BIO GRIP AND MACHINED TITANIIUM STIMULATE DENTAL PULP STEM CELLS TOWARDS OSTEOBLASTIC DIFFERENTIATION

S. FANALI[1], F. CARINCI[2], A. GIRARDI[3], A. PALMIERI[2], G. BRUNELLI[4], R. MONGUZZI[2]

[1]Department of Oral Science, Nano and Biotechnology, University "G. D'Annunzio", Chieti, Italy
[2]Department of D.M.C.C.C., Section of Maxillofacial and Plastic Surgery, University of Ferrara, Ferrara, Italy
[3]Department of Histology, Embryology and Applied Biology, Centre of Molecular Genetics, CARISBO Foundation, University of Bologna, Bologna, Italy
[4]Department of Dentistry and Maxillofacial Surgery, Don Orione Institute, Bergamo, Italy

Biomaterials have been utilized routinely during maxillofacial, craniofacial, and orthopaedic reconstructive surgical procedures. To investigate which one between BIO GRIP and machined titanium disk promote a greater osteoblast differentiation and proliferation, the expression levels of bone related genes (RUNX2, SP7, ALPL, SPP1, COL1A1, COL3A1 and FOSL1) and mesenchymal stem cells marker (ENG) were measured in dental pulp stem cells (DPSCs) after 15 and 30 days of treatment using real time Reverse Transcription-Polymerase Chain Reaction. Significantly differentially expressed genes among Bio Grip treated and untreated cells were ENG, FOSL1, RUNX2, COL3A1, BGLAP and SPP1 in the first 15 days of treatment and ENG, FOSL1, RUNX2,COL3A1 and BGLAP after 30 days. Conversely, all genes were differentially expressed among treated and untreated Machined Titanium DPSCs after 15 days. At the end of the exposure, SP7, COL3A1, COL1A1 and ALPL were the only gene differentially expressed. The present study demonstrated both biomaterials, are able to induce bone formation by influencing the expression pattern of gene involved in osteogenesis, extracellular matrix deposition and mineralization.

Advances in bone grafting are progressing with the evolution of biomaterials that permit the incorporation of osteoinductive and osteogenic proteins into osteoconductive composite scaffolds.

BIO GRIP is a SLA ("Sand-blasting and acid etching") treated surface, produced using a large-grit sandblasting technique which gives the surface a macro roughness. In-vitro studies show increased cell activities of SLA surfaces (1, 2) favoring a greater bone-implant contact at a shorter time.

Titanium and its alloys are employed as implant materials because of their biocompatibility and their desirable mechanical properties (3).

To investigate which one between BIO GRIP and machined titanium disk promote a greater osteoblast differentiation and proliferation, the quantitative expression of the mRNA of specific bone related genes, were examined in derived dental pulp stem cells treated with BIO GRIP and Machined Titanium surfaces.

Dental pulp represents an ideal source of stem cells because approachable niches containing a high number of stem cells compared to equal volumes of bone marrow (4-6).

In this study the expression levels of specific genes were examined by means of real time RT-PCR in DPSCs after treatment with BIO GRIP and machined titanium.

MATERIALS AND METHODS

Stem cells isolation from dental pulp and *flow cytometric analyses* were conducted as previously described (7).

Cell treatment

DPSCs were seeded on BIO GRIP and machined titanium disk contained in a multiweel. Another set of wells containing

Key Words: dental pulp stem cells, machined titanium, Bio Grip, osteoblast differentiation

Corresponding author: Prof. Francesco Carinci, M.D
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 Web: www.carinci.org
Phone: +39.0532.455874
Fax: +39.0532.455582

0393-974X (2011)

untreated cells were used as control.

The cells were maintained in a humidified atmosphere of 5% CO2 at 37°C.

Cells were harvested at two time points, 15 and 30 days, for RNA extraction.

RNA processing and *RT-PCR* were performed as previously described (7).

Statistical analyses

Comparison of the gene expression between DPSCs and NO was performed with "Two tailed ANOVA "statistic analyses using Excel spreadsheets (Microsoft Office 2003).

RESULTS

Cell cultures were phenotipically characterized by flow cytometric analyses as previously reported (7).

To investigate which one of the two biomaterials stimulate a greater osteoblasts differentiation and proliferation in DPSCs, several osteoblast genes and mesenchymal stem cells marker, were analyzed by quantitative real-time PCR after 15 and 30 days of treatment with both disk.

After 15 days of exposure to Bio Grip and Machined Titanium, DPSCc showed a similar pattern of gene expression, with both but one genes up-regulated. The only one gene down-regulated was SP7 (Fig. 1a and 1b).

After 30 days of treatment with Bio Grip, we noticed that the genes up-regulated were SP7, ENG, COL3A1, ALPL and RUNX2, while the down-regulated were FOSL1, COL1A1 and BGLAP. SPP1 was no more expressed (Fig. 2a).

In DPSCs treated with Machined Titanium for 30 days, all the genes resulted up-regulated (Fig. 2b)

Comparing by "Two tailed ANOVA" the relative expression of analyzed genes between BIO GRIP treated and untreated DPSCs we observed that significally differentially expressed genes after 15 days of treatment were ENG, FOSL1, RUNX2, COL3A1, BGLAP and SPP1 (Tab.I).

After 30 days of exposure to Bio Grip, we noticed that ENG, FOSL1, RUNX2, COL3A1 and BGLAP genes were differentially expressed among treated and untreated DPSCs (Tab. II).

The same analysis between Machined Titanium treated and untreated DPSCs showed that all genes were differentially expressed, after 15 days of treatment (Tab. III). After 30 days of exposure, the variation in gene expression was significant for SP7, COL3A1, COL1A1 and ALPL (Tab. IV).

DISCUSSION

In the past thirty years, a number of biomaterials have shown the ability to induce bone formation when implanted at heterotopic sites, an ability known as osteoinduction.

The SLA ("Sand-blasting and acid etching") surface treatment has as goal to control the surface roughness, to improve osseointegration and to increase the stability of bone-anchored dental implants. This treatment consists of bombarding the surface with a jet of Al2O3 particles followed by an acid etching.

Titanium is a very good material for this kind of implant due to its biocompatibility and has been

Table I. *Differentially expressed genes between Bio Grip treated and untreated DPSCs after 15 days of treatment*

Genes	Bio Grip (15 gg)	Differentially expressed genes
	Log10 RQ	**p<0,005**
SP7	-0,09	0,1543
ENG	1,10	**0,0018**
FOSL1	1,28	**0,0003**
RUNX2	0,37	**0,0128**
COL3A1	0,46	**0,0033**
COL1A1	0,06	0,8749
ALPL	0,28	0,2003
BGLAP	0,56	**0,0039**
SPP1	1,16	**0,0077**

Table II. *Differentially expressed genes between Bio Grip treated and untreated DPSCs after 30 days of treatment*

Genes	Bio Grip (30 gg)	Differentially expressed genes
	Log10 RQ	**p<0,005**
SP7	0,327	0,3354
ENG	0,71	**0,0018**
FOSL1	-0,40	**0,0093**
RUNX2	0,26	**0,0274**
COL3A1	0,09	**0,0105**
COL1A1	-0,67	0,0681
ALPL	0,12	0,1778
BGLAP	-1,02	**0,0082**
SPP1		

Table III. *Differentially expressed genes between Machined Titanium treated and untreated DPSCs after 15 days of treatment*

Genes	Machined Titanium (15 gg)	Differentially expressed genes
	Log10 RQ	**p<0,005**
SP7	-0,06	**0,0032**
ENG	0,80	**0,0001**
FOSL1	0,92	**0,0021**
RUNX2	0,47	**0,0046**
COL3A1	0,47	**0,0143**
COL1A1	0,22	**0,0052**
ALPL	0,26	**0,0544**
BGLAP	0,85	**0,0911**
SPP1	0,45	**0,0394**

Table IV. *Differentially expressed genes between Machined Titanium treated and untreated DPSCs after 30 days of treatment.*

Genes	Machined Titanium (30gg)	Differentially expressed genes
	Log10 RQ	**p<0,005**
SP7	0,26	**0,0173**
ENG	0,65	0,1284
FOSL1	0,31	0,3756
RUNX2	0,39	0,2688
COL3A1	0,65	**0,0950**
COL1A1	0,68	**0,0839**
ALPL	0,50	**0,0943**
BGLAP	0,68	0,1116
SPP1	0,62	0,1503

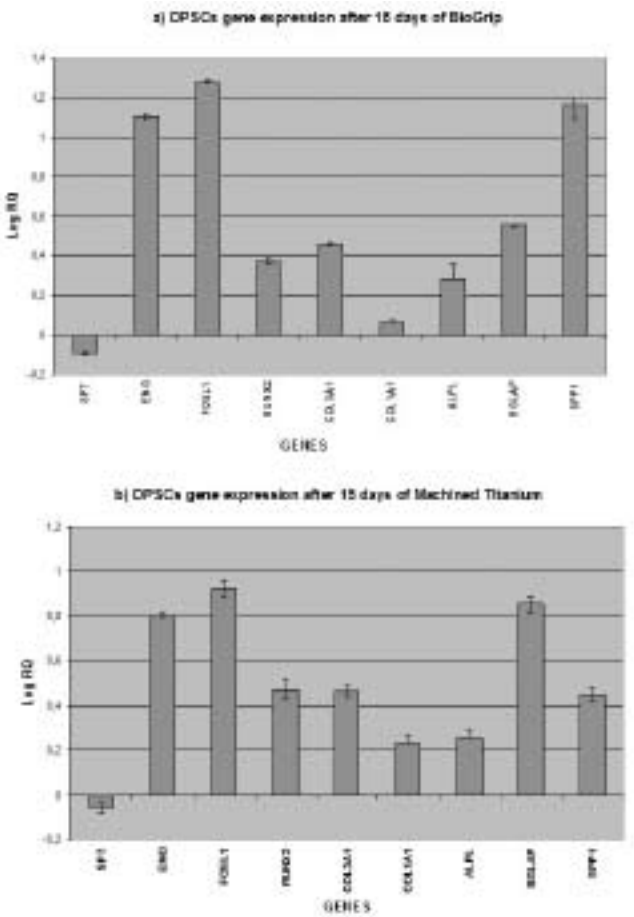

Fig.1. *Gene expression in DPSCs treated with BioGrip for 15 days (a) and with Machined Titanium for 15 days (b).*

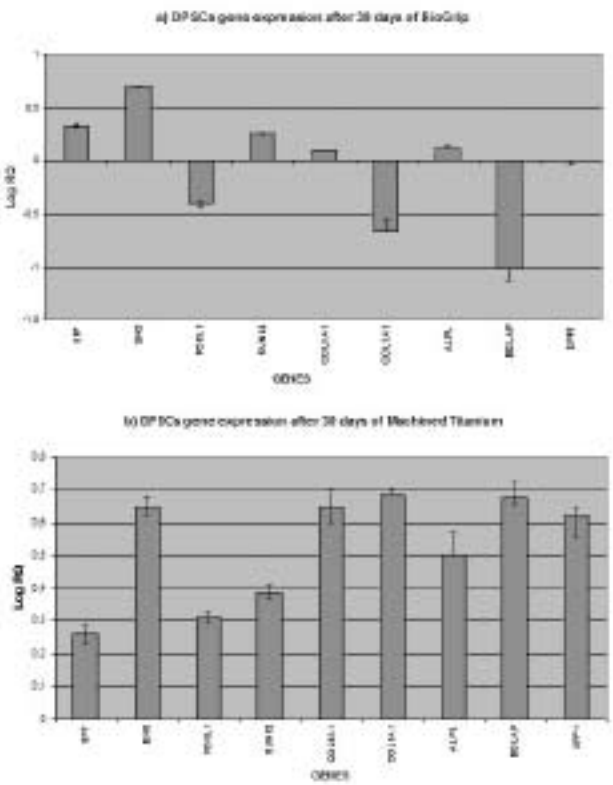

Fig.2. *Gene expression in DPSCs treated with BioGrip for 30 days (a) and with Machined Titanium for 30 days (b).*

demonstrated to be good for surgical increases implant osteointegration (8).

In order to compare the osteoinductive potential of Bio Grip SLA and machined titanium on DPSCs, changes in expression of bone related marker genes (RUNX2, SPP1, SP7, COLIA1, COL3A1, ALPL and FOSL1) and mesenchymal stem cells marker (ENG) were investigated by real-time RT–PCR.

After isolation by enzymatic digestion, DSCP were phenotipically characterized by flow cytometric analyses. Dental pulp derived cell were homogenously $CD105^{+}$, $CD90^{+}$, $CD34^{-}$, $CD45^{-}$, $CD14^{-}$, which is a typical mesenchymal stem cells surface antigen profile (7)

"Two tailed ANOVA" showed that the significantly differentially expressed genes among the Bio Grip treated and untreated DPSC were ENG, FOSL1, RUNX2, COL3A1 SPP1 and BGLAP after first 15 days of treatment. After 30 days of treatment, ENG, FOSL1, RUNX2, COL3A1, BGLAP and SPP1 were differentially expressed between treated cells and the control.

The same statistical analysis conducted among treated machinery titanium and untreated DPSC showed that all genes were differentially expressed after 15 of treatment. After 30 days, Machined Titanium caused a variation in SP7, COL3A1, COL1A1 and ALPL gene expression.

Both Bio Grip and Machined Titanium caused an up-regulation of RUNX2 for the entire treatment.

RUNX2 is the most specific osteoblast transcription factor, activated in the first stage of differentiation. It is a prerequisite for osteoblast differentiation and osteogenesis by binding to the promoter region of major osteoblast gene as ostecalcin and osteopontin. Its increase, in BioGrip treated DPSCs, is significant for the entire treatment, but not in Machined Titanium treated DPSCs.

Another transcriptional factor, SP7, that regulates bone formation and osteoblast differentiation and that is downstream of RUNX2 (9) was differentially expressed between ADSCs and NO.

SP7 (also named osterix) was down-regulated during the first 15 days of treatment in both the treatments but significally increased at 30 days of culture with both biomaterials.

SP7 is another important gene involved in osteoblast differentiation (10), and its overexpression guides DPSCs toward the osteoblastic lineage (11).

Bio Grip also modulates the expression of genes encoding for collagenic extracellular matrix proteins like collagen type 1α1 (COL1A1) and collagen type 3α1 (COL3A1). In fact, Bio Grip caused an up-regulation of COL3A1 during the first 15 days of treatment and a down-regulation of COL1A1 after 30 days of treatment, whilst Machined Titanium lead to an over-expression of both genes, for the entire treatment.

Another gene involved in osteoblast differentiation and modulated by Bio Grip was FOSL1. This gene was up regulated DPSCs during the first 15 days of Bio Grip exposure, but its expression was down-regulated after 30 days of treatment. Its expression increased during the first period of treatment probably because it is important in the first stage of mineralization.

ALPL is involved in bone mineralization and is over expressed for the entire treatment, with both biomaterials, but only in DPSC treated with Machined Titanium, this increased is significant.

SPP1 is significantly over-expressed in both cellular populations, for the first period of exposure to the biomaterials.

SPP1 is actively involved in bone resorbitive processes directly by ostoclasts. Osteopontin produced by osteoblasts, show high affinity to the molecules of hydroxylapatite in extracellular matrix and it is chemo-attractant to osteoclasts.

Seen that Runx2 induces the differentiation into preosteoblast, SP7 ensures their development into mature osteoblast, ALPL promotes matrix deposition and SPP1 is the most representative non collagenic component of extracellular bone matrix, we demonstrated that BioGrip and Machined Titanium have a similar biological effect on DPSCs. Both biomaterial, are able to induce bone formation by influencing the expression pattern of gene involved in osteogenesis, extracellular matrix deposition and mineralization. For these reasons, both can be employed as scaffold in regenerative medicine.

ACKNOWLEDGMENTS

This work was supported by FAR from the University of Ferrara (FC), Ferrara, Italy and by Don Orione Service s.r.l., Bergamo, Italy.

REFERENCES

1. Kieswetter K, Schwartz Z, Hummert TW, Cochran DL, Simpson J, Dean DD, Boyan BD. Surface roughness modulates the local production of growth factors and cytokines by osteoblast-like MG-63 cells. J Biomed Mater Res 1996; 32:55-63.
2. Martin JY, Schwartz Z, Hummert TW, Schraub DM, Simpson J, Lankford J, Jr., Dean DD, Cochran DL, Boyan BD. Effect of titanium surface roughness on proliferation, differentiation, and protein synthesis of human osteoblast-like cells (MG63). J Biomed Mater Res 1995; 29:389-401.
3. Brunette DM, Tengvall P, Textor M, Thomsen P. Titanium in medicine: material science, surface science, engineering, biological responde and medical application.

Berlin Springer; 2001.

4. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A 2000; 97:13625-30.
5. Srisawasdi S, Pavasant P. Different roles of dexamethasone on transforming growth factor-beta1-induced fibronectin and nerve growth factor expression in dental pulp cells. J Endod 2007; 33:1057-60.
6. Otaki S, Ueshima S, Shiraishi K, Sugiyama K, Hamada S, Yorimoto M, Matsuo O. Mesenchymal progenitor cells in adult human dental pulp and their ability to form bone when transplanted into immunocompromised mice. Cell Biol Int 2007; 31:1191-7.
7. Sollazzo V, Fanali S, Masiero E, Girardi A, Farinella F, Melloni L, Pezzetti FCI, Zauli G, Carinci F. Peptide-15 stimulates pupl stem cells towards osteoblasts differentiation. European Journal of Inflammation 2011; 9(Suppl. 1):125-30.
8. Szmukler-Moncler S, Testori T, Bernard JP. Etched implants: a comparative surface analysis of four implant systems. J Biomed Mater Res B Appl Biomater 2004; 69: 46-57.
9. Nishio Y, Dong Y, Paris M, O'Keefe RJ, Schwarz EM, Drissi H. Runx2-mediated regulation of the zinc finger Osterix/Sp7 gene. Gene 2006; 372:62-70.
10. Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR, de Crombrugghe B. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell 2002; 108:17-29.
11. Tai G, Polak JM, Bishop AE, Christodoulou I, Buttery LD. Differentiation of osteoblasts from murine embryonic stem cells by overexpression of the transcriptional factor osterix. Tissue Eng 2004; 10:1456-66.