IMPLANT SUFACE ACTIVATES FIBROBLASTS: AN IN VITRO STUDY

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Titanium (Ti) is that the most generally used material for dental, orthopedic and maxillofacial purposes thanks to its excellent biocompatibility and mechanical properties. Several data suggest that prosthesis anchorage to bone and soft tissue are often modulated by surface characteristics. Fibroblasts are the soft tissues cells concerned in producing extracellular matrix and collagen and their tight connection to implant neck is of paramount importance in preventing peri-implant infection. The aim of this work is to grow Human Fibroblast (HFb) for seven days in wells containing (or not) dental implants. The expression levels of some adhesion and traction-resistance related genes (COL11A1, COL2A1, COL9A1, DSP, ELN, HAS1, and TFRC) were analyzed using Polymerase Chain Reaction. Our results demonstrated that several genes encoding for extracellular matrix proteins are activated so giving more insight to the comprehension of the mechanism of cell to surface adhesion.

Titanium (Ti) is the most widely used material in implantology for dental, orthopedic and maxillofacial purposes due to their excellent biocompatibility and mechanical properties (1-21). In implantology a considerable number of implants failed due to the unsuccessful integration of the material with the surrounding tissue. The main causes of this bad outcome are inflammation, loss of supporting bone and soft tissue recession (22-31).

Cell colonization of biomaterials is characterized by the modification of cytoskeleton followed by activation of genes related to proliferation, migration and differentiation.

The surface-design enhanced tissue attachment, fibroblast proliferation and the formation of connective tissue fibers perpendicular to the implant (24, 25), with the formation of a biological seal that prevented bacterial colonization.

Different biocompatible materials are currently employed for implants. Among these, Ti is considered a "gold standard" because of its biocompatibility and good corrosion resistance (1,2). Recently, a new type of implant (Noris Medical Ltd., Nesher, Israel) with a spiral form has been produced. The aim of this work is to verify the effect of titanium layer using Human Fibroblast (HFb) culture. The expression levels of some adhesion and traction-resistance related genes (COL11A1, COL2A1, COL9A1, DSP, ELN, HAS1, TFRC) were analyzed using Polymerase Chain Reaction.

MATERIALS AND METHODS

Titanium implants

Four implants (Tuff type, Noris Medical Ltd. Nesher, Israel) were placed in wells.

Key words: titanium, implants, fibroblast, gene, expression

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Cells Culture

For the investigation, HFb at the second passage were seeded on wells (with or without implants) The medium was changed three times a week and the cells were maintained in a humified atmosphere of 5% CO2 at 37°C. Cells were trypsinized and lysed for RNA extraction, after 7 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., Austin, TX, USA).

Finally, the cDNA was amplified by real-time 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 PCR. The gene expression levels were normalized to the expression of the housekeeping gene TFRC and were expressed as fold changes relative to the expression of the untreated HFb.

Forward and reverse primers for the selected genes were designed using primer express software (Applied Biosystems, Foster City, CA, USA) and are listed in Table I.

Table I. *Primers sequences.*

Gene symbol	Primer sequence (5'>3')
COL11A1	Fw AGATGAGGCAAACATCGTTGA Rev ATCAGAATCCCTGCCGTCTA
COL2A1	Fw GCGACGACATAATCTGTGA Rev GTCCTTTGGGTCCTACAATA
COL9A1	Fw GTAACAGTGAAGGGGTCGTGA Rev TTGGCCAATCCTGATCTTTG
DSP	Fw ATGACCTGAGGAGAGGACGAA RevAGGCTCTCTCTTTCCTGTACCAC
ELN	Fw CTAAATACGGTGCTGCTGGC Rev CATGGGATGGGGTTACAAAG
HAS1	Fw CTCGGAGATTCGGTGGACTA Rev CGCTGATGCAGGATACACAG
TFRC	Fw CGCTGGTCAGTTCGTGATTA Rev GCATTCCCGAAATCTGTTGT

Real Time PCR

All PCR reactions were performed in a 20 µl volume using the ABI PRISM 7500 (Applied Biosystems, Foster City, CA, USA). Each reaction contained 10 µl 2X Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 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

Activation of extracellular matrix proteins were evaluated by measuring the gene expression levels after 7 days of treatment. Empty well was the control surface to compare the gene expression of implant surface. Fig. 1 showed the differentially expressed genes in the implants respect to the control surface.

DISCUSSION

A close joint between implant and surrounding tissues is essential for the maintenance and success of a dental implant in longevity. This result depends on the growth and adhesion of the cells of the periodontium to the tissue-implant interface.

The aim of this study was to compare fibroblasts behavior cultured in wells containing dental implants compared with empty wells.

After 7 days of treatment the expression levels of genes COL2A1, COL9A1, COL11A1, ELN, DSP and HAS1 were measured by relative quantification method using PCR. COL2A1, COL9A1 and COL11A1 are involved in conferring resistance to compressive forces (32). After 7 days of treatment we observed the up-regulation of all three collagens in fibroblast cultivated respect to control.

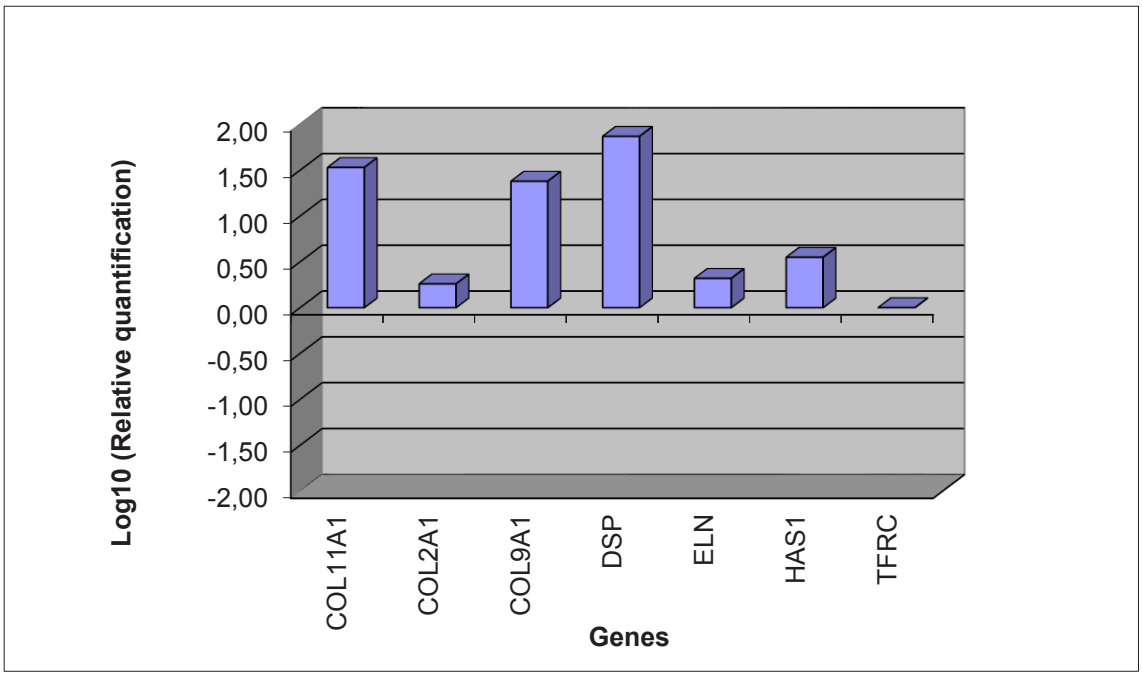

Fig. 1. *Gene expression in HFb*.

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

ELN gene encodes for elastin, the major component of the amorphous phase in elastic fibers of human gingival. Elastic fibers are the extracellular matrix structures responsible for the properties of resilience and elastic recoil in all elastic tissues (33, 34). This elastic tissue that surrounds the dental implant neck, named peri-implant, is critical for a successful implant osseointegration as it forms a biological seal at the gingival site.

Another gene up-regulated was HAS1, the hyaluron synthase. This gene is essential in the synthesis of high molecular-weight hyaluronic acid. Hyaluronic acid is a major glycosaminoglycan in the periodontal ligament and has important roles in cell adhesion, migration and differentiation mediated by various hyaluronon-binding proteins and cell-surface receptors (34).

Our preliminary results demonstrate that implant surface (after 7 day of fibroblast culturing) promotes 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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