu, Y. Oshida, Ilser Turkyilmaz, Eds. Osseointegration and Bioscience of Implant Surfaces Current Concepts at Bone-Implant Interface, Implant Dentistry - A Rapidly Evolving Practice. In Tech, 2011.

[3] M. S. Bryington, M. Hayashi, Y. Kozai, S. Vandeweghe, M. Andersson, A. Wennerberg, R. Jimbo, Dent. Mater. **29**, 514 (2013).

[4] J. L. Calvo-Guirado, M. Satorres-Nieto, A. Aguilar-Salvatierra, R. A. Delgado-Ruiz, J. E. Maté-Sánchez de Val, J. Gargallo-Albiol, G. Gómez-Moreno, G. E. Romanos, Clin. Oral. Investig. **19**, 509 (2015).

[5] J. E. Sundgren, P. Bodo, I. Lundstrom, J. Colloid Interf. Sci. **118**, 9 (1986).

[6] S. A. Yavari, R. Wauthle, J. Van der Stok, A. C. Riemslag, M. Janssen, M. Mulier, J. P. Kruth, J. Schrooten, H. Weinans, A. A. Zadpoor, Mater. Sci. Eng. C: Mater. Biol. Appl. **33**, 4849 (2013).

[7] H. Rotaru, R. Schumacher, S. G. Kim, C. Dinu, Maxillofac. Plast. Reconstr. Surg. **37**, 29 (2015).

[8] A. B. Novaes Jr, S. L. de Souza, R. R. de Barros,

K. K. Pereira, G. Iezzi, A. Piattelli, Braz. Dent. J. **21**, 471 (2010).
[9] T. Osathanon, K. Bespinyowong, M. Arksornnukit, H. Takahashi, P. Pavasant, J. Oral Sci. **53**, 23 (2011).
[10] K. Mustafa, A. Wennerberg, J. Wroblewski, K. Hultenby, B. S. Lopez, K. Arvidson, Clin. Oral Implants Res. **12**, 515 (2001).
[11] I. C. Brie, O. Soritau, N. Dirzu, C. Berce, A. Vulpoi, C. Popa, M. Todea, S. Simon, M. Perde-Schrepler, P. Virag, O. Barbos, G. Chereches, P. Berce, V. Cernea, J. Biol. Eng. **19**, 1754 (2014).
[12] K. J. Burg, S. Porter, J. F. Kellam, Biomaterials. **21**, 2359 (2000).
[13] T. L. Alzubaydi, S. S. Alameer, T. Ismaeel, A. Y. Alhijazi, M. Geetha. J. Mater. Sci. Mater. Med. **20**, 35 (2008).
[14] H. Rotaru, G. Armencea, D. Spîrchez, C. Berce, T. Marcu, D. Leordean, S. G. Kim, S. W. Lee, C. Dinu, G. Băciuţ, M. Băciuţ, Rom. J. Morphol. Embryol. **54**, 791 (2013).
[15] G. M. Dahab et al., J. Gastroenter. Hepat. **19**, 78 (2004).
[16] D. Gheban. J. Rom. Patologie, **8**,193 (2005).
[17] A. R. Biesbrock, M. Edgerton, A. R. Biesbrock, M. Edgerton, Int. J. Oral Maxillofac. Implants, **10**, 712 (1995).

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*Corresponding author: dr_brans@yahoo.com