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Original Article

Fibroblast behavior after titanium surfaces exposure

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ABSTRACT

Background: The main requirements for a good material are its ability to promote attraction and adhesion of bone precursor cells and their proliferation and differentiation. Different biocompatible materials are currently employed as scaffold. Among these, titanium is considered a "gold standard" because of its biocompatibility and good corrosion resistance.

Materials and Methods: The aim of this work was to compare two different AoN titanium layers (GR4 and GR5) to investigate which one had a greater osteoconductive power using human fibroblasts (HFb) culture at two different time-points. The expression levels of some adhesion and traction-resistance related genes (COL11A1, COL2A1, COL9A1, DSP, ELN, HAS1, and TFRC) were analyzed using real time reverse transcription-polymerase chain reaction.

Results: After 7 days of treatment with TiA 4GR, the only two up-regulated genes were COL2A1 and DSP. After 15 days of treatment, none of genes over expressed.

Conclusion: Our preliminary results suggest that neither AoN 4GR nor AoN 5GR are able to promote the production of protein involved in cell–cell and cell–matrix adhesion and in stress-resistance, required for a good outcome in dental implantology.

Key Words: Cell adhesion, gene expression, titanium alloys

INTRODUCTION

A rapid and good outcome osseointegration is a fundamental prerequisite 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.[2-4] The ability of the materials to reduce the space between implant and body tissues results in a successful superficial adhesion and cell proliferation. Cell recruitment, interaction, and adhesion play a crucial role in how cells react to the material to which they are attached. Fibroblasts are the soft tissues cells concerned in producing extracellular matrix and collagen. Upgrade those steps would improve vascularity at the implant surface and would decrease the risk of a bacterial infection.[5,6]

Several data suggest that prosthesis anchorage to bone and soft tissue can be modulated by surface characteristics. Having observed that smooth surfaces are less suitable to induce a similar behavior,[7] different treatments can be used to obtain surface roughness and promote fibroblast adhesion and colonization.

Different biocompatible materials are currently employed as scaffold. Among these, titanium is considered a "gold standard" because of its biocompatibility and good corrosion resistance.[8,9] With regard to this, titanium surfaces showed an excellent corrosion resistance and biocompatibility, when tested *in vitro*.[10] Titanium can be characterized by several degree of purity, depending on the relative percentage of different elements as iron, aluminum [Figure 1], vanadium, and molybdenum.[11]

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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) to investigate which one had a greater osteoconductive power using human fibroblast (HFb) culture at two different time-points. The expression levels of some adhesion and traction-resistance related genes (COL11A1, COL2A1, COL9A1, DSP, ELN, HAS1, and TFRC) were analyzed using real time reverse transcription-polymerase chain reaction (real time RT-PCR).

MATERIALS AND METHODS

AoN titanium implants

In this work we used two type of AoN alloy disk (named AoN 4GR and AoN 5GR), with a diameter of 5mm, that differed for chemical processing. AoN 4GR was treated with ortophosphoric acid and coated with calcium phosphate. AoN 5GR, on the other hand, having a different purity degree underwent a double sand-blasting process (corundum and aluminum).

Primary human fibroblast cells culture

Fragments of gingival tissue of healthy volunteers were collected during operation. The pieces were transferred in 75 cm^2 culture flasks containing DMEM (Dulbecco's Modified Eagle Medium) medium (Sigma Aldrich, Inc., St Louis, MO, USA) supplemented with 20% fetal calf serum and antibiotics (Penicillin 100 U/ml and Streptomycin 100µg/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 gingival tissue were removed from the culture flask. Cells were harvested after 30 days of incubation.

Cells culture

For the investigation, HFb 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 7 and 15 days of treatment.

RNA processing and real time PCR

Reverse transcription to cDNA was performed directly from cultured cell lysate using the TaqMAn Gene Expression Cells-to-Ct Kit (Ambion Inc., Austin, TX, USA) following manufacturer's instructions. Briefly, cultured cells were lysed with lysis buffer and RNA released in this solution. Cell lysate were reverse transcribed to cDNA using the RT Enzyme Mix and appropriate RT buffer (Ambion Inc.).Finally, the cDNA was amplified by real-time RT-PCR using Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and the specific assay designed for the investigated genes. Expression was quantified using real time RT-PCR. The gene expression levels were normalized to the expression of the house-keeping gene TFRC and were expressed as fold changes relative to the expression of the untreated HFb.Quantitation was carried out with the delta/delta calculation method.[12]

Forward and reverse primers for the selected genes were designed using primer express software (Applied Biosystems) and are listed in Table 1.

Real time RT-PCR

All PCR reactions were performed in a 20 µl volume using the ABI PRISM 7500 (Applied Biosystems). Each reaction contained 10µlof

Table 1: Primers sequences

Gene symbol	Gene name	Primer sequence (5′>3′)
COL11A1	Homo sapiens collagen, type XI, alpha 1	Fw: AGATGAGGCAAACATCGTTGA Rev: ATCAGAATCCCTGCCGTCTA
COL2A1	Homo sapiens collagen, type II, alpha 1	Fw: GCGACGACATAATCTGTGA Rev: GTCCTTTGGGTCCTACAATA
COL9A1	Homo sapiens collagen, type IX, alpha 1	Fw: GTAACAGTGAAGGGGTCGTGA Rev: TTGGCCAATCCTGATCTTTG
DSP	Homo sapiens desmoplakin	Fw: ATGACCTGAGGAGAGGACGAA Rev: AGGCTCTCTCTTTCCTGTACCAC
ELN	Homo sapiens elastin	Fw: CTAAATACGGTGCTGCTGGC Rev: CATGGGATGGGGTTACAAAG
HAS1	Homo sapiens hyaluronan synthase 1	Fw: CTCGGAGATTCGGTGGACTA Rev: CGCTGATGCAGGATACACAG
TFRC	Homo sapiens transferrin receptor protein 1	Fw: CGCTGGTCAGTTCGTGATTA Rev: GCATTCCCGAAATCTGTTGT

2 × Power SYBR® Green PCR Master Mix (Applied Biosystems, 400 nM concentration of each primer, and cDNA). All experiments were performed including non-template controls to exclude reagents contamination. PCRs were performed with two biological replicates.

RESULTS

Cell behavior was evaluated by measuring the gene expression levels of adhesion and traction-resistance related genes at two time-points (7 and 15 days). Real time RT-PCR data showed that after 7 days of treatment with TiA 4GR, the only two up-regulated genes were COL2A1 and DSP. After 15 days of treatment, no gene was overexpressed [Figure 1a and b].

Gene expression profile did not improve with AoN GR5 treatment. In fact, DSP expression was still greater than untreated cells, while COL2A1 and COL9A1were weakly up-regulated. Also in this case, all genes were under-expressed in 15 days treated cells than in untreated cells [Figure 2a and b].

DISCUSSION

A tight joint between implant and surrounding tissues is essential for a long-term successful and inflammation-free dental implant. Such a result depends on cell growth and adhesion at the tissue-implant interface. Recently, immediate loading seems to be the gold standard implant [Figure 3] used for edentulous patients to get a better immediate comfort.[13-16] The aim of this study was to compare fibroblasts behavior cultured on two different types of AoN titanium disks. AoN 4GR was treated with ortophosphoric acid and coated with calcium phosphate. AoN 5GR, having a different purity degree (as consequence of having a different chemical

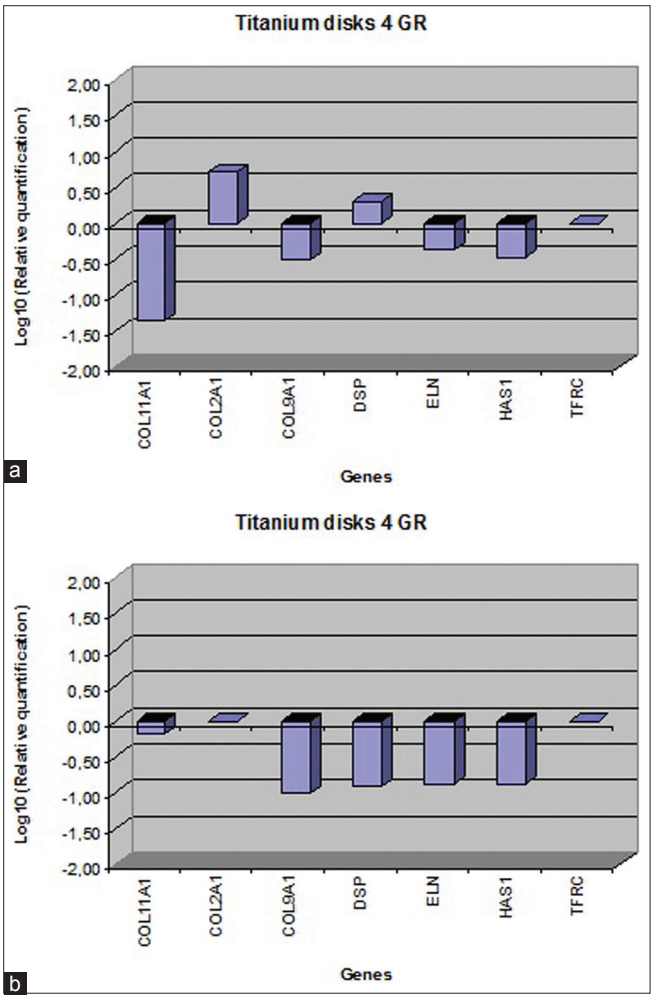

Figure 1: Human fibroblast (HFb) gene expression profile after 7 days (a) and 15 days (b) of treatment with AoN 4GR

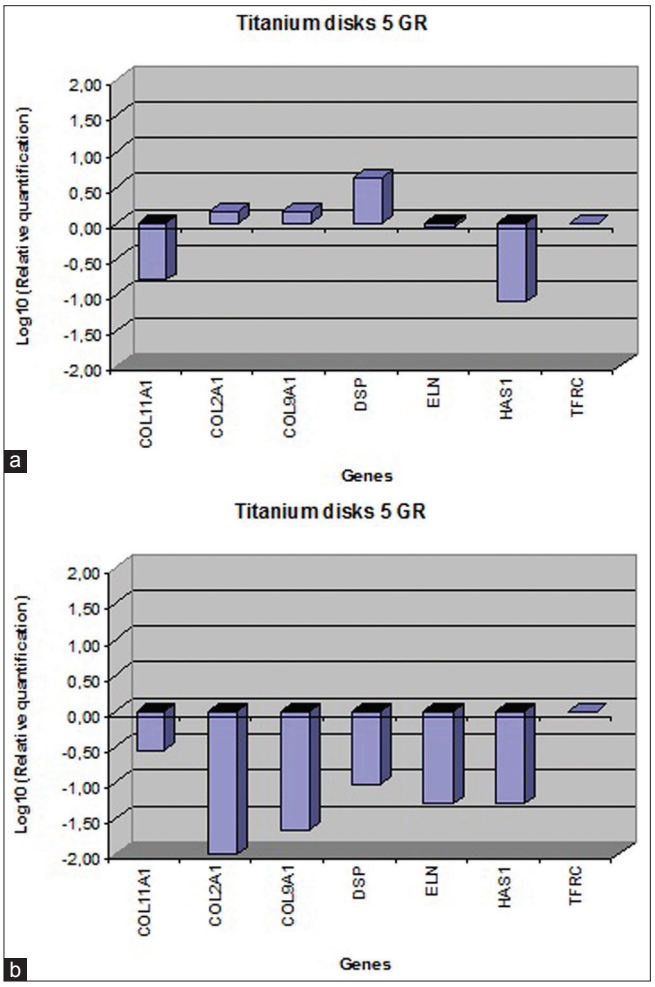

Figure 2: Human fibroblast (HFb) gene expression profile after 7 days (a) and 15 days (b) of treatment with AoN 5GR

Figure 3: Dental implant

composition), underwent a double sand-blasting process (corundum and aluminum).

After 7 and 15 days of treatment the expression levels of several genes were measured by relative quantitation method using real time RT-PCR in HFb.

After 7 days of exposure AoN 4GR caused up-regulation of COL2A1 and DSP. At the second time point (15 days) all genes resulted under-expressed.

COL2A1 gene promotes the production of the type 2 collagen, primarily found in cartilage, whose deficiency has been related to several skeletal diseases. Moreover, it is involved in conferring resistance to compressive forces.[17]

DSP encodes desmoplakine, an essential component of desmosomes that anchor intermediate filaments to desmosomal plaques. Its up-regulation suggest the involvement of this protein in cell–cell and cell–matrix adhesion.

The other type of titanium disks is AoN, which caused an up-regulation of DSP and just a weak over-expression of COL2A1 and COL9A by exposition to HFb. Their expression dropped dramatically after 15 days of exposure.

CONCLUSION

Our preliminary results suggest that neither AoN 4GR nor AoN 5GR are able to promote the production of protein involved in cell–cell and cell–matrix adhesion and in stress-resistance, required for a good outcome in dental implantology.

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