Dental Research Journal

Original Article

Titanium alloys (AoN) and their involvement in osseointegration

Matteo Danza[1], Ilaria Zollino[2], Valentina Candotto[2], Francesca Cura[3], Francesco Carinci[2]

[1]Departments of Oral Science, Nano and Biotechnologies, University of Chieti, Chieti, [2]Medical-Surgical Sciences of Communication and Behavior., Section of Maxillofacial and Plastic Surgery, University of Ferrara, Ferrara, [3]Histology, Embryology and Applied Biology, University of Bologna, Bologna, Italy

Received: August 2012
Accepted: October 2012

Address for correspondence:
Prof. Francesco Carinci, Department of D.M.C.C.C., Section of Maxillofacial and Plastic Surgery, University of Ferrara, Corso Giovecca 203,44100 Ferrara, Italy.
E-mail: crc@unife.it

ABSTRACT

Background: Osseointegration is essential for a long-term successful and inflammation-free dental implant. Such a result depends on osteoblastic cells growth and differentiation at the tissue-implant interface. The aim of this study was to compare two different AoN titanium layers (GR4 and GR5) to investigate which one had a greater osteoconductive power using human osteoblasts (HOb) culture at two different time-points.

Materials and Methods: The expression levels of some bone-related (ALPL, COL1A1, COL3A1, SPP1, RUNX2, and SPARC) were analyzed using real time reverse transcription-polymerase chain reaction (real time RT-PCR).

Results: Real-time RT-PCR data showed that after 3 days of treatment with TiA4GR, the genes up-regulated were COL3A1, ALPL, SPP1, and RUNX2. Moreover, no difference in gene expression was noticed 4 days later. On the other hand, the genes that overexpressed after 3 days of treatment with AoN5GR were ALPL, SPP1, and RUNX2. In both cases, the expression of COL1A1 and SPARC was negatively regulated.

Conclusion: Our data showed that both titanium surfaces led to osteoblasts recruitment, maturation, and differentiation, thus promoting osseointegration at the tissue-implant interface.

Key Words: Gene expression, osteoinduction, titanium alloys

INTRODUCTION

Osseointegration is a widely used term in dentistry and medicine fields and refers to the narrow junction between bone and artificial implant.

A rapid and good outcome in osseointegration is a fundamental pre-requisite for a successful dental implantation and depends on the shape, structure, and composition of the used surface.[1] The main requirements for a good material are its ability to promote attraction and adhesion of bone precursor cells and their proliferation and differentiation.[2] The ability of the materials to lead osteoinduction can be evaluated by some biological parameters such as alkaline phosphatase activity and up-regulation of several bone-related genes. Several data suggest that prosthesis anchorage to bone tissue can be modulated by surface characteristics.[3-5] Having observed that smooth surfaces are less suitable to induce a similar behavior,[6] different treatments can be used to obtain surface roughness and promote osteoblast adhesion and colonization.[7,8]

Different biocompatible materials are currently employed as scaffold. Among these, titanium is considered a "gold standard" because of its biocompatibility and good corrosion resistance.[9,10]

Titanium can be characterized by several degree of purity, depending on the relative percentage of different elements as iron, aluminium, vanadium, and molybdenum. Moreover, its excellent corrosion resistance and biocompatibility, have been largely shown.[11]

Access this article online

Website: www.drj.ir

Recently, a new type of implant with a spiral form has been produced (Ultimate, AoN, Grisignano di Zocco, VI). The aim of this work was to compare two different AoN titanium layers (GR4 and GR5) and to investigate which one had a greater osteoconductive power using human osteoblasts (HOb) culture at two different time-points. The expression levels of some bone-related (ALPL, COL1A1, COL3A1, SPP1, RUNX2, and SPARC) were analyzed using real time reverse transcription-polymerase chain reaction (real time RT-PCR).

MATERIALS AND METHODS

AoN titanium implants

In this study, we used two types of AoN alloy disk (named AoN4GR and AoN5GR), with a diameter of 5 mm that differed for chemical processing. AoN4GR was treated with ortophosphoric acid and coated with calcium phosphate. AoN5GR, on the other hand, having a different purity degree underwent a double sand-blasting process (corundum and aluminium).

Primary human osteoblast cells culture

Fragments of bone derived from skull of healthy volunteers were collected during operation. The pieces were transferred in 75 cm^2 culture flasks containing DMEM (Dulbecco's Modified Eagle Medium) (Sigma Aldrich, Inc., St Louis, MO, USA) supplemented with 20% fetal calf serum, antibiotics (penicillin 100 U/ml and streptomycin 100 μg/ml; Sigma Aldrich, Inc.).

Cells were incubated in a humified atmosphere of 5% CO_2 at 37°C. The medium was changed the next day and twice a week. After 15 days, the pieces of bone tissue were removed from the culture flask. Cells were harvested after 30 days of incubation.

Cells culture

For the investigation, HOb at the second passage were seeded on two different types of AoN titanium dishes (4GR and 5GR). A set of untreated cells were used as controls. The medium was changed three times a week and the cells were maintained in a humified atmosphere of 5% CO_2 at 37°C. Cells were trypsinized and lysed for RNA extraction, after 3 and 7 days of treatment.

RNA processing and real time PCR

Reverse transcription to cDNA and gene expression quantification were performed as previously discussed.[4] In Table 1 were listed primer and probe sequences for the amplification of the bone related genes.

RESULTS

Osteoinductive properties of two different titanium disks were evaluated by measuring the gene expression levels of bone-related genes in treated HOb, at two time-points (3 and 7 days).

Real-time RT-PCR data showed that after 3 days of treatment with TiA4GR, the genes up-regulated were COL3A1, ALPL, SPP1, and RUNX2. Moreover, no difference in gene expression was noticed 4 days later. On the other hand, the genes that overexpressed after 3 days of treatment with AoN5GR were ALPL, SPP1, and RUNX2. In both cases, the expression of COL1A1 and SPARC was negatively regulated [Figure 1a and 1b].

HOb after 7 days of treatment with AoN5GR showed overexpression of COL3A1, RUNX2, SPP1, and SPARC. Instead ALPL and COL1A1 were the under-regulated genes [Figure 2a and b].

DISCUSSION

Osseointegration is essential for a long-term successful and inflammation-free dental implant [Figure 3].[12,13]

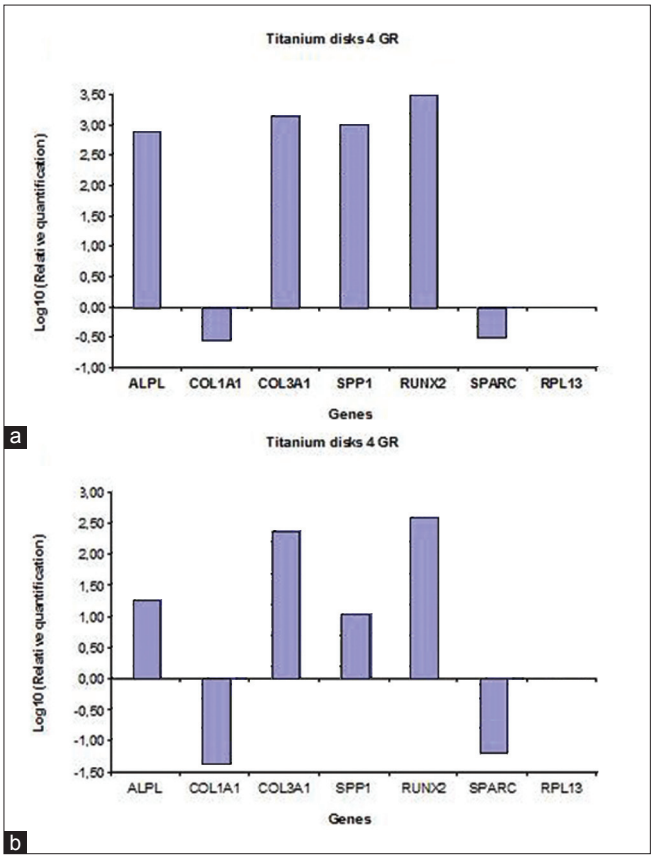

Figure 1: Human osteoblasts gene expression profile after 3 days (a) and 7 days (b) of treatment with AoN 4GR

Table 1: Primer and probes used in real time polymerase chain reaction

Gene symbol	Genename	Primersequence (5′>3′)	Probesequence (5′>3′)
SPP1	Osteopontin	F-GCCAGTTGCAGCCTTCTCA R-AAAAGCAAATCACTGCAATTCTCA	CCAAACGCCGACCAAGGAAAACTCAC
COL1A1	Collagen type I alpha1	F-TAGGGTCTAGACATGTTCAGCTTTGT R-GTGATTGGTGGGATGTCTTCGT	CCTCTTAGCGGCCACCGCCCT
RUNX2	Runt-related transcription factor 2	F-TCTACCACCCCGCTGTCTTC R-TGGCAGTGTCATCATCTGAAATG	ACTGGGCTTCCTGCCATCACCGA
ALPL	Alkaline phospatase	F-CCGTGGCAACTCTATCTTTGG R-CAGGCCCATTGCCATACAG	CCATGCTGAGTGACACAGACAAGAAGCC
COL3A1	Collagen, type III, alpha 1	F-CCCACTATTATTTTGGCACAACAG R-AACGGATCCTGAGTCACAGACA	ATGTTCCCATCTTGGTCAGTCCTATGCG
SPARC	Osteonectin	F-GCCTGATGAGACAGAGGTGG R-CTGGACAGGATTAGCTCCCA	TGGAAGAAACTGTGGCAGAGGTGA
RPL13A	Ribosomal protein L13	F-AAAGCGGATGGTGGTTCCT R-GCCCCAGATAGGCAAACTTTC	CTGCCCTCAAGGTCGTGCGTCTG

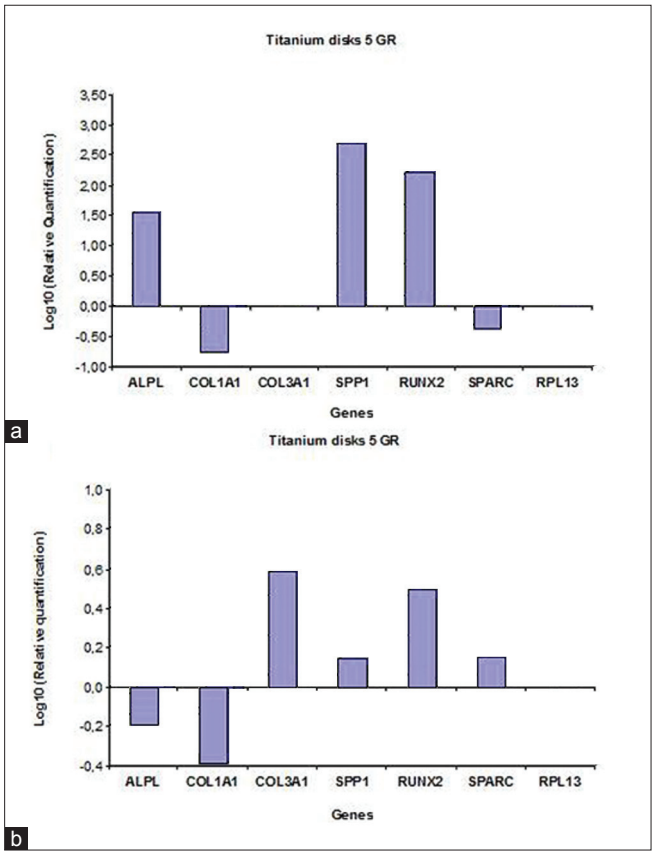

Figure 2: Human osteoblasts gene expression profile after 3 days (a) and 7 days (b) of treatment with AoN 5GR

Immediate loading implants are gold standard to improve comfort of edentulous patients.[14,15] Such a result depends on osteoblastic cells growth and differentiation at the tissue-implant interface.

The aim of this study was to compare osteoblasts behavior, cultured on two different types of AoN titanium disks. AoN4GR was treated with ortophosphoric acid and coated with calcium phosphate. AoN5GR, having a different purity degree (as consequence of having a different chemical composition), underwent a double sand-blasting process (corundum and aluminium).

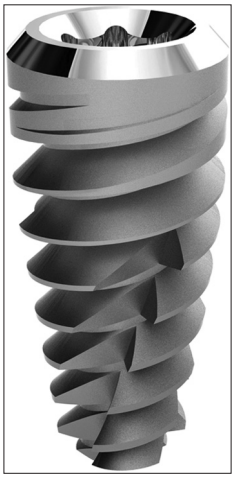

Figure 3: Dental implant

After 3 and 7 days of treatment the expression levels of bone-related genes were measured by relative quantification method using real-time RT-PCR in HOb.

AoN4GR caused up-regulation of ALPL, COL3A1, SPP1, and RUNX2 and down-regulation of COL1A1 and SPARC both after 3 days than after 7 days of treatment.

Alkaline phosphatase (ALPL) plays a critical role in bone-matrix mineralization. Its activity is required for the normal mineralization of the osteoid during bone modelling and remodelling. An increased expression of this protein can be used as marker of calcification and osteoblasts differentiation.[16]

RUNX2 is a transcription factor required at the early stage of osteoblast differentiation and it can induce

ALPL activity.[17] Moreover, it has been demonstrated that RUNX2 is involved during cell migration. Its up-regulation may be involved in pre-osteoblasts attraction and in their subsequent maturation and differentiation.

SPP1 encodes osteopontin, the most important non-collagenic component of extracellular bone matrix. Weber *et al*. demonstrated an involvement of this protein in cell adhesion and migration, suggesting the interest of this protein also, in recruitment of immature cells around the implant surface and in extracellular matrix deposition.[18]

AoN GR4 also modulates the expression of COL3A1. Type 3 collagen is a protein that gives strength and support to several body tissues.

The other type of titanium disks is AoN which caused an up-regulation of ALP, SPP1, and RUNX2 and a down-regulation of COL1A1 and SPARC on exposition to Hob after 3 days of treatment.

Expression of ALPL decreased after 7 days of exposure, whereas we registered an increased expression of SPARC and COL3A1.

Osteonectin (SPARC), a multi-functional protein, plays a double action in modulating cell-cell and cell-matrix interactions. Moreover, it has a recognized role in osteoblastic functional differentiation of bone cells.

CONCLUSION

Our data showed that both titanium surfaces led to osteoblasts recruitment, maturation, and differentiation, thus promoting osseointegration at the tissue-implant interface.

ACKNOWLEDGMENTS

This work was supported by the University of Ferrara (F.C.), Ferrara, Italy and by PRIN 2008 (20089MANHH_004).

REFERENCES

1. Tsukimura N, Kojima N, Kubo K, Att W, Takeuchi K, Kameyama Y, *et al*. The effect of superficial chemistry of titanium on osteoblastic function. J Biomed Mater Res A 2008;84:108-16.
2. Scarano A, Sinjari B, Di Iorio D, Murmura G, Carinci F, Lauritano D. Surface analysis of failed oral titanium implant after irradiated with Ercr: YSGG2780 laser. Eur J Inflamm 2012;10:49-54.
3. Dea L. Osteobiol enhances osteogenic differentiation in bone marrow derived stem cells. Eur J inflamm 2012;10:83-8.
4. Lauritano D, Carinci F, Zollino I, Hassanipour A, Saggese V, Palmieri A, *et al*. Osteoplant acts on stem cells derived from bone marrow inducing osteoblasts differentiation. Eur J Inflamm 2012;10:89-94.
5. Lauritano D, Carinci F, Zollino I, Hassanipour A, Saggese V, Palmieri A, *et al*. P15 induces RUNX2 in bone marrow derived stem cells. Eur J Inflamm 2012;10:95-100.
6. Khang D, Carpenter J, Chun YW, Pareta R, Webster TJ. Nano technology for regenerative medicine. Biomed Micro devices 2010;12:575-87.
7. Brunelli G, Carinci F, Zollino I, Candotto V, Scarano A, Lauritano D. Peri-implantitis: A case report and literature review. Eur J Inflamm 2012;10:1-6.
8. Brunelli G, Carinci F, Zollino I, Candotto V, Scarano A, Lauritano D. SEM evaluation of 10 infected implants retrived from man. Eur J Inflamm 2012;10:7-12.
9. Park JB, Lakes RS. Biomaterials: An Introduction. 2nd. ed. New York: Plenum Publishing; 1992.
10. Rolf S. The corrosion properties of titanium and titanium alloys. In: Brunette DM, Tengvall P, Textor M, Thomsen P, editors. Titanium in Medicine. Berlin: Springer-Verlag; 2001. p. 145.
11. Fathi H, Mortazavi V. Tantalum, niobium and titanium coatings for biocompa improvement of dental implants. Dent Res J 2007;4:74-82.
12. Fanali S, Carinci F, Zollino I, Brugnati C, Lauritano D. One-piece implants installedin restored mandible: A retrospective study. Eur J Inflamm 2012;10:37-41.
13. Lucchese A, Carinci F, Saggese V, Lauritano D. Immediate loading versus traditional approach in functional implantology. Eur J Inflamm 2012;10:55-8.
14. Fanali S, Carinci F, Zollino I, Brugnati C, Lauritano D. A retrospective study on 83 one-piece implants installed in resorbed maxilla. Eur J Inflamm 2012;10:55-8.
15. Scarano A, Murmura G, Carinci F, Lauritano D. Immediately loaded small diameter dental implants: Evaluation of retention, stability and comfort for the edentulous patient. Eur J Inflamm 2012;10:19-24.
16. Zernik J, Twarog K, Upholt WB. Regulation of alkaline phosphatase and alpha 2(I) procollagen synthesis during early intramembranous bone formation in the rat mandible. Differentiation 1990;44:207-15.
17. Harada H, Tagashira S, Fujiwara M, Ogawa S, Katsumata T, Yamaguchi A, *et al*. Cbfa1 isoforms exert functional differences in osteoblast differentiation. J Biol Chem 1999;274:6972-8.
18. Weber GF, Ashkar S, Glimcher MJ, Cantor H. Receptor-ligand interaction between CD44 and osteopontin (Eta-1). Science 1996;271:509-12.

How to cite this article: Danza M, Zollino I, Candotto V, Cura F, Carinci F. Titanium alloys (AoN) and their involvement in osseointegration. Dent Res J 2012;9:S207-10.

Source of Support: This work was supported by the University of Ferrara (F.C.), Ferrara, Italy and by PRIN 2008 (20089MANHH_004). **Conflict of Interest:** None declared.