Effects of Fluoride-Modified Titanium Surfaces on Osteoblast Proliferation and Gene Expression

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Purpose: *The objective of this study was to test the hypothesis that fluoride-modified titanium surfaces would enhance osteoblast differentiation. Osteoblast growth on a moderately rough etched fluoride-modified titanium surface (alteration in cellular differentiation) was compared to osteoblast growth on the same surface grit-blasted with titanium dioxide. The potential role of nanometer-level alterations on cell shape and subsequent differentiation was then compared.* ***Materials and Methods:*** *Human embryonic palatal mesenchymal (HEPM) cultures were incubated on the respective surfaces for 1, 3, and 7 days, followed by analysis for cell proliferation, alkaline phosphatase (ALP) -specific activity, and mRNA steady-state expression for bone-related genes (ALP, type I collagen, osteocalcin, bone sialoprotein [BSP] II, Cbfa1, and osterix) by real-time polymerase chain reaction (PCR).* ***Results:*** *The different surfaces did not alter the mRNA expression for ALP, type I collagen, osterix, osteocalcin, or BSP II. However, Cbfa1 expression on the fluoride-modified titanium surface was significantly higher (*$P < .001$*) at 1 week. The number of cells on this surface was 20% lower than the number of cells on the surface* TiO_2*-blasted with 25-*μ*m particles but not significantly different from the number of cells on the surface* TiO_2*-blasted with 125-*μ*m particles. Cells grown on all the titanium surfaces expressed similar levels of ALP activity.* ***Conclusions:*** *The results indicated that a fluoride-modified surface topography, in synergy with surface roughness, may have a greater influence on the level of expression of Cbfa1 (a key regulator for osteogenesis) than the unmodified titanium surfaces studied.* (Basic Science) Int J Oral Maxillofac Implants 2006;21:203–211

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Considerable differences have been observed in the differentiation and mineralization of osteoblasts grown on different dental implant surfaces in vitro.[1–3] These differences have been attributed to varying surface chemistries and topographies. In general, increased surface roughness is associated with decreased cell proliferation and increased differentiation. However, it is important to realize that the reaction of cells to a material is dependant on the cellular maturation stage, indicating that the surface may modulate maturation of the cells.[4] The behavior of osteoblasts on artificial surfaces is also dependent on the culture systems and experimental cell culture conditions.[5] Therefore, results may be contradictory.

It has been proposed that differences in surface properties may have profound effects on extracellular matrix (ECM) protein adhesion and subsequent cell attachment. Surface effects are mediated through integrins, the cell surface receptors that recognize and bind to a specific motif in ECM attachment proteins. The association of integrin receptors with the underlying cytoskeleton has been shown to influence such cell properties as cell shape, differen-

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tiation, proliferation, survival, and gene expression.[6] Signals from the extracellular environment can be transferred to the cell as a consequence of the interaction of integrins with both extracellular and intracellular proteins; consequently, cells assume a variety of morphologies or cell shapes upon attachment. This results in new gene transcription as well as new protein synthesis. The production of new extracellular matrix in response to different surfaces may contribute to the cessation of proliferation and the acquisition of different cell phenotypes.

For maximum bone cell differentiation and bone formation, rough surfaces with isotropic and evenly spaced indentations, such as are produced by blasting with particles of titanium dioxide (TiO_2) 60 to 90 μm wide (ie, surfaces with S_a values of around 0.7 μm), appear to be optimal.[7] It has also been suggested that rougher surfaces, such as are produced by blasting surfaces TiO_2 particles 300 μm wide (ie, surfaces with S_a values of around 1.4 μm), may have no further advantage with respect to bone formation, as the optimal roughness value may have been exceeded.[7] One theory is that, relative to an average osteoblast size of 10 to 12 μm, there exists a certain range of roughness that can be perceived by the cell.[7] If roughness is perceived, the cell cannot flatten and spread and therefore differentiates. Hence topographical characteristics of a titanium surface may influence final cell differentiation. However, this theory is based on an in vitro study that used laboratory-produced surfaces that were not equivalent to commercially produced surfaces.

Surface chemistry also plays an important role in the reaction of bone cells to the implant, since it influences charge and wettability. Surface wettability is largely dependent on surface energy and influences the degree of contact with the physiologic environment. Increased wettability enhances interaction between the implant surface and the biologic environment.[8] Improved bone bonding and accelerated bone formation seems possible with roughened surfaces further modified with certain acid treatments. The sandblasted and acid-etched surface has been demonstrated to enhance bone apposition in histomorphometric and removal torque analyses.[9,10] These studies indicate that a subtractive surface modification (ie, acid etching) has a positive effect on the strength of ossointegration and that a synergistic mechanism exists that enhances bone formation with the right combination of macro-topographic modification (eg, blasting) and microtexture modification (ie, acid etching) of the implant.[11,12]

It is known that fluoride can stimulate the production of new bone, in part, by stimulating the proliferation of osteoblasts.[13,14] The fluoride concentration on the surface is thought to be released upon a phosphate exchange reaction during initial exposure to the wound healing environment. In this way, the surface acts as a site for calcium and phosphate precipitation, allowing for increased bone contact and thus implant stability.[15] Therefore, fluoride modification of implant surfaces presents an approach to improve osseointegration. In vivo research has indicated that following a 3-month healing period, fluoride-modified implant surfaces demonstrated significantly higher bone-to-metal contact and retention to bone compared to implants with a similar surface roughness.[16] Surface modification of titanium with fluoride changes the chemical structure of the surface, resulting in an increased affinity to the TiO_2 surface for calcium and phosphate ions. The capability to adsorb calcium and phosphates promotes bone formation and the process of bone bonding in vitro and in vivo.[17,18]

In vitro studies have shown that differences in the microtopography of an implant surface can affect the expression of key bone matrix-related proteins and osteogenic transcription factors that will enhance osteogenesis on implant surfaces.[2,19–21] Different bone cell responses associated with different implant surface roughnesses may be explained, in part, by the modulated expression of the selected ECM-related genes and transcription factors, in particular Cbfa1. The aim of the present study was to examine bone cell responses to fluoride-modified titanium surfaces as compared to standard TiOBlast surfaces and non–fluoride-modified rough titanium surfaces.

MATERIALS AND METHODS

Cell Culture and Titanium Implant Specimens

Commercially pure grade IV titanium disks 12.0 mm in diameter and 2.0 mm high were used in the study. The disks were grit-blasted with TiO_2 particles with an average size of 25 μm to create a roughened surface topography. Previous studies[7,16] have characterized the surface roughness properties of these disks having an average height deviation from the mean value (R_a) of 1.12 ± 0.24 μm. This level of implant surface roughness was defined by Albrektsson and Wennerberg as "moderately rough."[22] Disks were also further grit-blasted with 125 μm TiO and then kept at a constant temperature and pH using a continuous hydrofluoric acid rinse protocol designed to prevent aggressive etching of the metal surface. This resulted in a surface with a low level[23] of titanium-fluoride (0.5 to 3 atomic %). A previous study[16] showed that this process results in an average sur-

face roughness of R_a = 0.91 ± 0.14 μm. Disks were also prepared with large-grit 125-μm TiO blasting alone (Rough Blast [RB]). Grit blasting with the large particle size resulted in a deeper average surface roughness (Sa ~ 3.8 ± 1.1 μm).[24] The disks were provided by Astra Tech (Mölndal, Sweden). According to the manufacturer, the control titanium dioxide specimens (TO) and the fluoride-modified specimens (OsseoSpeed [OS]) were prepared using the same procedures used for the preparation of clinical implants.

Scanning Electron Microscopy of the Surfaces

Surfaces were analyzed using scanning electron microscopy (SEM) (S-4000 SEM; Hitachi, Tokyo, Japan) to determine their microtopographic properties. The disks were viewed at magnifications of 1,000× and 10,000×. Selected samples for SEM were also taken of the human embryonic palatal mesenchymal (HEPM) cells on the surfaces after 3 days of culture. Samples were fixed in 3% glutaraldehyde, washed twice in 0.1 mol/L sodium-cacodylate buffer (pH 7.4), and dehydrated using a graded series of ethanols. After being dried with hexamethyldisilazane (HMDS), samples were sputter-coated with gold and photographed using an Amray 1820 SEM (Bedford, MA) with an acceleration voltage of 20 kV.

Cell Proliferation

Human embryonic palatal mesenchymal cells (HEPM 1486; ATCC, Manassas, VA) were cultured in Eagle's minimum essential medium (EMEM) supplemented with Earle's salts, L-glutamine (2 mmol/L), nonessential amino acids (0.1 mmol/L), sodium pyruvate (1 mmol/L), 10% fetal bovine serum, and 25 μg/mL penicillin/streptomycin. HEPM cells were plated at high-density micromass conditions in triplicate (50,000 cells/10 μL droplet culture) onto the TO and OS surfaces and onto tissue culture plastic (TCP) in 24-well culture plates and placed into a 37°C incubator. After 1 hour of attachment, 1 mL of medium supplemented with ascorbate (50 μg/mL) was added to the cultures. Cultures were maintained for 24 hours, 3 days, or 1 week. For the 1-week cultures, the medium was changed on the third day. At 24 hours, 3 days, and 1 week, the medium was removed from the wells, and cell proliferation was estimated by quantifying the attached cells with the model ZM Coulter counter (Beckman Coulter, Fullerton, CA).

Alkaline Phosphatase–Specific Activtiy

Triplicate samples of normalized protein lysates (Pierce BCA Protein Assay, Pierce, Rockford, IL) were used for measuring the alkaline phosphatase–specific (ALP) activity using a commercial kit (Malachite Green Phosphate Assay Kit; BioAssay Systems, Hayward, CA). This assay is based on quantification of the complex formed between Malachite Green, molybdate, and free orthophosphate. The enzymatic reaction between the Malachite Green and 10 μL of the samples lysates was determined by spectrophotometry at 650 nm with a PowerWave 200 scanning microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT) in Costar UV-transparent microplates (Corning, Corning, NY). Data was captured using KC4 microplate data acquisition software (Bio-Tek Instruments) and compared with the phosphate standards. Enzymatic activity was measured as the phosphate concentration per culture (pmol/750 μL culture).

Real-Time Reverse Transcriptase Polymerase Chain Reaction

Osteoblast genotypic markers tested were ALP, collagen type I, osteocalcin, Cbfa1 isoform 3, bone sialoprotein (BSP II) and osterix. RNA extracts from HEPM cells grown in micromass cultures as described on TO, OS, and RB surfaces were analyzed in triplicate at 1 day, 3 days, and 7 days using quantitative real-time multiplex reverse transcriptase polymerase chain reaction (RT-PCR) with a Taqman Gold RT-PCR kit (PerkinElmer, Wellesley, MA). On days 1, 3, and 7, total RNA was isolated by means of the RNeasy Mini Kit (Qiagen, Valencia, CA). The concentration of RNA in each sample was determined by light absorbance at 260 nm, and dilution with RNase/DNase free water (Qiagen) was carried out where necessary to correct the RNA concentrations of the samples. A total of 3 μL of the normalized RNA concentrations from each sample was reverse transcribed into cDNA with Taqman reverse transcription reagents (Applied Biosystems, Foster City, CA).

RT reactions were performed in a PTC-200 Peltier Thermal Cycler (Biorad/MJ Research, Hercules, CA). Multiplex real-time PCR reactions were performed in 96-well optical reaction plates (PerkinElmer) in an ABI Prism 7700 sequence detection (PerkinElmer). Steady-state mRNA levels of each gene were normalized to 18s rRNA and calculated by the comparative method of relative quantification in multiplex reactions. Primers were designed with the Software Primer Express (Applied Biosystems).

Statistical Analysis

For the cell attachment, proliferation, and gene expression assays, results from the 3 different experimental runs were pooled (n = 9, 3 groups and 3 repetitions). Differences between experimental groups were calculated using a 1-way analysis of variance (ANOVA) with a Tukey multiple comparisons test to a confidence level of $\alpha < .05$.

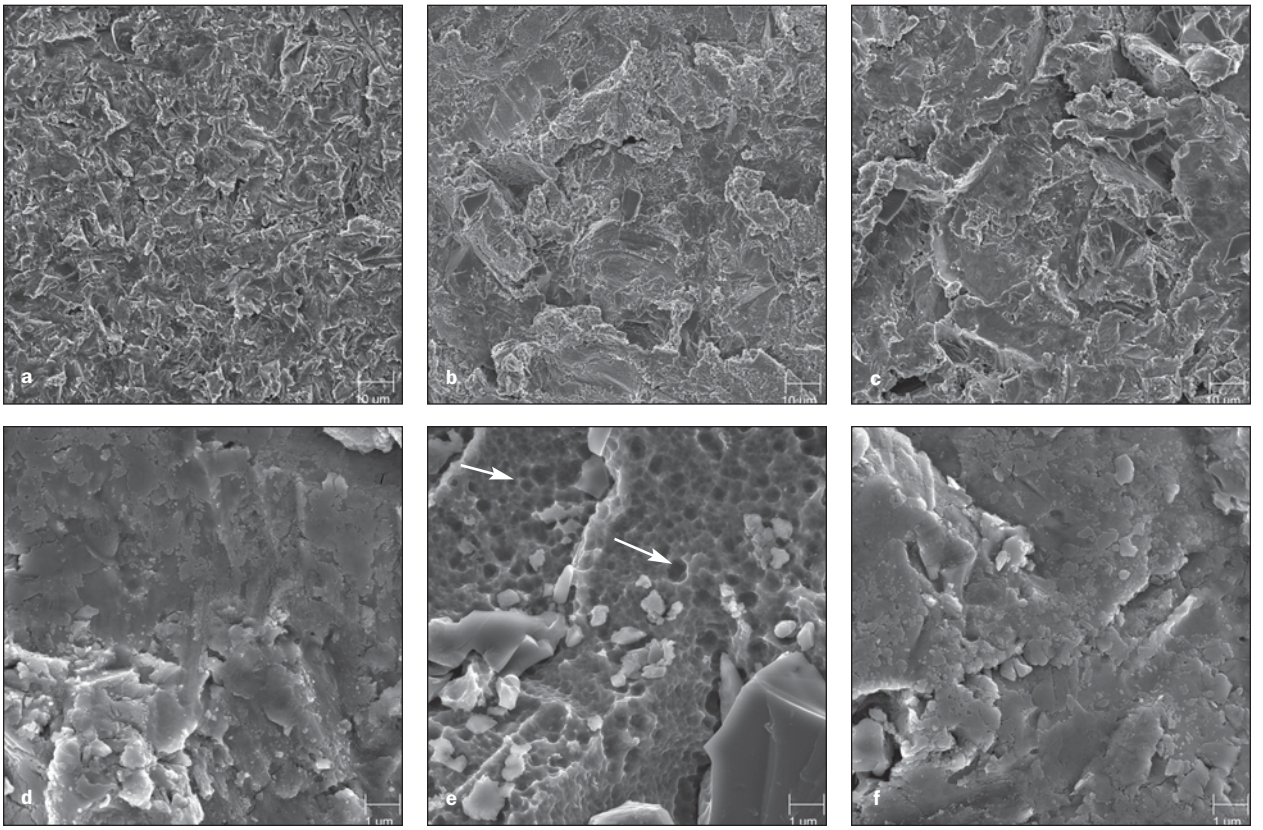

Fig 1 SEM images of *(a)* the TO, *(b)* the OS, and *(c)* the RB titanium surfaces (original magnification ×1,000; bar = 10 μm). The same surfaces—*(d)* TO, *(e)* OS, and *(f)* RB—are also shown at a higher magnification (original magnification ×10,000; bar = 1 μm). The nanopores present in the OS surface *(white arrows)* were caused by the acid treatment.

RESULTS

SEM

The results of the SEM analyses of the surfaces studied are displayed in Figs 1a to 1c. SEM showed that all the surfaces demonstrated isotropic properties possessing no dominating surface structures. The TO surface was the smoothest; the OS and RB surfaces appeared morphologically similar. At a higher magnification (Figs 1d to 1f), numerous secondary nanopores, less than 1.0 μm in diameter, were seen on the OS surface.

Cell Attachment and Morphology at 3 Days

The SEM observation of cell morphology on the titanium surfaces at 3 days shows that in the center of the micromass cultures, the cells were well spread; they presented with a predominantly flattened morphology and followed a continuous multilayered conformation. Cells at the edge of the micromass were seen to bridge across the peaks of all the surfaces in all directions (Figs 2a to 2c). The cell morphology was comparable between the surfaces, ie, cells were flattened out and spanned over the peaks of the surface.

Osteoblast Proliferation

The number of cells on the surfaces after plating depended on the surface (Fig 3). On all the disks, there was a progressive increase in cell number during the 7 days of culture. At day 1, the number of cells on all the surfaces was comparable. In cultures grown on the TO surface, the number of cells was comparable to TCP at days 1 and 7. On day 3, the number of cells on TCP was significantly higher than the number of cells on any of the titanium surfaces ($P < .001$); the titanium surfaces were all comparable to each other with respect to number of cells. At day 7, there was no significant difference in the number of cells on TO compared to TCP, whereas the number of cells on the OS and RB surfaces was reduced by 20% compared to the levels seen on TCP and TO ($P < .001$). There was no significant difference in cell proliferation between the OS and RB surfaces.

ALP-Specific Activity

No significant differences were observed in the level of ALP-specific activity of cells grown on the OS surface versus the RB surface at any time (Fig 4). Significant differences were seen only between ALP activity

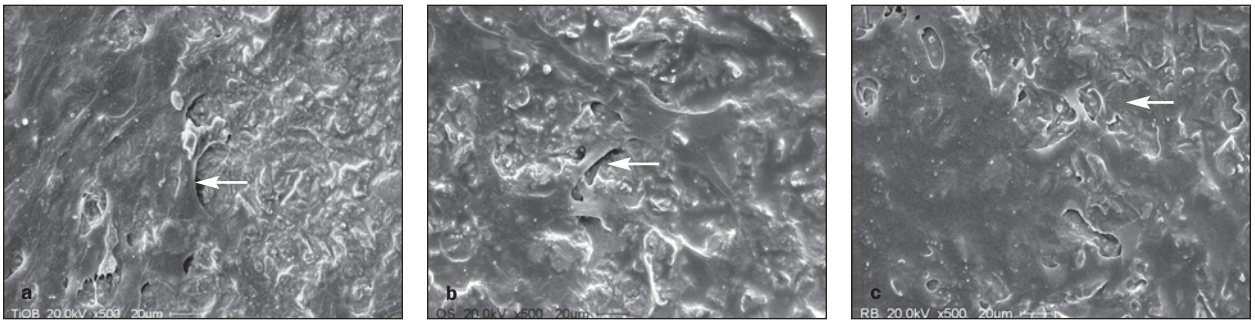

Fig 2 SEM of HEPM cells cultured for 3 days on *(a)* the TO, *(b)* the OS, and *(c)* the RB surfaces (original magnification ×500; bar = 20 μm). Cells on all the titanium surfaces were spread and stretched over the tops of the surfaces *(white arrows)*.

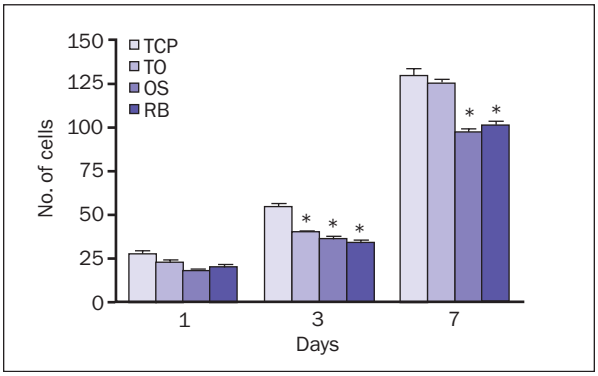

Fig 3 Number of HEPM cells on different surfaces over time. Means ± SDs shown for 9 specimens. *Significant difference ($P < .001$) between the number of cells on a given titanium surface compared to the number of cells on TCP for each culture period.

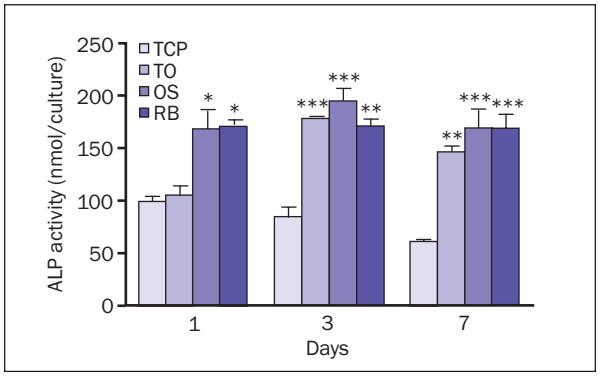

Fig 4 ALP-specific activity of HEPM cells cultured on different surfaces over time. Means ± SDs shown for 9 specimens. *, **, and *** denote the significance levels ($P < .05$, $P < .01$, and $P < .001$, respectively) of the differences in activity on the titanium surfaces compared to activity on TCP.

on TCP and on the titanium surfaces as a group and between the ALP activity on TO and the 2 rougher OS and RB surfaces at 24 hours of culture. At day 1, ALP activity on TCP and TO surfaces was significantly lower than on OS and RB surfaces ($P < .05$). At day 3, ALP activity on TCP was significantly lower than on all the titanium surfaces ($P < .01$ for RB surfaces and $P < .001$ for TO and OS). By day 7, ALP activity on TCP was significantly lower than on TO ($P < .01$), OS ($P < .001$), or RB ($P < .001$). There was no significant difference in the ALP activity between any of the titanium surfaces at days 3 and 7, indicating increased expression of the osteoblast phenotype on all the titanium surfaces compared to TCP at the longer time points.

Osteoblast Gene Expression

Markers associated with osteoblasts were present on all the surfaces at all time points (Fig 5). The mRNA levels for ALP, BSP II, and osterix were equivalent at all time points for all the surfaces tested (Figs 5a to 5c). For osteocalcin, gene expression increased significantly ($P < .05$) from day 3 to day 7 on all the surfaces (Figs 5d and 5e). In contrast, significantly different Cbfa1 gene expression ($P < .001$) was detected between all the 3 surfaces at the same time point and at different times (Fig 5f). Cbfa1 expression was significantly higher ($P < .001$) on the OS surface compared to the other titanium surfaces at 1 week of culture. Cbfa1 gene expression increased 2-fold from day 3 to day 7 on OS and TO surfaces but not on the RB surface. Differences in gene expression were not related to cell number differences as seen in Figs 3 and 6. Cell number actually decreased at time points that correlated to increased gene expression. An additional control was performed with multiplex reactions using an internal control for normalization.

DISCUSSION

To enhance osseointegration, dental implant surfaces could possess the ability to stimulate differentiation of osteogenic cells and matrix formation. Surface chemistry, surface morphology, and roughness

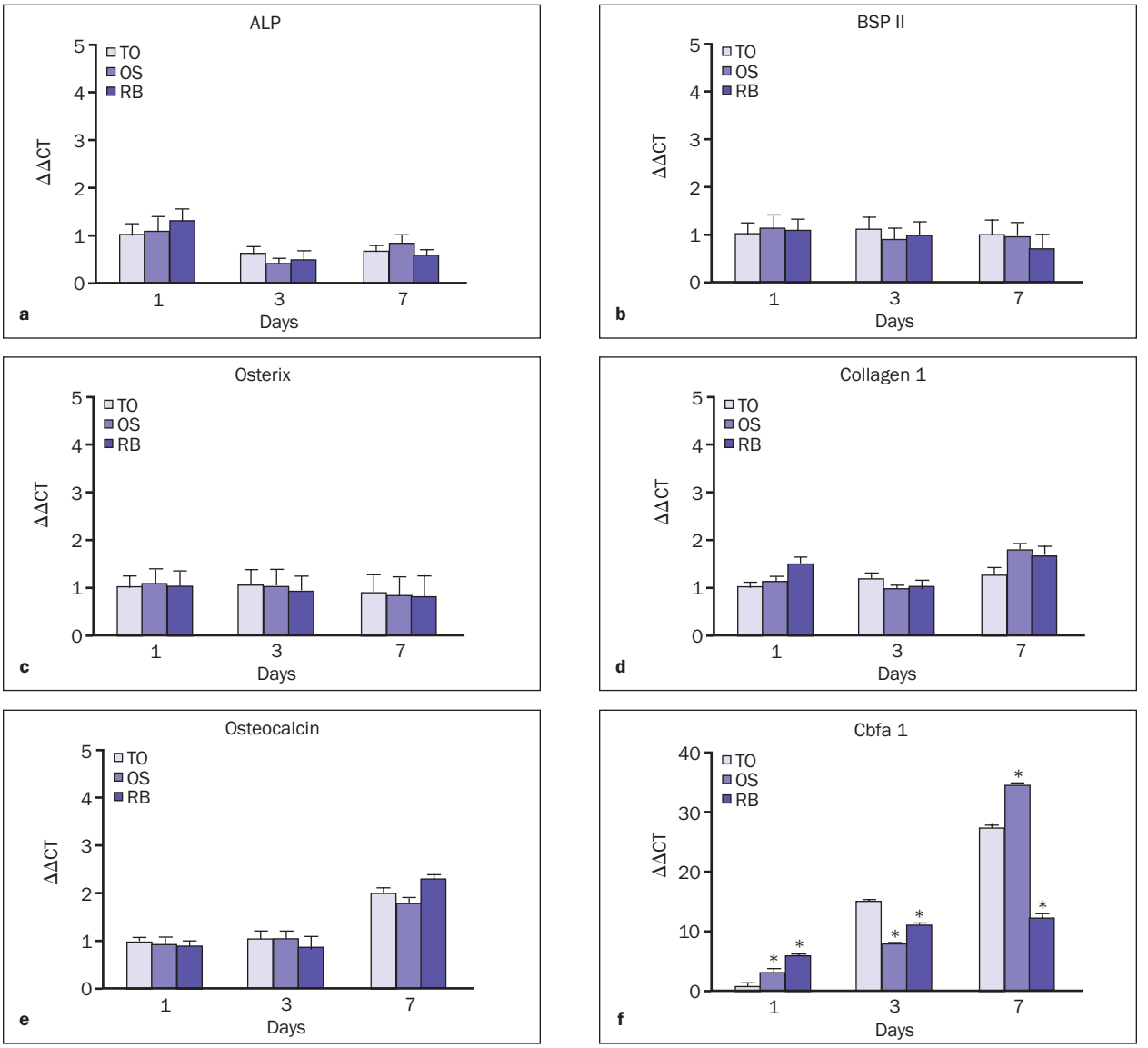

Fig 5 Gene expression of HEPM cells on different surfaces over time. Means ± SDs shown for 9 specimens per group. * denotes the significance level ($P < .001$) of the difference in gene expression on OS and RB surfaces compared to TO for each culture period. No significant differences in cell loss were evident between any of the surfaces tested. ΔΔCT = fractional cycle number of mRNA.

are important properties which influence cell-biomaterial interactions and bone formation on titanium implants of various designs and surface preparations.[11] It has been demonstrated that surface topography and surface roughness can modulate the phenotypic expression of osteoblastlike cells.[25] Quantitative studies conducted for 1 and 3 months in vivo have suggested that there exists an upper and a lower limit of surface roughness for optimal bone response to implants.[7,26]

Surface texturing of implant surfaces by acid etching has been shown to have superior bone cell responses compared with surfaces blasted with large particles alone; such surfaces induced a more pronounced proliferation and increased differentiation of the cells.[27] Excessive etching, however, can lead to a loss of surface features. The use of large-particle grit blasting materials can lead to reduced or unpredictable surface topography.[11] Viornery and associates[28] found that titanium with ethane-1,1,2-triphos-

phonic acid modification may be preferable to unmodified titanium, as the total amount of synthesized type I collagen was found to be significantly higher. However, it was not conclusively determined whether the effect of surface chemistry is more significant than the effect of surface roughness; the effect of surface chemistry may be synergistic.[29]

Generally, oral implants are introduced clinically without adequate clinical documentation before product launch.[30] The TiOblast implant has more than 10 years of recorded follow-up with respect to survival and more than 7 years with respect to success.[31] Animal studies have demonstrated that modifying a moderately roughened implant surface with hydrofluoric acid allows improved bone-to-implant attachment in a shorter healing time, even though the surface roughness is slightly reduced by the acid treatment.[16,24] The purpose of this study was to investigate whether a moderately rough fluoride-modified titanium surface (OS) would induce a substantial enhancement in osteoblast differentiation and growth of preosteoblasts in culture as compared to a conventional non–fluoride-modified surface (TO) and a non–fluoride-modified surface grit-blasted with larger-diameter particles (RB) than conventional blasted surfaces.

Although the influence of topography of metallic substrates on human osteoblast proliferation has been largely demonstrated, sometimes surface roughness alone may not significantly affect bone cell responses,[32] and this could explain the similarity in the cell morphology at 3 days of culture. Bigerelle and Anselme[33] demonstrated that the classically described effects of roughness on morphology and proliferation may be related more to surface morphology than to surface amplitude.

At the end of 7 days of culture, the cell number on the rougher titanium surfaces was lower than on the smoother TCP and TO surfaces. Cell attachment, spreading, migration, and proliferation require signals from the extracellular matrix, and these signals are transduced by integrin receptors and their association with the intracellular actin cytoskeleton.[34,35] Lian and Stein[36] observed that decreased proliferation of osteoblast cells precedes the expression of the more differentiated phenotype in culture. Roughened surfaces resulted in stronger adhesion to the surface and hence less cell migration and proliferation, but the cells exhibited a more differentiated phenotype. ALP-specific activity is an early marker of osteoblast differentiation. Although not significantly different, ALP activity on the OS and RB surfaces was higher than on the smoother TO surface, indicating a higher rate of cellular differentiation on the OS and RB surfaces.

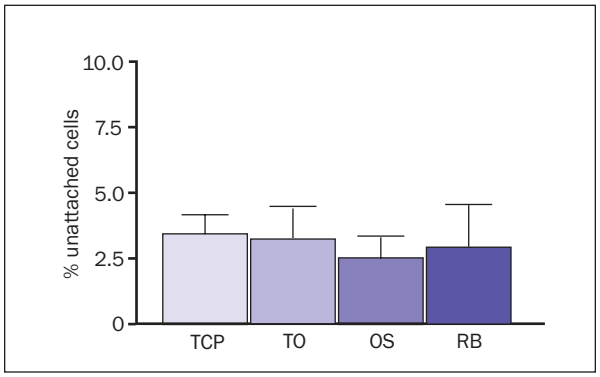

Fig 6 Unattached cells after 24 hours in culture on surfaces expressed as percentage of the initial number of cells plated. Means ± SDs shown for 9 specimens. No significant differences in cell loss were evident between any of the surfaces tested.

HEPM cells have been shown to express osteoblastic phenotypes and to mineralize in culture.[2,37] In the present study, the response of the HEPM cells cultured on the surfaces studied showed that all disks, independent of surface chemical composition or surface roughness, allowed cell attachment, cell proliferation, and osteoblastic differentiation, expressed as mRNA bone-related gene and transcription factor expression. ALP and type I collagen were highly expressed in the early stage of bone maturation, whereas osteocalcin and BSP II were expressed mostly late in osteogenesis. Cbfa1 is a transcription factor that is essential for the maturation and differentiation of mesenchymal stem cells and is differentially regulated in primary human osteoblasts depending on stage of maturation.[38] Osterix is a protein needed for osteoblast differentiation; it acts downstream of Cbfa1.[39]

Although mRNA expression for bone cell osteogenic markers was expressed equally on all the surfaces at days 1 and 3, at day 7, the number of cells on OS and RB surfaces was lower than the number of cells on TO surfaces, indicating that stimulation of cell differentiation over proliferation had occurred on the OS and RB surfaces. As Cbfa1 expression was higher on the OS surfaces than on the RB surfaces, this suggests that the surface chemistry of the OS surface interrelated with surface topographical features may have a greater influence on the differences observed than surface roughness alone, although the precise effect of surface chemistry and roughness on cell genotypic expression is not clear.

Collagen type I and osteocalcin gene expression (at 1 week) was not affected by the different surfaces. Cbfa1 mRNA expression, though higher on the RB surface than on the OS surface at day 1 and day 3, was

highest at day 7 on the OS surface. If there is a stimulating effect to the cells with increasing surface roughness, the highest levels of osteocalcin gene expression and Cbfa1 mRNA expression should have been found in the cells on the RB surfaces. However, at day 7 the osteocalcin gene expression on the RB surfaces was not significantly different from expression on the TO or OS surfaces. Cbfa1 expression was the lowest on the RB surface at 7 days (Fig 5f), although the cell density was not significantly different from that on the OS surface. In the present study, no differences in osteocalcin or BSP II gene expression were found at 1 week between the samples. The different surfaces were not observed to induce a difference in the mineralization process, at least not during the first week. For the tested bone-related markers, it is not clear whether maximal expression had already reached. However, the experiments had been performed with high seeding densities (micromass cultures) to accelerate the cell differentiation process, as it is known that the cellular response to the type of surface is dependent on cell maturation state.[1,40]

Masaki and associates[41] studied gene expression of HEPM cells cultured for 72 hours on different titanium surfaces, including the OS and TO surfaces. Among other differences, they found that the mRNA Cbfa1 level was significantly higher on the OS and TO surfaces compared to the rougher sandblasted, acid-etched surfaces. The present results show that at 72 hours, the mRNA Cbfa1 level for the TO surface was significantly higher than the levels on the rougher OS and RB surfaces. However, at day 7, Cbfa1 expression on the OS surface was higher than on the TO and RB surfaces. The difference between the results may have been caused by differences in the methods used to prepare the surfaces, differences in the surfaces used for comparison, and the fact that different time points were studied.

The present results indicate that fluoride-modified titanium surfaces result in improved osteoblast response as compared to TO or RB surfaces. The TO and OS surfaces differed with respect to roughness; it is likely that this difference contributed to the different osteoblast response noted on the OS surface. The OS and RB surfaces also differed with respect to roughness, although this difference was smaller than the roughness difference between OS and TO. However, surface roughness differences alone may not have an effect on the observed difference in osteoblast response, as titanium with increased surface roughness alone is not more efficient in enhancing osteoblast proliferation and differentiation than machined titanium.[42] The results also indicate that fluoride modification of the surface may be more important for osteoblastic cell responses than differences in the S_a values from 1.3 μm to 1.5 μm (the variation in roughness values between the OS and RB surfaces).

This finding suggests that fluoride modification of the titanium surface appeared to be an important factor in the regulation of Cbfa1 expression of HEPM cells. Although the present results cannot be directly extrapolated to the clinical setting, it may be suggested that differences in the Cbfa1 expression may partially account for the greater percentage of metal-to-bone contact and the greater removal torque seen with fluoride-modified implants as compared to rough non–fluoride-modified implants.[16] Thus, modification of titanium surfaces with fluoride in synergy with a moderate surface roughness may be a useful method to enhance osteogenesis and improve bonding between the implant surface and bone.

CONCLUSIONS

All the titanium surfaces studied supported cellular growth and the temporal expression of an array of bone-related genes and transcription factors. The surfaces induced differential expression of Cbfa1 on the different surfaces right from day 1 of culture. At day 7, the OS surface induced greater expression of Cbfa1 than TiOB and RB surfaces, suggesting that the OS surface may possess a higher potency to enhance osteogenesis. For this reason, the moderately roughened fluoride-modified surface appears to be a better surface for the support and promotion of cell proliferation and differentiation than the smoother and rougher non–fluoride-modified surfaces.

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