Dental Implants Osteogenic Properties Evaluated by cDNA Microarrays

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Objective: *This study aims at applying cDNA microarray analysis* in vitro *for establishing and comparing the osteogenic properties of dental implants with different surface characteristics.*

Materials and Methods: *Saos-2 osteoblasts were cultured in bottom-cone tubes in presence of 5 different dental implants with various surface characteristics. Cells adherent to dental implants were detached and RNA purified. The expression of 18,401 genes was tested by cDNA microarray.*

Results: *The number and viability of cells adherent to different dental implants varied but without any significant statistical difference. Conversely, gene expression was revealed to be a more sensitive biomarker being remarkably different in cells adherent to different implants. The 5 dental implants significantly modulated the expression of 14 osteogenic activities mainly including bone morphogenetic proteins, osteomodulin, and osteoprotegerin.*

Conclusion: *Despite no significant differences having been found in in vitro cell number and viability, cells adherent to 5 differently surfaced implants showed different gene expression profiles. Thus, to evaluate osteogenesis as related to dental implants, it is important to analyze not only the number of adherent cells but also the activation of genes encoding for osteogenic activities. (Implant Dent 2011;20:299–305)*

Key Words: titanium implant, gene expression, cDNA microarray, osteogenesis

Several studies have described the influence of a titanium implant's surface on peri-implant tissues behavior,[1–3] especially in the first phases of healing.[4] In fact, cell migration, adhesion, spreading, proliferation, and differentiation, which are key points to achieve osseointegration,[5] may be influenced by implant surface characteristics.[6] Surface roughness seems to influence cell behavior. In particular, it has been reported that osteoblast-like cells show a tendency to attach more quickly to surfaces with a rougher microtopography.[7] Moreover, osteoblast-like cells seem to be more differentiated on rougher surfaces.[8]

Titanium surfacing also seems to have a direct genetic effect on osteoblasts and to induce several bone-related genes.[9] It has been demonstrated that rough surfaces (average roughness [Ra], 3–4 μm) were superior to smooth surfaces (Ra, 0.2 μm) in promoting osteogenic induction of the human osteosarcoma cell line MG63, as determined by observing increased production of osteocalcin, osteoprotegerin, and TGF-β.[10]

Recent studies have supposed that not only microtopography but also nanotopography can influence osteoblast adhesion and differentiation and that nanostructuration can improve osteoprogenitor cell differentiation toward an osteoblastic phenotype.[11–15]

However, when comparing different surface treatments and their biologic effects at the bone-implant interface, it is not possible to implicitly assume that surface topography is the only variable involved. In fact, the treatment used to obtain a given surface topography also affects the surface chemistry of the implants. As a consequence, the effects related to surface chemistry can operate over and above surface topography.[16,17]

Surface chemical composition, hydrophilicity, and roughness are parameters that may play a role in implant-tissue interaction and osseointegration. It is not clear, however, which characteristics of the surface are involved in different cellular responses. Accordingly, the *in vitro* comparative evaluation of osteogenic properties of titanium implants still remains a challenge.

Microarray methods provide a formidable tool to comparatively analyze the effect of titanium implants on

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ISSN 1056-6163/11/02004-299
Implant Dentistry
Volume 20 • Number 4
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DOI: 10.1097/ID.0b013e318225f22b

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osteoblasts providing a great mass of mechanistic data; thus, basing the results of the comparative analysis on ten thousands of gene expression pathways and not only on a few selected activities. The aim of this study is to evaluate the *in vitro* genetic expression of osteoblasts grown in contact with different surfaced implants.

MATERIALS AND METHODS

Cells

Saos-2 osteoblasts were obtained from the Cell bank, National Institute for Research on Cancer, Genoa, Italy. Cells were cultured in a monolayer Dulbecco's Modified Eagle Medium supplemented with 10% (v/v) fetal calf serum, 2 mM L-Glutamine, 100 IU mL^{-1} penicillin, and 100 mg mL^{-1} streptomycin. Cells were grown in 25 cm^2 culture flasks in a humidified 5% CO_2 atmosphere at 37°C and supplied with fresh culture medium every 48 hours. When an 80 to 90% of confluence was reached, the monolayer were subcultured and used for experiments by means of 0.25% trypsin—1 mM ethylenediaminetetraacetic acid.

The cells were seeded in bottom-cone glass tubes containing the dental implant (6×10^5 cells/tubes). Cells were incubated in 2 mL of complete medium. After 72 hours, the medium was removed, and cells nonadherent to the dental implant were gently washed away with phosphate-buffered saline washing. Cells adherent to the dental implant were detached by trypsin, and collected cells were counted and subsequently used for gene expression analysis by cDNA microarray. In fact, under this condition, cell growth was maximized, as controlled in preliminary experiments.

Dental Implants

Five different commercial screw-shaped dental implants with different surface modifications were investigated in this study:

Tapered Internal (BioHorizons Implant Systems, Inc., Birmingham, AL) and Resorbable Blast Texturing with Laser-Lok collar; (Ra, not available).

Nanotite (3i Implant Innovations, Inc., Palm Beach Gardens, FL), osseotite surface combined with a discrete crystalline deposition of nanometer scale calcium phosphate; (Ra, 0.28 ± 0.06 μm).[18]

Full Osseotite (FOSS; 3i Implant Innovations, Inc.), osseotite surface: dual acid-etched with HF and then HCl/H_2SO_4; (Ra, 0.86 ± 0.14 μm).[19]

Straumann SLActive Standard Implant (Institut Straumann AG, Basel, Switzerland), sandblasted with large grits (0.25–0.50 mm), acid-etched with HCl/H_2SO_4, rinsed under N_2 protection, and preserved in isotonic NaCl solution; (Ra, 2.93 ± 0.46 μm).[19]

SwissPlus (Zimmer Dental, Inc., Carlsbad, CA), grit-blasted with hydroxylapatite (Microtextured Titanium, MTX). As referring control, cells grown in absence of dental implant were used (1–2 μm, as provided by the manufacturer).

Quantification of Cellular Attachment to Dental Implants

Attachment of Saos-2 cells to dental implant was quantified by using the 3-[4,5-dimethylthiazol-2yl]-2,5 diphenyl tetrazolium bromide (MTT; Sigma–Aldrich) at 72 hours of cell growth. MTT test evaluate cell viability as related to the mitochondrial capacity to reduce MTT salts. Two implants for each type were used.

Briefly, the 6 samples of cells were resuspended in Dulbecco's Modified Eagle Medium added with MTT (5 mg mL^{-1}) and incubated for 4 hours at 37°C in 5% CO_2. Samples were then centrifuged at 152*g* for 10 minutes, medium discarded, and the solubilization solution (HEPES 50 mM/ethanol 1:9, pH 8) added. MTT reduction was detected by quantifying its reduced derivative by evaluating absorbance at 590 nm using a fiber optic spectrophotometer (Nanodrop-ND1000; NanoDrop products, Wilmington, DE). MTT results are expressed as percentage activity when compared with control samples and standardized taking into account cell number.

Gene Expression Analysis by cDNA Microarray

The expression of 18,401 human genes was tested in cell attached to den-

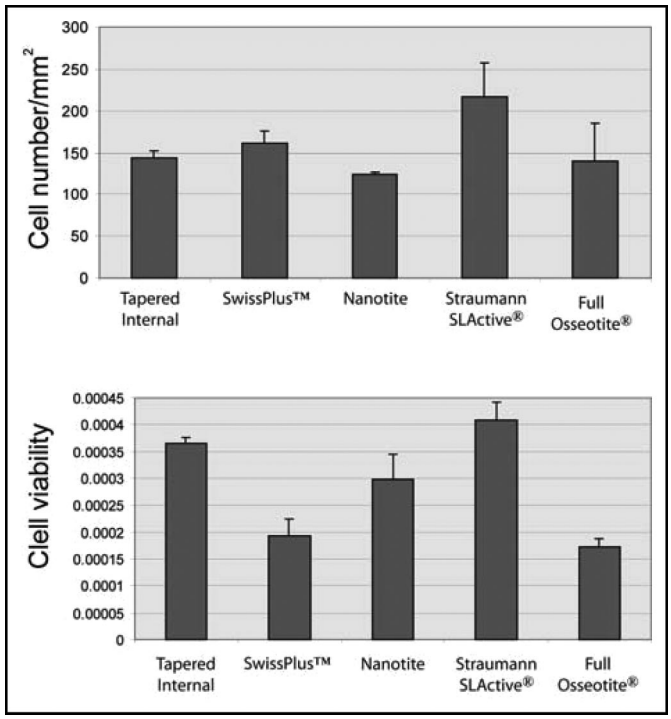

Fig. 1. Number of cells adherent to each type of dental implant as evaluated after trypsin detachment (upper panel). Cell viability of implant adherent cells was evaluated by MTT test (lower panel).

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tal implants by cDNA microarray. Custom microarray as made available from the Microarray Department, University of Amsterdam, was used. The whole list of spotted genes is available at the website http://www.micro-array.nl/libraries.html. Extracted RNA underwent reverse transcription and preplateau amplification using quantitative real-time PCR before probe synthesis for array hybridization as previously reported.[20] Hybridized microarrays were then analyzed in a laser scanner acquiring signal from each spot (ScanArray; PerkinElmer, Waltham, MA).

Data analysis was performed subtracting for each microarray the local spot background from raw spot intensity, log transformation, normalization per chip, and per array (GeneSpring software version 7.2; Agilent Technologies, Santa Clara, CA). Data generated for each mRNA were compared among the various experimental groups by volcano-plot analysis taking into account as thresholds of 2-fold variation and statistical significance $P < 0.05$ as evaluated by analysis of variance (ANOVA) after Bonferroni multiple testing correction. Global mRNA expression profiles were compared by hierarchical cluster analysis (HC) and bidimensional principal component ANOVA (PCA). Forty-nine genes specifically involved in osteogenesis were identified by analyzing gene ontology using the GeneSpider GeneSpring function referring to UniGene database.

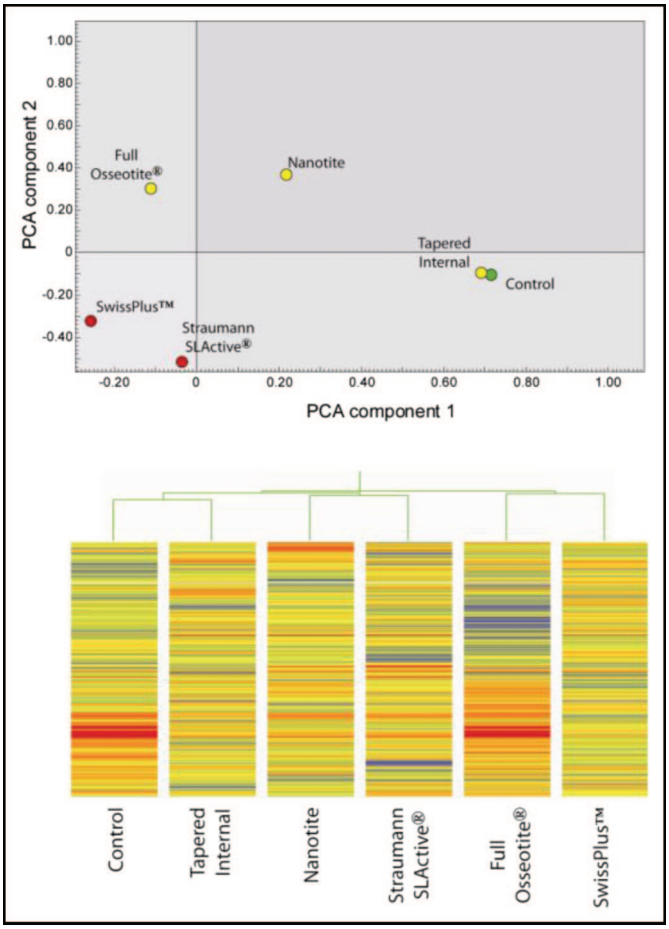

Fig. 2. Expression profile of 18,401 genes as modulated by various dental implants in comparison with control. Gene expression pathways are compared among different experimental condition by supervised PCA (upper panel) and unsupervised HC analysis (lower panel). In PCA, each expression profile corresponding to a specific experimental condition is located in a bidimensional space depending on the overall trend of all tested genes. Yellow dots identify expression profiles similar to control and red dots different from control. In HC, each column represents an expression profile corresponding to a specific experimental condition clustered with other expression profiles depending on the similarity of the expression trend of tested genes. A color scale identifies the expression level of each gene: from blue (low) to red (high). Similar expression profiles cluster nearby in the hierarchical tree.

RESULTS

Figure 1 summarizes the numbers of adherent cells after 72 hours of culture on the respective dental implants. No significant differences in numbers of adherent cells were observed even though in Straumann SLActive dental implant, the greatest number of cells as related to surface was counted (218 cells/mm^2). The viability of adherent cells as tested by MTT test is reported in lower panel of Figure 1. Again, no significant difference was recorded although Straumann SLActive and Tapered Internal implants gave the highest cell viabilities.

Figure 2 reports gene expression profile of osteoblast-like cells adherent to each type of dental implant when compared with controls (cells grown in absence of dental implant) as analyzed both by HC and PCA. HC indicates that the gene expression profile mostly resuming those of control cells is attributable to Tapered Internal implant, which clusters together control samples in the hierarchical tree. Other dental implants are ranked, basing on an increasing difference when compared with control, in the following order: Nanotite, Straumann SLActive, Full Osseotite, and SwissPlus.

PCA indicate that Full Osseotite, Nanotite, and Tapered Internal have a similar expression profile to control, whereas SwissPlus and Straumann SLActive, basing on their very different gene expression profile, are located far away from control.

Figure 3 reports variations in the expression of 49 genes involved in osteogenesis in cells adherent to each type of dental implant when compared with control. The identity and function of the 14 genes specifically varying their expression as related to the type of dental implant are reported in Table 1. Both Tapered Internal and Nanotite increased the expression of 4 osteogenic activities, whereas decreasing only 1. Full Osseotite is in an intermediate situation increasing 2 osteogenic

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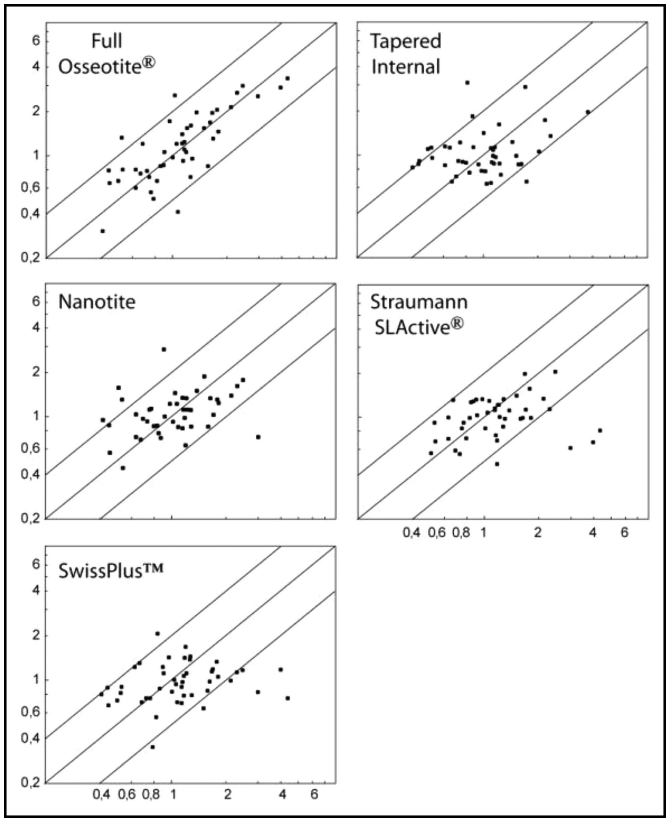

Fig. 3. Comparison of expression of 49 genes involved in osteogenesis between cells adherent to various dental implants (vertical axes) and controls (horizontal axes) by scatter plot analysis. Diagonal lines refer to the 2-fold variation interval assumed as not significant. Dots falling outside this interval indicate significant variation either in the sense of upregulation (scatter-plot upper part) or downregulation (scatter-plot lower part).

activities and decreasing only 1. Conversely, Straumann SLActive and SwissPlus inhibited the expression of 4 and 5 osteogenic genes, respectively, whereas SwissPlus activated only 1.

On the whole, gene expression data indicate that Tapered Internal and Nanotite have the most potent osteogenic activities, Full Osseotite has an intermediate efficacy, whereas Straumann SLActive and SwissPlus have the lowest ability in inducing osteoblast growth.

DISCUSSION

Microarray technology provides information on the expression of thousands of genes in a single experiment. In this investigation, microarray technology was applied to profile the gene expression occurring during *in vitro* osteoblasts adhesion, proliferation, and differentiation around dental implants with different surface characteristics.

No data were found in the literature about gene expression profiles of cells grown in contact with the implants tested except for Straumann SLActive and Full Osseotite implants. The analysis of these implants influence on osteoblast gene expression is therefore a novelty of this investigation.

In this *in vitro* investigation, no significant differences were found in cell number and viability of cells adherent to 5 differently surfaced implants. All the implants tested allowed cell adhesion, proliferation, and differentiation toward an osteogenic phenotype. Conversely, gene expression was revealed to be a more sensitive biomarker being remarkably different in cells adherent to different implants. In fact among 49 selected genes involved in osteogenesis, 14 genes significantly changed their expression profiles when comparing implants that have undergone different surface treatments.

These data indicate a reasonable homogeneity in cell response to different titanium surfaces. Classical endpoints (proliferation and viability) did not differ among the implants tested, and only 14 osteogenic genes were found to be differently modulated by the implants tested.

Gene expression data indicate that Tapered Internal implant induced the gene expression profile most similar to the control and increased the expression of the greatest number of osteogenic genes (4) together with Nanotite implant. Also MTT test, measuring cell viability and mitochondrial function, indicates that cells adherent to Tapered Internal implant possess a higher viability when compared with cells adherent to other implant types except Straumann SLActive, which showed the highest viability values. Tapered Internal implant presents a surface sandblasted with hydroxyapatite particles and a Laser-Lok collar with 10^{-12} μm grooves. The Laser-Lok microtexturing on the implant collar was designed to provide a physical attachment of collagen fibres, which was demonstrated in previous studies.[21,22] In this investigation, the different osteogenic characteristics of the Laser-Lok collar and of the remaining hydroxyapatite-sandblasted surface were not specifically investigated. Therefore, the outcomes for this implant relates both to the Laser-Lok collar and to the sandblasted surface.

Straumann SLActive implant allowed the adherence of the greatest number of cells with the highest viability rate, although differences did not reach the statistical significance threshold. However, gene expression results provide evidence that cells adherent to Straumann SLActive implant have a decreased expression of 3 bone morphogenetic proteins (BMP 5, 6, and 7) and collagen type I.

This is in contrast with other *in vitro* investigations[10,23] where the upregulation of collagen type I and osteogenesis-related factors (ALP, OPG, osteocalcin, and glyceraldehyde-3-phosphate-dehydrogenase) was highest on SLActive surfaces than on the other surfaces tested (smooth, acid-etched, grit blasted, and acid-etched).

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Table 1. Identity and Function of Genes Significantly ($P < 0.05$) Varying Their Expression in Cells Growing on Each Type of Dental Implant

Gene Name	Gene Bank Code	Gene Ontology	Fold Variation Versus Controls				
			Full Osseotite	Tapered Internal	Nanotite	Straumann SLActive	Swiss Plus
Bone morphogenetic protein 5	M60314	Induces bone formation	↓2.6	—	—	↓2.8	—
Osteoglycin (osteoinductive factor, mimecan)	NM_014057	Induces ectopic bone formation	−2.5	—	—	—	—
Osteomodulin	NM_005014	Implicated in bone mineralization processes. Has a function in binding of osteoblasts via the alpha(V)beta(3)-integrin	−2.6	−2.2	−2.5	—	—
Bone morphogenetic protein receptor, type IB	NM_001203	BMPs are involved in endochondral bone formation. These proteins transduce their signals through the formation of heteromeric complexes of 2 different types of serine (threonine) kinase receptors	—	↓2.6	—	—	—
Oteoclast stimulating factor 1	NM_012383	Induces bone resorption through enhancing osteoclast formation and activity	—	−2.1	−3.2	—	—
Osteoprotegerin ligand	AF053712	Acts as decoy receptor for RANKL and thereby neutralizes its function in osteoclastogenesis. Inhibits the activation of osteoclasts and promotes osteoclast apoptosis in vitro. Bone homeostasis seems to depend on the local RANKL/OPG ratio	—	−2.3	−3.1	—	—
Bone morphogenetic protein 7	NM_001719	Induces bone formation. Osteoinductive factor responsible for epithelial osteogenesis	—	−2.1	—	↓2.7	—
Bone morphogenetic protein 6	NM_001718	Induces bone formation	—	—	↓3.8	↓5.9	↓3.3
Bone morphogenetic protein 15	NM_005448	Induces bone formation	—	—	−3.9	—	—
Alpha-2 collagen type I	L47668	Abundant in bone. Mutations in this gene are associated with osteogenesis imperfecta	—	—	—	↓2.0	—
Osteopontin (bone sialoprotein I)	NM_000582	Binds tightly to hydroxyapatite. Appears to form an integral part of the mineralized matrix	—	—	—	—	↓2.1
Osteoprotegerin	NM_002546	Inhibits the activation of osteoclasts and promotes osteoclast apoptosis	—	—	—	—	↓2.2
Bone morphogenetic protein 1	NM_006129	Cleaves the C-terminal propeptides of procollagen I, II and III Inducing bone formation	—	—	—	—	↓2.1
Osteoblast cadherin 11	NM_001797	Calcium dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells; thus contributing to the osteoblast sorting	—	—	—	—	↓2.3
Bone sialoprotein II	NM_004967	Binds tightly to hydroxyapatite. Appears to form an integral part of the mineralized matrix. Important to cell-matrix interaction. Promotes Arg-Gly-Asp-dependent cell attachment	—	—	—	—	−2.2

—, not significant variation.

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Moreover, in *in vivo* studies, implants with SLActive surfaces have been proven to promote bone regeneration and increase implant stability values with respect to the well-documented traditional SLA surface.[24,25]

Full Osseotite implant presents the Osseotite surface, which is realized by a dual acid-etching (DAE) protocol. DAE surfaces are reported to promote osseointegration and show good performances in *in vivo* investigations,[26–28] but only a few data are available in the literature on gene expression profile of osteoblast grown into contact with DAE surfaces. An *in vitro* study by Nakamura et al[29] showed that collagen I, osteopontin, and osteocalcin genes were upregulated in dental pulp cells grown on DAE surfaces compared with machined surfaces within the first week of culture through day 28. In this investigation, machined surfaces were not tested, and the expression of the mentioned genes did not significantly differ between Full Osseotite and the other implants examined at 72 hours of growth. A comparison among the 2 investigations outcomes is not possible because of the differences in the study methods (type of cells, timing, etc.). In any case, the gene expression patterns that were found support the positive *in vivo* outcomes for DAE surfaces.

Nanotite implant was the only implant with a nanomodified surface. Nanotite implant presents the microtopography of the DAE surface combined with a nanometer-scale discrete crystalline deposition of calcium phosphate, which creates a more complex surface topography (Ra on a micrometer level: 0.20 μm; Ra on a nanometer level: 0.023 μm).[18] Recent studies reported that surface modifications at the nanometer level could improve implant performance.[11–15] On the basis of this investigation, it is not possible to attribute to nanostructuration or calcium phosphate rather than some other characteristic the osteogenic properties of Nanotite.

CONCLUSION

These findings indicate that to determine the osteogenic ability of dental implants, it is fundamental not only to quantify adherent cells but also to evaluate from a qualitative standpoint their commitment to induce osteogenesis by analyzing gene expression signature. In fact, while no significant differences were found in cell number and viability, tested implants exhibited different osteogenetic properties, inducing different gene expression profiles of attached cells.

Furthermore, the herein presented results identify 14 osteogenic activities whose expression is commonly targeted by dental implants. Accordingly, these activities may be proposed as molecular biomarkers to comparatively test the osteogenic ability of dental implants both in *in vitro* and in *in vivo* studies.

Disclosure

The authors claim to have no financial interest in any company or any of the products mentioned in this article.

REFERENCES

1. Le Guèhennec L, Soueidan A, Layrolle P, et al. Surface treatments of titanium dental implants for rapid osseointegration. *Dent Mater*. 2007;23:844-854.

2. Shalabi MM, Gotermker A, Van'T Hof MA, et al. Implant surface roughness and bone healing: A systematic review. *J Dent Res*. 2006;85:496-500.

3. Ellingsen JE, Thomsen P, Lyngstadaas SP. Advances in dental implant materials and tissue regeneration. *Periodontol* 2000. 2006;41:136-156.

4. Deligianni DD, Katsala N, Ladas S, et al. Effect of surface roughness of the titanium alloy Ti-6Al-4V on human bone marrow cell response and on protein adsorption. *Biomaterials*. 2001;22:1241-1251.

5. Brånemark PI, Breine U, Adell R, et al. Intra-osseous anchorage of dental prostheses. 1: Experimental studies. *Scand J Plast Reconstr Surg*. 1969;3:81-100.

6. Bächle M, Kohal RJ. A systematic review of the influence of different titanium surfaces on proliferation, differentiation and protein synthesis of osteoblast-like MG63 cells. *Clin Oral Implants Res*. 2004;15:683-692.

7. Bowers KT, Keller JC, Randolph BA, et al. Optimization of surface micromorphology for enhanced osteoblast responses in vitro. *Int J Oral Maxillofac Impl*. 1992;7:302-310.

8. Martin JY, Schwartz Z, Hummert TW, et al. 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.

9. Arcelli D, Palmieri A, Pezzetti F, et al. Genetic effects of a titanium surface on osteoblasts: A meta-analysis. *J Oral Science*. 2007;49:299-309.

10. Zhao G, Raines AL, Wieland M, et al. Requirement for both micron- and submicron scale structure for synergistic responses of osteoblasts to substrate surface energy and topography. *Biomaterials*. 2007;28:2821-2829.

11. Le Guèhennec L, Martin F, Lopez-Heredia MA, et al. Osteoblastic cell behavior on nanostructured metal implants. *Nanomed*. 2008;3:61-71.

12. Dalby MJ, McCloy D, Robertson M, et al. Osteoprogenitor response to semi-ordered and random nanotopographies. *Biomaterials*. 2006;27:2980-2987.

13. Mereilles L, Arvidsson A, Albrektsson T, et al. Increased bone formation to unstable nano rough titanium implants. *Clin Oral Implants Res*. 2007;18:326-332.

14. Mereilles L, Curire F, Jacobsson M, et al. The effect of chemical and nanotopographical modifications on the early stages of osseointegration. *Int J Oral Maxillofac Implants*. 2008;23:641-647.

15. Mereilles L, Melin L, Peltola T, et al. Effect of hydroxyapatite and titania nanostructures on early in vivo bone response. *Clin Implant Dent Rel Res*. 2008;10:245-254.

16. Morra M, Cassinelli C, Bruzzone G, et al. Surface chemistry effects of topographic modification of titanium dental implant surfaces. 1: Surface analysis. *Int J Oral Maxillofac Impl*. 2003;18:40-45.

17. Cassinelli C, Morra M, Bruzzone G, et al. Surface chemistry effects of topographic modification of titanium dental implant surfaces. 2: In vitro experiments. *Int J Oral Maxillofac Impl*. 2003;18:46-52.

18. Svanborg LM, Andersson M, Wennerberg A. Surface characterization of commercial oral implants on the nanometer level. *J Biomed Mater Res*. 2010;92B:462-469.

19. Sammons RL, Lumbikanonda N, Gross M, et al. Comparison of osteoblast spreading on microstructured dental implant surfaces and cell behavior in an explants model of osseointegration. A scanning electron microscopic study. *Clin Oral Impl Res*. 2005;16:657-666.

20. Izzotti A, Pulliero A, Orcesi S, et al. Interferon-related transcriptome alterations in the cerebrospinal fluid cells of Aicardi-Goutières patients. *Brain Pathol*. 2009;19:650-660.

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21. Nevins M, Nevins ML, Camelo M, et al. Human histologic evidence of a connective tissue attachment to a dental implant. *Int J Periodontics Restorative Dent*. 2008;28:111-121.

22. Shapoff CA, Lahey B, Wasserlauf PA, et al. Radiographic analysis of crestal bone levels around Laser-Lok collar dental implants. *Int J Periodontics Restorative Dent*. 2010;30:129-137.

23. Qu Z, Rausch-Fan X, Wieland M, et al. The initial attachment and subsequent behaviour regulation of osteoblasts by dental implant surface modification. *J Biomed Mater Res*. 2007;82A: 658-668.

24. Schwarz F, Sager M, Ferrari D, et al. Bone regeneration in dehiscence-type defects at non-submerged and submerged chemically modified (SLActive) and conventional SLA titanium implants: An immunohistochemical study in dogs. *J Clin Periodontol*. 2008;35:64-75.

25. Schätzle M, Männchen R, Balbach U, et al. Stability change of chemically modified sandblasted/acid-etched titanium palatal implants. A randomized-controlled clinical trial. *Clin Oral Implants Res*. 2009;20:489-495.

26. Testori T, Woolfe S, Porter SS, et al. A prospective multicenter clinical study of the Osseotite implant: A four-year interim report. *Int J Oral Maxillofac Implants*. 2001;16:193-200.

27. Trisi P, Lazzara R, Rebaudi A, et al. Bone-implant contact after 2 months of healing in the human maxilla. *J Periodontol*. 2003;74:945-956.

28. Khang W, Feldman S, Hawley CE, et al. A multi-center study comparing dual acid-etched and machined-surfaced implants in various bone qualities. *J Periodontol*. 2001;72:1384-1390.

29. Nakamura H, Saruwatari L, Aita H, et al. Molecular and biomechanical characterization of mineralized tissue by dental pulp cells on titanium. *J Dent Res*. 2005; 84:515-520.

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