Classification and Effects of Implant Surface Modification on the Bone: Human Cell–Based In Vitro Studies

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Implant surfaces are continuously being improved to achieve faster osseointegration and a stronger bone to implant interface. This review will present the various implant surfaces, the parameters for implant surface characterization, and the corresponding in vitro human cell–based studies determining the strength and quality of the bone-implant contact. These in vitro cell-based studies are the basis for animal and clinical studies and are the prelude to further reviews on how these surfaces would perform when subjected to the oral environment and functional loading.

Key Words: titanium, dental implant, implant surface morphology, osseointegration, surface topography

Introduction

Titanium and its alloys are established materials for dental implants because of their physical strength, material stability, and tissue compatibility.[1] The first generation of dental implants wa machined with a smooth surface and was used successfully clinically.[2] However, the healing period for these implants may be as long as 6–9 months before they are osseointegrated enough to be loaded. Osseointegration is dependent on the direct interaction between the bone cells and the titanium surface. The design of a new and efficient implant material requires an understanding of the adhesion of osteoblasts at the bone-implant interface. Thus, the search continues for an ideal implant surface modification that can osseointegrate to bone faster and with a stronger bone-to-implant interface.

This review will present (1) the various implant surfaces and how each is achieved, (2) the parameters to characterize the implant surface morphology and how they are evaluated, and (3) the human cell–based studies to determine the strength and quality of the bone-implant contact resulting from each surface modification.

Classification of Implant Surfaces

The adhesion and differentiation of osteoblastic cells are influenced by the surface properties of the dental implant.[3,4] Surface properties include chemical composition, surface energy, roughness, and topography. In dental implants, surface roughness are often modified to modulate bone apposition.[5,6] Surface roughness can be described as macro-, micro-, and nanometer-sized texture. Macro- and micrometer roughness facilitates mechanical anchorage to bone.[5] Nanometer roughness affects the adsorption of proteins and the adhesion of osteoblastic cells. It can modulate the rate of ossteointegration.[7,8] A variety of surface treatments (Table 1) can be used to produce the desired surface topography. Commercially available implants vary in titanium composition and surface modifications (Table 2), having an understanding of these differences can help clinicians make an informed choice in implant selection for their patients.

Methods of Implant Surface Alteration: Physical and Subtractive

Machined

The machined implant is turned, milled, or polished. It is minimally rough, with a surface area roughness (S_a) value of 0.3–1.0 μm.[9] The surface morphology is determined by the manufacturing tools used, the implant material, the lubricant, and the speed at which it is machined.

Sandblasted

The sandblasted implant is grit blasted by small particles (alumina or titanium oxide), which creates craters and ridges on impact. The surface morphology is determined by the following particle characteristics: its material, size (25, 75, and 250 μm), shape, density, and speed at which it is propelled.[10]

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DOI: 10.1563/aaid-joi-D-16-00079

Table 1

Classification of surface treatment for dental implants

Physical (and Subtractive) Surface Treatment	Chemical (and Additive) Surface Treatment	Biological/Biomimetic Surface Treatment
1. Machined surface	1. Acid etching	1. Bioactive coatings
2. Abrasive/sandblasting with grit media	2. Alkaline treatment	2. Attachment of peptides
3. Laser etching	3. Anodization of the surface	3. Attachment of antibiotics
4. Nanoparticle compaction	4. Peroxidation	4. Attachment of growth factors
5. Porous tantalum trabecular metal	5. Fluoride treatment	5. Attachment of bone remodeling agents
	6. Vacuum treatment	
	7. Plasma coatings and surface treatments	

Laser etched

Laser-etched implants uses lasers as a micromachining tool to produce selective modification and generation of complex microstructures at micrometer and nanometer level. Advantages of the laser technique include the absence of chemicals and the convenience of being able to incorporate it into the routine manufacturing.

Nanoparticle compaction

Compaction of nanoparticles on the implant surface conserves the chemistry of the underlying surface while changing or modifying the chemistry and structure of the outer surface layer.[11]

Porous tantalum trabecular metal

Porous tantalum trabecular metal has recently been designed and developed to enhance secondary stability via bone ingrowth, and it is incorporated on the surface of the titanium dental implants.[12] It is not a regular coating or surface treatment. The titanium alloy and the porous tantalum trabecular metal of the implant are prepared separately. The porous vitreous carbon scaffold acts as a second layer on the titanium implants. Tantalum is deposited onto the vitreous carbon scaffolds using chemical vapor deposition or infiltration and then laser welded onto the titanium alloy core.[13,14] The porous layer, with a structure similar to trabecular bone, is used to improve the bonding between the osseous tissue and the dental implants through osseointegration.[12,15,16]

Methods of Implant Surface Alteration: Chemical and Additive

Acid etched

The acid-etched implant is pitted by acid (HCl/H_2SO_4 mixture or 2% HF/10% HNO_3) removal of grains and grain boundaries, which are more sensitive to etching. This selective removal of the implant surface is minimally rough (S_a value of 0.3–1.0 μm).[17] The surface morphology is determined by the implant material, the microstructure of the surface, the type of acid, and the soaking duration. To further improve the implant surface, the implants are blasted by particles before acid etching. This subsequent etching removes embedded blasted particles and gives a dual surface roughness (S_a value of 1–2 μm).[18]

Alkaline treatment

Alkaline oxidation can be achieved by soaking the implant in high alkaline solutions followed by heat treatment. There are several examples of such methods (eg, soaking in 4–5 M sodium hydroxide solution and heat treatment at 600°C for 24 hours or soaking in boiling alkali solution of 0.2 M sodium hydroxide and heat treatment at 1400°C for 5 hours). The alkaline treatment can be preceded by acid etching to increase the porosity of the titanium surface.[19]

Anodized

The anodized implant undergoes anodic oxidation, an electrochemical process in an electrolyte that results in a microstructure surface with micrometer-sized open pores. This process involves passing a current through the implant as the anode with phosphoric acid as the electrolyte to form the surface oxide. The surface morphology could be modified by varying the anode potential, the electrolyte composition, the temperature, and the current.[20] Ions such as phosphorus,[21] calcium,[22] and magnesium[23] can also be integrated into the implant surface via modification of the electrolyte composition.

Peroxidation

Peroxidation of the implant surface produces a titania gel layer through treatment with a perioxide-based chemical agent. Chemical treatment of implant surfaces with hydrogen peroxide results in chemical dissolution and oxidation of the titanium surface. When titanium surfaces react with hydrogen peroxide, titania gel layers are formed. The thickness of titania layer formed can be controlled by adjusting the treatment time, and it has been demonstrated that when immersed in simulated body fluid, thicker layers of titania gel are more favorable for the deposition of apatite.[24]

Fluoride modified

This is a fluoride-modified nanostructure implant surface. The surface modification involved blasting with titanium oxide (TiO_2) and treating with dilute hydrofluoric acid. The optical interferometer microscopy data of the fluoride-modified implant surface showed a mean surface area roughness of 1.24–1.26 μm.[25]

TABLE 2

Surface treatment and dental implants

	Surface	Implant Systems
Physical (and subtractive) surface treatment	Machined	Brånemark
		Astra Tech
	Sand blasted	Biohorizons Tapered (blasted with resorbable blasted media)
		Astra Tech Tioblast (blasted with titanium oxide)
		Zimmer Dental MTX SwissPlus (blasted with hydroxylapatite)
		Inclusive tapered implants
	Laser etched	Brånemark BioHelix
		Biohorizon Laserlok
	Nanoparticle compaction	Nil
	Porous tantalum trabecular metal	Zimmer Trabecular Metal
Chemical (and additive) surface treatment	Acid etched	Biomet 3i OsseoTite
	Chemical reaction with surface elements	Nil
	Anodization	Nobel biocare TiUnite
	Peroxidation	Nil
	Fluoride modified	Astra Tech OsseoSpeed
	Vacuum treatment	Nil
	Plasma coating	Dentsply Frialit
		Adin Touareg CloseFit
		Adin Touareg-OS
		Straumann ITI titanium plasma-sprayed (TPS)
		Hiossen HS II
		Hiossen HG II/ III
Biological/biomimetic surface treatment	Bioactive coating/HA	Zimmer Screw Vent
		Zimmer Dental MP-1
	Attachment of peptides	Nil
	Attachment of enzymes	Nil
	Attachment of BMP	Nil
Combination of surface treatment	Sandblasted and acid etched	Straumann SLA
		Straumann SLA active
		Dentsply Ankylos Plus
		Camlog Promote
		Adin Touareg-S
		Adin Swell
		Adin One
		Hiossen ET III SA
		DentiumUSA SuperLine
		DentiumUSA Implantium
		DentiumUSA SimpleLine II
	Sandblasted, acid etched, chemical modified	Biomet 3i NanoTite
		Astra Tech OsseoSpeed

Vacuum treatment

Vacuum treatment of the implant surface can be achieved by glow-discharge deposition of coating material from a solid target or by reactions in the gas phase. It can also be achieved by exposing the titanium surface to a glow-discharge of energetic ions that specifically modify the surface properties by bombardment.

Another method in vacuum treating implants is the ion implantation method; this involves the bombardment of high energy ions, which penetrate the surface of the implant.[26] It can be controlled by varying the concentration and the energy of the ions and can increase the corrosion resistance by forming a titanium-nitrogen (Ti-N) surface.[27] This method can also be used to develop antimicrobial surfaces on the implant via deposition of fluoride and silver (Ag) ions on implant surface without toxicity.[26]

Plasma coated

Plasma sprayed calcium phosphate-coated implants have improved bioactivity. Hydroxyapatite (HA) is a form of calcium phosphate coating. Plasma spraying with HA can increase the surface area, as well as increase the average surface roughness (R_a value 5.0 $\pm$ 1.0 μm).[28]

METHODS OF IMPLANT SURFACE ALTERATION: BIOLOGICAL/BIOMIMETIC SURFACE ALTERATION

Bioactive coatings

The bioactive coating of titanium implants involves precipitation of calcium phosphate apatite crystals on the titanium surface. The deposition of calcium phosphate onto titanium surfaces can be achieved by using a titanium cathode and a platinum anode to generate a current producing a brushite coating, which is hydrothermally processed to apatite on the implant surface.[29,30] It can also be achieved by immersing in stimulated body fluids and producing a heterogeneous nucleation and growth of bone-like calcium phosphate crystals on the implant surface.[31,32] Calcium phosphate apatite

formation on titanium metals in stimulated body fluids can be further enhanced by heat treatment after exposure to strongly acidic or alkaline solutions.[33,34]

Attachment of peptides

This involves the coating of titanium implant surface with synthetic arginylglycylaspartic acid (RGD) peptides that contain binding sites for integrin receptors.[35]

Attachment of antibiotics

Antibiotics such as cephalothin, carbenicillin, amoxicillin, cefamandol, tobramycin, gentamicin, and vancomycin can bind to calcium-based coatings of implants, as well as be released from it. This antibiotic-releasing coating also retains its antimicrobial properties.[36]

Attachment of growth factors

The implant surface can be coated with osteogenesis-stimulating agents to accelerate angiogenesis and bone formation around implants. These growth factors coating the implant can be bone morphogenetic proteins (BMPs), transforming growth factor β1 (TGF-β1), vascular endothelial growth factors (VEGFs), platelet-derived growth factors (PDGFs), or insulin-like growth factors (IGFs). BMPs can be directly incorporated into the implant surface,[37] or they can be incorporated via the use of a plasmid containing the BMP-encoding gene.[38]

Attachment of a bone remodeling agent

The implant surface can also be coated with bone remodeling-associated agents like bisphosphonates. Bisphosphonates have a great chemical affinity for calcium phosphate molecules and thus can be incorporated via the biomimetic coating procedure. Bisphosphonates can also be coupled with RGD peptides and chemically absorbed on titanium to produce synergistic osteogenic effects.[39]

Methods and Techniques to Characterize Modified Surfaces

Surface topography

Wennerberg et al[40] published guidelines for surface topography measurement. To obtain reliable quantification, methods for topographic measurement should have both excellent vertical and lateral resolution, as well as a reasonable analysis area. A scanning electron microscope (SEM) can qualitatively evaluate surface structure in both the micro- and nano-range. Scanning electron microscopy can also obtain quantitative topographic data via stereo imaging and image analysis.

Surface elemental composition

Different spectroscopic techniques used to evaluate the surface elemental composition include Auger electron spectroscopy (AES), energy dispersive X-ray spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), and secondary ion mass spectroscopy (SIMS). It is important to note that the surface composition of the implant differ from that of the corresponding bulk material.

Surface phase composition

Techniques enabling phase identification of implant surface crystal structure include Raman spectroscopy, high-resolution transmission electron microscopy (HRTEM), electron diffraction, X-ray diffraction, and electron backscattering diffraction.

Surface energy

Surface energy is commonly measured by the contact angle between the implant surface and liquids of differing surface tension. The surface tension is then calculated from a Zisman plot of the measured contact angle. A more simple analysis can also be done using the water contact angle as a representative parameter of the surface tension.

Effect of Different Surface Modification Techniques on the Surface Properties of the Titanium Surface

Different surface modification techniques have been mainly used to improve the surface roughness and hydrophilicity. Some modified surface compositions could also contain bioactive substances. Implant morphology such as grooves, ridges, and tool marks can influence the interaction between the bone and the implant. The implant morphology can also increase the overall surface area available for osseointegration. Rougher surfaces can stimulate attachment, differentiation, and proliferation of bone cells, thus increasing bone growth and mineralization. Rougher surfaces with an open structure have been shown to induce faster and more effective osseointegration. Unfortunately, this rougher surface substrate tends to accumulate bacteria.

Most techniques are applied to change the surface roughness. Surface roughening can be induced by machining, blasting, laser etching, acid/alkaline etching, anodization, and coatings. The roughness of implant surfaces treated via machining is normally less than 1 μm. The surfaces treated via blasting and plasma spraying result in the highest roughness. The size of the blasting particles determines the roughness. However, coating machined titanium or sandblasting with acid-etched titanium surfaces with polyelectrolytes does not alter the surface roughness.[41] There is no consensus on the optimal implant roughness that produces the best effects on bone.

Anodized titanium substrates have numerous nanoscale surface features.[42] Anodization in acetic acid can produce intensely etched interlaced grooves that are distributed homogeneously on the surface. Further high density submicron scale pores can be developed with sulphuric acid anodization. When both treatments are combined, the surface created is a multilevel surface-porous anodic layers consisting of interlaced macroscopic grooves overlaid with submicrometer pores.[43] Furthermore, elements like calcium, oxygen, phosphorous, and sodium can be introduced onto the titanium surface during anodization.[44]

Hydrophilicity contributes to the wettability and the surface energy of the implant surface and is affected by the surface

TABLE 3

In vitro human cell–base studies on modified surfaces for dental implants

Study	Evaluated Surfaces	Cell Type Used
Acevedo-Morantes et al[58]	Combinatorial physical vapor deposition technique on titanium alloy with: tin (Sn), chromium (Cr), or niobium (Nb) Titanium alloy (Ti6Al4V) control	Human fetal osteoblastic cell line (hFOB 1.19)
Arciniegas et al[59]	Ni-free titanium alloys: Ti19.1Nb8.8Zr (main surface oxide layer is TiO_2 followed by Nb_2O_5 and ZrO_2) Ti41.2Nb6.1Zr (main surface oxide layer is Nb_2O_5 followed by TiO_2 and ZrO_2)	Preosteoblastic MG-63 cells
Bagno et al[66]	Sandblasted Sandblasted and acid treated Sandblasted, acid treated and RGD-containing $(GRGDSP)_4$ K peptide Sandblasted, acid treated and (351-359)HVP sequence mapped on Human Vitronectin Peptide	Human osteoblast
Bagno et al[67]	Oxidized Silanized Peptide-grafted (sequence 351–359 mapped on Human Vitronectin Peptide)	Human osteoblast
Baldi et al [71]	Five different implants: Tapered Internal (BioHorizons Implant Systems) with resorbable blast texturing and Laser-Lok collar Nanotite (3i Implant Innovations), osseotite surface combined with nanometer scale crystalline deposition of calcium phosphate; (R_a, 0.28 $\pm$ 0.06 μm)[33] Full Osseotite (FOSS; 3i Implant Innovations), osseotite surface dual acid-etched with HF and HCl/H2SO4; (R_a, 0.8 $\pm$ 0.14 μm)[34] Straumann SLActive Standard Implant (Institut Straumann AG), sandblasted with large grits (0.25– 0.50 mm), acid-etched with HCl/H2SO4, rinsed under N_2 protection, and preserved in isotonic NaCl solution; (R_a, 2.93 $\pm$ 0.46 μm)[34] SwissPlus (Zimmer Dental), grit-blasted with hydroxylapatite (Microtextured Titanium, MTX) Control, cells grown in absence of dental implant	Human osteoblast-like cells, Saos-2
Berardi et al[82]	Commercially pure titanium sandblasted and with laser-produced holes with diameters of 5, 10, and 20 μm sandblasted control	Human osteoblast-like cells, Saos-2
Brama et al[60]	Titanium grade 2 carbide coated (coating process used pulsed laser deposition technology to produce surfaces of titanium carbide) Uncoated titanium	Human fetal osteoblast line (hFOB 1.19) Primary human osteoblasts (hOB)
Bucci-Sabattini et al[51]	Sandblasted/acid-etched surface as control Calcium phosphate (CaP) low impregnated surface (Ossean) as test group	Human Saos-2 osteoblasts Bone mesenchymal stem cells
Cecchinato et al[52]	Magnesium-loaded mesoporous titanium oxide coating Magnesium-loaded mesoporous titanium oxide coating with 3D nanostructure	Transfected Human Fetal Osteoblast (hFOB)
Cei et al[96]	Sandblasted 5-, 10-, and 20-μm laser engineered micropore	Osteoblast-like cells harvested from 6 healthy patients
Conserva et al[83]	Six types of implants with 4 different surface treatments: Turned Sandblasted Acid etched Anodized	Osteoblast-like Saos-2 cells

TABLE 3

Extended

Evaluated Parameters	Conclusion
Cell adhesion Cell proliferation Protein adsorption	Of the 3 surface treatments, Nb promoted stronger osteoblast attachment and spreading Sn- and /Nb-rich regions showed the highest osteoblast proliferation rate and Cr-rich regions the lowest
Cell adhesion Cell proliferation Alkaline phosphatase activity	Cell adhesion response (after 4 and 8 hours of incubation) in both tested titanium alloys was not statistically different from the titanium control After longer incubation times, 2 nm thick layer of amorphous niobium oxide (Nb_2O_5) detected on both alloys, has a significant effect favoring cell adhesion and differentiation
Cell adhesion Adhesion strength	Presence of peptide promote cell adhesion The bioactive peptide covalently bond to the sandblasted and acid etched surface to improve cell adhesion Surface with the HVP-related peptide exhibited the highest adhesion capacity followed by the surface with RGD-containing peptide when compared with sandblasted, and sandblasted and acid etched
Cell adhesion Adhesion strength	Peptide grafted surface increased cell adhesion compared with other surfaces Peptide grafted surface promoted the fastest increase in cell adhesion
Cell adhesion Bone-related protein: Bone morphogenic proteins Osteomodulin Osteoprotegerin Osteoglycin Osteoclast stimulating factor Osteopontin Osteoblast cadherin Bone sialoprotein Alpha-2 collagen type I others	No significant difference in cell adhesion and viability in the 5 implants Fourteen osteogenic genes differently modulate implants tested: Tapered Internal implant and Nanotite implant induced the expression of 4 osteogenic genes and decreased expression of 1 osteogenic gene. Full Osseotite increased 2 osteogenic genes and decreased 1 osteogenic gene. Straumann SLActive and Swiss Plus decreased the expression of 4 and 5 osteogenic gene respectively, with SwissPlus activating 1 osteogenic gene
Cell adhesion Bone-related protein: Alkaline phosphatase activity	At days 3 and 7, the laser-pitted surface stimulated a higher production of alkaline phosphatase compared with control At day 10, there was no significant difference between the test and control
Cell proliferation Bone-related protein: Alkaline phosphatase A2 pro-collagen type 1 Osteocalcin Bone morphogenic protein-4 Transforming growth factor β Core binding factor-1/osteoblast specific factor 2	Coating of titanium implants with a layer of titanium carbide increases the biocompatibility of titanium, stimulates proliferation, adhesion and differentiation of osteoblasts Expression of genes central to osteoblast differentiation were up-regulated in primary human osteoblasts and hFOB1.19 grown on carbide coated titanium compared with uncoated titanium Genes involved in modulation of osteoclastogenesis and osteoclast activity were unchanged compared with control
Cell adhesion Cell proliferation Bone-related protein: Alkaline phosphatase activity	Test surface induced significantly higher cell differentiation levels than the control Specific alkaline phosphatase activity was higher on the Ossean surface than on the control surface
Cell morphology Cell viability Gene expression of bone markers Cell mineralization	Osteoblasts viability, adhesion, and gene expression were unaffected by the addition of magnesium, but were enhanced by the 3D nanostructure of the titanium oxide layer
Cell morphology Cell viability Cell proliferation	Laser-engineered porous titanium surfaces promote cell viability and proliferation Hemispherical porosity of 20 μm seems to trigger greater cell response
Cell adhesion Cell proliferation Cell viability Bone-related protein: Alkaline phosphatase	All the implants tested supported cell adhesion, proliferation and differentiation with no cytotoxicity effects SaOS-2 cells spread more rapidly on sandblasted surfaces Turned surfaces showed the lowest cell proliferation Sandblasted surfaces showed the greatest alkaline phosphatase activity values per cell

TABLE 3

Continued

Study	Evaluated Surfaces	Cell Type Used
Córdoba et al[65]	Quercitrin-nanocoated titanium surfaces Aminosilanized surfaces (3-aminopropyl)triethoxysilane) a cross-linker molecule between titanium and quercitrin Control titanium	Human bone marrow stromal cells (hMSCs)
Cunha et al[111]	Laser-induced periodic surface structures Nanopillars Microcolumns covered with laser-induced periodic surface structures	Human mesenchymal stem cells
De Peppo et al[75]	Nanopatterned model surfaces Surfaces covered by polystyrene nanoparticles	Human mesenchymal stem cells
Deng et al[74]	Titanium with nanotopography prepared with high-energy shot-peening (HESP) technique: shot peened for 3 minutes, shot peened for 5 minutes Untreated control	Preosteoblastic MG-63 cells
Dimitrievska et al[86]	Titania–hydroxyapatite nanocomposite coating (10% wt hydroxyapatite sprayed by high-velocity oxy-fuel) Standard FDA-approved single-phase hydroxyapatite coating Pure titanium	Human mesenchymal stem cells Human mesenchymal stem cell-derived osteoblasts
Fassina et al[95]	Control titanium alloy Ti6Al4V sandblasted by Al2O3 powder Sandblasted by Al_2O_3 powder then plastically and deformed with a punching process	Human Saos-2 osteoblasts
Fritsche et al[112]	Polished titanium alloy (Ti6Al4V)	MG-63 osteoblastic cells
Gittens et al[88]	Commercially pure titanium treated to produce microsmooth machined and pickled "pre-treatment" disks Commercially pure titanium treated to produce microrough "sandblasted large-grit acid-etched" disks Above mentioned specimens were further processed using a simple oxidation treatment to superimpose nanostructures on the surface to yield nanomodified microsmooth or nanomodified microrough specimens	Human osteoblast-like MG63 cells Human mesenchymal stem cells
Györgyey et al[113]	Commercially pure titanium rods, sandblasted and acid etched: control Commercially pure titanium rods, sandblasted and acid etched, and ablation with Nd:YAG laser Commercially pure titanium rods, sandblasted and acid etched, and ablation with KrF excimer laser	MG-63 osteoblastic cells
Hélary et al[68]	Polished titanium Chemically oxidized titanium Electrochemically oxidized titanium Grafting of bioactive polymer to titanium obtained by radical copolymerization of sodium styrene sulfonate and pendant methacrylic functions linked to the titanium oxide layer	MG-63 osteoblastic cells

Table 3

Extended Continued

Evaluated Parameters	Conclusion
Cell adhesion and metabolic activity Cell mineralization Bone-related protein: Alkaline phosphatase activity	Quercitrin-nanocoated surfaces showed a faster stem cell adhesion than control surfaces Quercitrin-nanocoated surfaces enhanced the mineralization of the cells after 21 days of cell culture
Cell adhesion Cell spreading Cell differentiation	Cells spreading was more significant on polished surfaces than on laser-textured surfaces Average focal adhesion area was significantly larger for cell cultured on polished surfaces than on textured surfaces Matrix mineralization and bone-like nodule formation were enhanced by the presence of laser-induced periodic surface and nanopillars in comparison with cells seeded on microcolumn-textured surfaces
Cell morphology Cell proliferation Osteogenic differentiation	The morphology, proliferation, and osteogenic differentiation of human mesenchymal stem cells could be adjusted by the size of nanoparticles on surfaces
Cell adhesion Cell viability Bone-related protein: Alkaline phosphatase	Compared to the untreated control, the nanotopographic surface increased the adhesion, viability, and differentiation of MG-63 cells
Cell adhesion and proliferation Cell differentiation	There were no significant differences in the cell adhesion on the different coatings
Cell maturation and morphology Mineralization Bone-related protein: Osteocalcin Alkaline phosphatase activity	At the alkaline phosphatase activity peak (7 days), the activity on the titania–hydroxyapatite nanocomposite coatings was significantly higher than the activity on the pure hydroxyapatite and titanium coated substrates At the osteocalcin expression peak (day 21) the expression was higher on the titania–hydroxyapatite nanocomposite than on the pure hydroxyapatite and titanium coated substrates
Cell proliferation Protein coating DNA content	In comparison with sandblasted titanium surface, the plastic deformation increased the cell proliferation and the surface coating of bone matrix
Cell adhesion Adhesion force	A statistically significant increase in shear stress was observed during the first 6 hours After 12 hours of adhesion a distinct loss in adhesion strength was detected Another increase in average shear stress to was detected after 48 hours
Surface wettability Bone-related protein: Osteocalcin Osteoprotegerin Vascular endothelial growth factors	Nanostructures superimposed onto microrough titanium surfaces synergistically enhance MG63 osteoblastic maturation, but suppress human mesenchymal stem cell osteoblastic differentiation MG63s and human mesenchymal stem cell on microrough surfaces also had higher levels of osteocalcin compared with controls, and was also significantly higher than on the nanomodified surfaces MG63s produced higher levels of osteoprotegerin and vascular endothelial growth factors on both microrough groups compared with microsmooth controls, with the highest levels found on the combined nanostructured surfaces Human mesenchymal stem cells produced slightly lower levels of osteoprotegerin on the different microrough groups compared with controls, with the lowest levels found on the nanostructured surfaces Human mesenchymal stem cells produced higher levels of vascular endothelial growth factors on SLA surfaces compared controls, while the levels on nanostructured surfaces were slightly lower than controls
Cell viability Cell attachment and proliferation Bone-related protein: Alkaline phosphatase	No significant differences between the laser-ablated surfaces and the sand-blasted and acid-etched surfaces on cell attachment and proliferation After 7 days of incubation, there was no statistical differences in the secretion of ALP in the control compared with the laser-treated groups
Cell attachment Bone-related protein: Alkaline phosphatase activity	Cell attachment was improved on grafted titanium samples Alkaline phosphatase activity and calcium nodules formation were significantly enhanced on grafted titanium surfaces compared with unmodified surfaces

TABLE 3

Continued

Study	Evaluated Surfaces	Cell Type Used
Hempel et al[102]	Polished titanium Sandblasted/hot acid etched titanium surfaces Sandblasted/hot acid etched titanium surfaces that were partly alkaline treated	MG-63 SaOS-2 Human mesenchymal stromal cells
Heo et al[94]	Smooth machined 75 μm Al_2O_3 blasted 125 μm Al_2O_3 blasted Anodized	Human adult periodontal ligament stem cell
Herrero-Climent et al[57]	Machined commercially pure grade 3 titanium Commercially pure grade 3 titanium acid-etched in hydrofluoric acid Commercially pure grade 3 titanium grit-blasted with alumina particles Commercially pure grade 3 titanium grit-blasted with acid-etched treatment	MG-63 osteoblastic cells
Kaluderovic et al[114]	Commercially pure titanium Coating of commercially pure titanium in 1.25 M NaOH electrolyte Coating of commercially pure titanium with $Ca(H_2PO_4)_2$ electrolyte Coating of commercially pure titanium in the 1.25 M NaOH electrolyte and second in the 0.02 M $Ca(H_2PO_4)_2$ electrolyte Coating of commercially pure titanium in 1.25 M NaOH electrolyte with additional soaking in a 0.02 M $Ca(H_2PO_4)_2$ solution for the infiltration of calcium and phosphate ions Commercially pure titanium etched with a mixture of equal parts of concentrated hydrochloric acid and sulphuric acid	Primary human osteoblast cells from 1 male human mandibular bone sample without any pathologic clinical or radiographic evidence
Khan et al[115]	Polished titanium surface Rough-hydrophobic titanium surface Rough-hydrophilic titanium surface Tissue culture plastic	Human bone marrow derived stromal cells from 3 unrelated donors
Kim et al[64]	Magnesium (Mg) implantation in titanium surfaces treated with sand blasting using large grit and acid etching Titanium surfaces treated with sand blast using large grit and acid etching	Human mesenchymal stem cells

TABLE 3

Extended Continued

Evaluated Parameters	Conclusion
Cell morphology	Impaired spreading was found on both sandblasted and etched surfaces
Metabolic activity	Proliferation after 4 and 7 days increased on polished surface compared with both sandblasted and etched surfaces
Bone-related protein:	
Alkaline phosphatase activity	Alkaline phosphatase activity of human mesenchymal stromal cells and MG-63 was increased on polished surface compared with both sandblasted and etched surfaces
Prostaglandin E2	Prostaglandin E2 formation of human mesenchymal stromal cells and MG-63 was significantly higher in both sandblasted and etched surfaces compared with polished surfaces
Cell proliferation	Cell proliferation was higher on rough surface than other surfaces, especially in 75 μm Al_2O_3 blasted surfaces
Bone-related protein:	
Osteocalcin	Osteocalcin highly expressed on Al_2O_3 blasted surfaces
Osteopontin	
Type I collagen	
Glyceraldehyde 3-phosphate dehydrogenase	
Cell adhesion	The surfaces with increasing roughness show more osteoblastic adhered cells
	This effect was most pronounced on samples blasted and blasted with acid-etching
	The acid-etching treatment did not improve the osteoblastic adhesion in comparison with the control
	The grit-blasted with acid etching surface produced higher cell adhesion compared with only grit-blasted surfaces
	Cell adhesion were statistically significant on grit-blasted and the grit-blasted with acid-etched surfaces versus smooth and acid-etched surface
Cell proliferation	There were higher cell proliferation on the surface made from commercially pure titanium
Cell morphology	
Bone-related proteins:	Commercially pure titanium etched with a mixture of equal parts of concentrated hydrochloric acid and sulphuric acid induced the lowest production of osteocalcin, the lowest production of sialoprotein, and the lowest proliferation rate
sialoprotein	
osteocalcin	
Cell proliferation	A higher numbers of cells were present on polished titanium than rough surfaced titanium at all time points
Osteogenic mineralization	
Bone-related protein:	By day 21, rough-hydrophilic titanium surface compared with all other surfaces produced significantly higher levels of osteocalcin, osteoprotegrin, growth differentiation factor 15, and calcium deposition
Runt-related transcription factor 2	
Osteopontin	
Bone sialoprotein type 2	By 21 days, alkaline phosphatase activity decreased on all titanium surfaces compared with 14 days
Osteocalcin	
Osteoprotegrin	Osteogenic differentiation of bone marrow derived stromal cells is a dynamic process affected by extrinsic factors
Growth differentiation factor 15	
Alkaline phosphatase activity	Surface modifications of titanium implants form micro rough substrates that enhance osteogenic differentiation and function in uncommitted human bone marrow derived stromal cells
Cell proliferation	Cell proliferation was lower on sandblasted and acid-etched titanium surface than on Mg implanted sandblasted and acid-etched titanium surface observed up to 15 days
Cytotoxicity	
Cell adhesion	
Calcium accumulation	Cell adhesion was higher on Mg implanted sandblasted and acid-etched titanium surfaces than on sandblasted and acid-etched titanium surface
Bone-related protein:	
Alkaline phosphatase activity	Alkaline phosphatase activity and calcium accumulation was higher in Mg implanted sandblasted and acid-etched titanium surfaces than sandblasted and acid-etched titanium surface

TABLE 3

Continued

Study	Evaluated Surfaces	Cell Type Used
Kim et al[99]	Titanium surface modified with heparin and then immobilized with bone morphogenetic protein-2 Titanium surface with bone morphogenetic protein-2 Unmodified titanium surface	Human osteosarcoma MG-63 cell line
Kim et al[90]	Untreated titanium Ultraviolet (UV) radiation-treated titanium implants Alendronate sodium trihydrate-soaked titanium implants UV radiation and alendronate sodium trihydrate treated titanium implants	Human osteosarcoma MG-63 cell line
Klein et al[92]	Smooth pretreatment surfaces Rough sandblasted/acid-etched surfaces Rough sandblasted/acid-etched surfaces further processed to provide a highly hydrophilic surface Tissue culture polystyrene served as control	Commercial hipbone-derived human osteoblastic cell line
Klinger et al[101]	Machined Sandblasting and acid etching Sandblasting, acid etching and hydrofluoric acid treatment (fluoride modified)	Human osteoblast-like cells, Saos-2
Knabe et al[80]	Smooth machined Acid etched and sandblasted surface Titanium plasma-sprayed coating Plasma-sprayed porous HA coating	Human bone derived cells
Kohal et al[104]	Machined titanium Anodized titanium Sandblasted and acid-etched zirconia Machined zirconia	Human osteoblast
Kopf et al[81]	Blood coagulated on a micro-roughened hydrophobic titanium surface Blood coagulated on a hydrophilic micro-roughened titanium surface with nanostructures Whole human blood preincubation on a micro-roughened hydrophobic titanium surface Whole human blood preincubation on a hydrophilic micro-roughened titanium surface with nanostructures	Primary human bone cells
Lavenus et al[76]	Vapour deposition of: 30-nm nanpores 15-nm nanpores 300-nm nanpores	Human mesenchymal stem cells

Table 3

Extended Continued

Evaluated Parameters	Conclusion
Cell adhesion Cell proliferation Calcium concentration Bone-related protein: Alkaline phosphatase activity Osteocalcin Osteopontin	Cell proliferation was significantly increased in MG-63 cells on titanium substrates containing bone morphogenetic protein-2 compared with the unmodified titanium group at 3 and 7 days of culture At 3 and 7 days, cell proliferation was also significantly increased in MG-63 cells on titanium surface modified with heparin and then immobilized with bone morphogenetic protein-2 compared with titanium surface with bone morphogenetic protein-2 Osteoblasts cultured on titanium surface modified with heparin and then immobilized with bone morphogenetic protein-2 produced increased alkaline phosphatase activity, calcium deposition, osteocalcin and osteopontin levels compared with titanium surface with bone morphogenetic protein-2 or unmodified titanium surface
Cell proliferation Cytotoxicity Cell adhesion Osteoblast differentiation Bone-related protein: Alkaline phosphatase activity	MG-63 cells cultured on UV radiation and alendronate sodium trihydrate treated titanium implants showed significantly higher cell proliferation, cell attachment, cell growth alkaline phosphatase activity, and calcium mineralization than those cultured on other implants The treatment of titanium surfaces with UV and alendronate sodium trihydrate may synergistically enhance osteoblastic differentiation and mineralization
Cellular proliferation Cell adhesion and differentiation Bone-related protein: Integrin subunits β1 and αv Runt-related transcription factor 2 -Collagen type Iα Alkaline phosphatase Osteocalcin	Smooth pretreatment surfaces resulted in an immature, dividing osteogenic phenotype which was high in proliferation rates, low in integrin levels, and low in specific osteogenic cell differentiation Rough sandblasted/acid-etched surfaces especially the hydrophilic surfaces promoted both cell adhesion as well as the maturation of osteogenic precursors into post-mitotic osteoblasts In the first 48 hours, hydrophilic rough sandblasted/acid-etched surfaces resulted in lowest cell proliferation rates but exhibited highest levels of the investigated integrins, runx-2, collagen type Iα, alkaline phosphatase, and osteocalcin
Cell proliferation Cell differentiation Bone-related protein: Alkaline phosphatase activity Osteocalcin	Cell proliferation, alkaline phosphatase activity and osteocalcin expression was not statistically different between machined and rough surfaces Cell proliferation and osteocalcin expression was not statistically different between fluoride treated and not fluoride treated Alkaline phosphatase activity was significantly higher in fluoride modified surface than not fluoride treated
Cell proliferation Cell differentiation Bone-related protein: Type I collagen Osteocalcin Osteopontin Osteonectin Alkaline phosphatase Bone sialoprotein	HA-coated titanium has the most effect on cell differentiation and osteogenic gene expression compared with the others tested Acid etched and sandblasted surface induced higher cell proliferation and differentiation than plasma sprayed surface
Cell proliferation Bone-related protein	Cell proliferation was retarded in the anodized surface during the first 7 days After 28 days, cell proliferation was at the same level for all surfaces At 21 days, up-regulation of bone related genes was seen in sandblasted and acid-etched zirconia
Cell adhesion Cell mineralization Bone-related protein: Alkaline phosphatase Collagen type I	More cells were significantly attached on the whole blood preincubated hydrophilic micro-roughened titanium surface with nanostructures compared with other surfaces with or with preincubation with blood The overall expression of alkaline phosphatase and collagen type I was increased in titanium surfaces preincubated with blood compared with surfaces without blood pretreatment The preincubation with blood resulted in a dense fibrin network over the entire surface of the hydrophilic micro-roughened titanium surface Blood pretreatment promoted an earlier and enhanced mineralization of human bone cells cultivated on hydrophilic micro-roughened titanium surface with nanostructures compared with micro-roughened hydrophobic titanium surface
Cell adhesion Cell differentiation Bone-related protein: Runt-related transcription factor 2 Collagen type I alpha 1 Bone sialoprotein Osteocalcin Alkaline phosphatase and others	Expression of integrins was significantly modulated by titanium nanopore size Cell differentiation was more potent in 30- and 150-nm nanopore surface than 300-nm surface

TABLE 3

Continued

Study	Evaluated Surfaces	Cell Type Used
Lewallen et al[100]	Surgical-grade porous titanium implanted with adipose-tissue-derived mesenchymal stromal/stem cells Standard tissue culture plastic as control	Adipose-tissue-derived mesenchymal stromal/stem cells from several different patients
Liang et al[97]	Commercially pure titanium Zinc implanted pure titanium (Implantation times: 20, 40, 60, and 80 minutes)	MG-63 human osteosarcoma cells
Lin et al[46]	Polished grade 4 titanium Grit-blasted and acid etched grade 4 titanium Grit-blasted and acid etched grade 4 titanium, then treated by the electrochemical anodization	MG63 cells
Longo et al[61]	Zirconia-blasted grade 2 titanium using Ion Plating Plasma Assisted technology to coat titanium implants with a thin but hard nanostructured layer composed of titanium carbide and titanium oxides, clustered around graphitic carbon Zirconia-blasted grade 2 titanium	Saos-2 clonal human osteosarcoma cell line
Mamalis et al[108]	Commercially pure grade 4 titanium Commercially pure grade 4 titanium standard sandblasted with large grits and acid etched to produce a rough surface (SLA) chemically modified sandblasted and acid etched (SLActive)	Human bone marrow-derived mesenchymal cells

TABLE 3

Extended Continued

Evaluated Parameters	Conclusion
Cell attachment	Porous titanium implants can be biologically enhanced using adipose-tissue-derived mesenchymal stromal/stem cells
Cell proliferation and multipotency,	
Cell differentiation phenotypes	Cells achieve greater proliferation and sustain extracellular matrix production longer when seeded on porous titanium implanted with adipose-tissue-derived mesenchymal stromal/stem cells
Bone-related protein:	
Alkaline phosphatase	
Integrin-binding sialoprotein	The production of mRNAs for extracellular proteins by cells grown on porous titanium implanted with adipose-tissue-derived mesenchymal stromal/stem cells is robust and follows temporal patterns distinct from cells grown on standard tissue culture plastic
Matrix gla protein	
Aggrecan	
Adiponectin	
Biglycan	
Decorin	
Collagenous proteins	
Bone morphogenic protein	
Runt-related transcription factor 2	
Genes involved in cell cycle progression, mesenchymal lineage identity , osteoblastic transcription factors, and epigenetic regulators	
Cell morphology	After 24 hours, a higher density of cells was present on the zinc implanted pure titanium surfaces compared with the commercially pure titanium surface
Cell differentiation	
Cell proliferation	Increased zinc implantation resulted in a greater density of attached cells
	There is significantly greater MG-63 cell growth on Zn-modified titanium surfaces than on pure titanium surfaces
	The modified zinc implanted pure titanium surfaces may inhibit MG-63 cell apoptosis and promote proliferation
Cell adhesion and morphology	Electrochemical functionalization can modify not only the surface chemistry but also wettability
Bone-related protein:	
Alkaline phosphtase	The grit-blasted and acid-etched titanium surface presented the most hydrophobic surface property
	The most hydrophilic material was the grit-blasted and acid-etched titanium treated by the electrochemical anodization
	Polished titanium presented intermediate hydrophilicity compared with the grit-blasted and acid-etched titanium surface
	The hydrophilic grit-blasted and acid-etched titanium surface highly affected the blood compatibility of titanium when contacted with blood
	The hydrophilic grit-blasted and acid-etched titanium surfaces exhibited highest alkaline phosphatase activity, followed by the grit-blasted and acid-etched titanium surface and the polished titanium surface
	The alkaline phosphatase activity was surface roughness dependent
Cell adhesion	The proliferation, adhesion, and spreading of cells cultured on coated titanium samples are higher than on uncoated titanium
Cell proliferation	
Bone-related protein:	The nanostructured layer caused an overexpression of many early genes correlated to proteins involved in bone turnover and an increase in the number of surface receptors
Alkaline phosphatase	
Transforming Growth Factor-β1	
Osteocalcin	The chemistry of the layer induces a better formation of blood clots and a higher number of adhered platelets, compared with the uncoated cases, and these are useful features to improve the speed of implant osseointegration
Collagen 1a2	
Paxillin	
Integrin α3β1	
Four and Half LIM domains protein	
Runt related transcription factor 2	
Cell attachment and proliferation	Hydrophilic chemical modification decreases cell attachment and proliferation; and up-regulates early osteoblastic differentiation gene
Bone-related proteins and transcription genes that control of osteogenic differentiation	Nineteen genes were significantly up-regulated when human mesenchymal cells were cultured on the SLA surfaces and 27 genes were significantly up-regulated when human mesenchymal cells were cultured on the SLActive surfaces

TABLE 3
Continued

Study	Evaluated Surfaces	Cell Type Used
Mangano et al[87]	Laser sintered Ti–6Al–4V alloy implant surface modification Acid-etched commercially available implant	Human alveolar osteoblasts Dental pulp stem cells extracted from teeth of healthy adults
Mansell et al[84]	Lipid-functionalized solid titanium (covalent modification of titanium with lysophosphatidic acid using different mechanisms including use of thermal initiator and azobisisobutyronitrile enabled lysophosphatidic acid attachment to the metal surface) Unmodified titanium (control) Piranha-treated titanium (control) Silane-treated titanium (control)	Human osteoblast-like MG63 cells
Mendonça et al[78]	Machined Acid etched Sol-gel derived nanostructured surface (Titania-Anatase, Titania-Rutile, Alumina and Zirconia nanocoating)	Human mesenchymal stem cells
Min et al[50]	Commercially pure titanium without any modification (control) Titanium surface sandblasted with large grit and acid etched Anodized titanium surface Titanium surface coated with calcium phosphorus Cell attachment test was performed to select 2 candidate surfaces for laminin-derived functional peptide coating: the anodized and commercially pure titanium surfaces were selected for peptide application	Human osteosarcoma osteoblast-like cells
Minagar et al[79]	Titania–zirconia–zirconium titanate nanotube with diverse nanoscale dimensional characteristics fabricated via anodization at applied potentials of 20, 25, 30, and 35 V (The annealed nanotubes exhibited a mean inner diameter of 40 ± 12, 59 ± 17, 64 ± 23, and 82 ± 26 nm when anodized at applied potentials of 20, 25, 30, and 35 V respectively)	SaOS2 cells
Morra et al[53]	Grade 2 titanium samples treated by galvanostatic anodization Titanium implants modified by acrylic acid surface grafting-collagen I coupling	Bone-marrow-derived human mesenchymal cells
Nayab et al[49]	Calcium ion-implanted titanium Nonimplanted titanium control	MG-63 cells

Table 3

Extended Continued

Evaluated Parameters	Conclusion
Cell adhesion	There was no significant difference in adhesion potential between the surfaces regardless of dental pulp stem cells or osteoblasts
Bone-related protein:	
Osterix	Both surfaces showed a considerable fitness for cell adhesion at all times compared with control surface
Runt-related transcription factor 2	
Bone morphogenic protein	Dental pulp stem cells expressed osteocalcin only during their differentiation into osteoblasts
Vascular endothelial growth factors	
	An increased osteocalcin expression indicated that more cells have switched toward the osteogenic lineage
	At day 15, cells grown on laser sintered titanium alloy surface showed a higher expression of osteocalcin than the acid-etched titanium surfaces
	Vascular endothelial growth factor mRNAs are highly expressed by both dental pulp stem cells and osteoblasts on all the surfaces
	All the surfaces lead to the formation of blood vessels in association with bone
Cell proliferation	The piranha and untreated control samples yielded more hydrophilic surfaces than lipid-functionalized solid titanium and silane treated surfaces
Surface wettability	
Bone-related protein:	The lipid-functionalized solid titanium form an effective covalent attachment
Alkaline phosphatase	The lipid-functionalized solid titanium exhibit a greater capacity to support human osteoblast maturation compared with controls, as supported by marked, synergistic increases in total alkaline phosphatase activity
Cell differentiation	Nanoscale Alumina surface promoted greater cell differentiation and osteoblastic gene expression than machined and acid-etched surfaces
Bone-related protein:	
Alkaline phosphatase	
Bone sialoprotein	
Runt-related transcription factor 2	
Osteocalcin	
Osteoprotegerin	
Osterix mRNA	
and a panel of 76 genes related to osteogenesis	
Cell attachment	The anodized titanium surface had a significantly higher cell attachment than any of the other investigated surfaces
Bone-related protein:	
Alkaline phosphatase activity	On the commercially pure titanium surface, the laminin-derived functional peptide coating significantly increased cellular alkaline phosphatase activity and the expression levels of alkaline phosphatase and bone sialoprotein mRNA when compared with the scrambled peptide-coated and uncoated surfaces
Bone sialoprotein	
Cell adhesion and spreading	The lowest density of SaOS2 cells arose on the nanotubular surface with inner diameter of 59 ± 17 nm because of its high roughness amplitude parameters
	The next lowest density evolved on the nanotubular surface with inner diameter of 64 ± 23 nm because this has the lowest surface energy and the lowest roughness spacing parameters
	The highest density of SaOS2 cells was on the nanotubular surface with inner diameter of 40 ± 12 nm because of the optimum nanospacing
	The second highest density appeared on the nano-tubular surface with inner diameter of 82 ± 26 nm because of the existence of proteins on the tube walls with thicknesses of 24 ± 7 nm
Cell adhesion and growth	Titanium implants modified by acrylic acid surface grafting-collagen I coupling enhanced cell adhesion compared with galvanostatic anodization
	At each experimental times, significant increases in cell density were found in titanium implants modified by acrylic acid surface grafting-collagen I coupling when compared with anodized titanium
Cell activity	Surfaces implanted with calcium ions can enhance the expression of certain bone-associated components in vitro
Bone-related protein:	
Alkaline phosphatase	
Bone morphogenetic protein receptor-1β	
Bone sialoprotein	
Osteonectin	
Osteopontin	

TABLE 3

Continued

Study	Evaluated Surfaces	Cell Type Used
Nayab et al[63]	Calcium ion-implanted titanium Nonimplanted titanium control	MG-63 cells
Olivares-Navarrete et al[91]	Pretreatment grade 2 unalloyed titanium Grade 2 unalloyed titanium sandblasted and acid etched Hydrophilic modified grade 2 unalloyed titanium sandblasted and acid etched Standard tissue culture plastic as control Cover-glass control	Mesenchymal stem cells MG63 cell line
Olivares-Navarrete et al[45]	Grade 2 unalloyed pretreatment titanium Grade 2 unalloyed pretreatment titanium grit blasted followed by acid etching in HCl/H_2SO_4 Grade 2 unalloyed pretreatment titanium further coated with amorphous graphitic carbon films produced by a DC-magnetron sputtering system Grade 2 unalloyed pretreatment titanium grit blasted followed by acid etching in HCl/H_2SO_4 then further coated with amorphous graphitic carbon films produced by a DC-magnetron sputtering system Standard tissue culture plastic as control	Human osteoblast-like MG63 cells Human bone marrow mesenchymal stem cells
Rausch-Fan et al[105]	Controls (no specimens) Hydrophobic acid-etched Hydrophobic coarse-grit blasted, acid-etched surfaces Hydrophilic acid-etched Hydrophilic coarse-grit-blasted	Osteoblast-like cell MG-63 Primary human alveolar osteoblasts
Rosales-Leal et al[56]	Polished Titanium hydrofluoric acid (HF) etched Al_2O_3 blasted Al_2O_3 blasted +HF etched	Osteoblast-like osteoscarcoma cell MG-63
Pareta et al[70]	Ionic plasma deposited polymeric and metallic coatings on titanium or titanium alloys Direct nitrogen ion immersion plasma deposited polymeric and metallic coatings on titanium or titanium alloys Titanium or titanium alloy controls	Human osteoblasts
Park et al[41]	Chitosan coated machined titanium surface or sandblasted/acid etched titanium surface Poly(L-glutamic acid) coated machined titanium surface or sandblasted/acid etched titanium surface Poly(L-lysine) coated machined titanium surface or sandblasted/acid etched titanium surface	MG-63 cells
Pei et al[62]	Recombinant human dentin matrix protein 1 modified titanium Polished titanium Polished titanium treated with alkaline and water	MG-63 cells
Ramaglia et al[77]	Sandblasted and acid-etched commercially pure titanium Smooth machined commercially pure titanium	SaOS-2 human osteoblast-like cells

TABLE 3

Extended Continued

Evaluated Parameters	Conclusion
Immunostaining of the cell proliferation-associated nuclear Ki-67 antigen	Calcium ion-implanted titanium enhanced expression of the cell proliferation-associated nuclear Ki-67 antigen and increased numbers of mitotic cells
DNA content and synchronization of MG-63 cells Measurement of S phase	Cell grown on calcium ion-implanted titanium synchronized at the G1/S boundary, more rapidly re-entered and progressed through the S and G2/M phases of the cell cycle than on titanium
Cell differentiation Bone-related protein: Osteocalcin Osteoprotegerin Vascular endothelial growth factor-A Transforming growth factor β1 Alkaline phosphatase specific activity and protein levels α2β1 integrin signaling Dkk2 expression and levels	Hydrophilicity increased the expression of alkaline phosphatase, osteoprotegerin, and osteocalcin but significantly decreased the expression of vascular endothelial growth factor-A and TGF-β1 α2β1 integrin mediated osteoblast response to microstructured titanium helped induce co-cultured mesenchymal stem cells differentiation to osteoblasts On the titanium substrates, secreted Dkk2 acted both on the osteoblasts in an autocrine manner and on the distal mesenchymal stem cells to promote their differentiation in the osteoblast lineage
Cell attachment Surface wettability Bone-related protein: Osteocalcin Osteoprotegerin Vascular endothelial growth factor-A Active transforming growth factor β1 Latent transforming growth factor β1	In MG63 and human mesenchymal stem cells cultures, cell number was decreased and alkaline phosphatase specific activity increased on titanium substrates especially grit blasted and acid etched titanium with no difference between carbon coated titanium or uncoated In MG63 and human mesenchymal stem cells cultures, osteocalcin, osteoprotegerin, vascular endothelial growth factor-A, and active transforming growth factor β1 increased with surface roughness and was highest on grit blasted and acid etched titanium Osteogenic differentiation was greater on rough surfaces compared with smooth regardless of surface chemistry Integrins β1, α1, or α2 supports osteoblast maturation on rough surfaces regardless of surface chemistry Integrin αv supports osteoblast maturation only on graphitic carbon-coated surfaces, and not on titanium
Cell proliferation Bone-related protein: Alkaline phosphatase activity Osteocalcin Osteoprotegerin Transforming growth factor-beta1 (TGF-β1) Vascular endothelial growth factor (VEGF)	Cell proliferation in MG-63 and primary alveolar osteoblast was highest in controls followed by hydrophobic acid-etched, hydrophilic acid-etched, hydrophobic coarse-grit-blasted and acid-etched, and hydrophilic coarse-grit-blasted and acid-etched surfaces Hydrophilic coarse-grit-blasted and acid-etched exhibited the highest alkaline phosphatase and osteocalcin production followed by hydrophobic coarse-grit-blasted and acid-etched, hydrophilic acid-etched, and hydrophobic acid-etched
Cell adhesion Cell proliferation	Cell adhesion was greater on Al_2O_3 blasted and Al_2O_3 blasted +HF etched surfaces compared with the other tested surfaces Cell proliferation was improved in hydrofluoric acid (HF) etched surfaces followed by Al_2O_3 blasted +HF etched surfaces
Cell adhesion	Osteoblast adhesion was increased by ionic plasma deposited and direct nitrogen ion immersion plasma deposited coatings These coatings modify the surface roughness and surface energy
Cell proliferation Gene expression Integrin expression Bone-related protein: alkaline phosphatase activity	Increase alkaline phosphatase activity on polyelectrolyte coated surfaces Enhanced osteocalcin and osteoprotegerin production on chitosan coated surface Integrin subunit expression was sensitive to surface chemistry and roughness
Cell attachment Cell proliferation Bone-related protein: alkaline phosphatase activity	Recombinant human dentin matrix protein 1 coating improved attachment, proliferation and alkaline phosphatase activity
Cell morphology Cell adhesion Cell proliferation Expression of bone differentiation markers and extracellular matrix components	Surface topography may influence the phenotypical expression of human osteoblast-like cells Collagen I deposition and α2-β1 receptor expression was significantly increased Cell proliferation was not affected, but cell adhesion was increased

TABLE 3
Continued

Study	Evaluated Surfaces	Cell Type Used
Ramis et al[48]	Polished titanium Fluoride modified titanium Grit blasted titanium Grit blasted and fluoride treated titanium	Normal human osteoblasts (NHOst cell system)
Ravanetti et al[69]	Acid-etched Anodic spark deposition and alkali etching	Human primary osteoblasts
Ravichandran et al[93]	Poly-lactic-co-glycolic acid nanofibers coated on pure titanium or titanium alloy Poly-lactic-co-glycolic acid/collagen nanofibers coated on pure titanium or titanium alloy Poly-lactic-co-glycolic acid/nano-hydroxyapatite nanofibers coated on pure titanium or titanium alloy Poly-lactic-co-glycolic acid/collagen/nano-hydroxyapatite nanofibers coated on pure titanium or titanium alloy	Human mesenchymal stem cells
Santander et al[44]	Electrical deposited calcium phosphate coating to form a biomimetic advanced surface Polished titanium	Human mesenchymal stem cells
Shi et al[110]	Acid-etched Hydrophilic acid etched Coarse-grit blasted and acid-etched Hydrophilic coarse-grit blasted and acid-etched	Human umbilical vein cell MG-63 cells
Si et al[85]	Plasma sprayed micro-roughened titanium dioxide/zirconia coating Titanium dioxide coating	Human mesenchymal stem cells
Smith et al[42]	Nanostructured poly-lactic-co-glycolic acid coated Anodized titanium Titanium control	Previously characterized human osteoblasts (ATCC CRL 11371)
Uggeri et al[103]	Sandblasted with ZrO_2 + acid-etched (soft-SLA) Sandblasted with Al_2O_3 + acid-etched (hard-SLA) Control: machined smooth surface	Jaw osteoblasts
Wall et al[89]	Rough hydrophobic surface that was sand-blasted and acid-etched Rough hydrophobic surface that was sand-blasted and acid-etched then further chemically modified to have high wettability/hydrophilicity Control: smooth polished	Human mesenchymal stem cells
Xie et al 2011[43]	Anodized porous titania layer following an anodizing regimen with pure titanium anodized at 100, 150, and 180 V Directly anodized without the following the anodizing regimen as control Some surfaces following the anodizing regimen at 15 0V was further subjected to hot water treatment	MG-63 cells

TABLE 3

Extended Continued

Evaluated Parameters	Conclusion
Gene expression	Toll-like receptor 3, ankylosis-progressive homolog, decorin, osteocalcin, and runt-related transcription factor-2 were responsive genes to roughness Distal-less homeobox-2 and Tuftelin-1 were responsive genes to fluoride treatment Responsive genes to both fluoride treatment and roughness were collagen type I, parathyroid hormone-like hormone, hairy and enhancer of split-1, follistatin, ectonucleotide pyrophosphatase /phosphodiesterase-1, and thyroid hormone receptor-alpha
Cell morphology Cell adhesion Cell proliferation Cell metabolic activity	Enhanced osteoblast adhesion and higher metabolic activity for the anodic spark deposition and alkali etching treated group
Cell proliferation Cell mineralization Bone-related protein: alkaline phosphatase activity	Cell adhesion and proliferation were highest in poly-lactic-co-glycolic acid/collagen/nano-hydroxyapatite nanofibers coated surfaces Rate of proliferation was significantly higher on the poly-lactic-co-glycolic acid/nano-hydroxyapatite and poly-lactic-co-glycolic acid/collagen/nano-hydroxyapatite scaffolds compared with the poly-lactic-co-glycolic acid and poly-lactic-co-glycolic acid/collagen fibers on both pure titanium and titanium alloy Cell differentiation and mineralization was enhanced by mineralized poly-lactic-co-glycolic acid /collagen nanofibers surfaces
Cell adhesion Cell proliferation Gene expression	Improved adhesion and proliferation Induces the expression of RUNX2 and Osteopontin
Cell co-culture of human umbilical vein cell with MG63s Cell proliferation Expression of angiogenesis-related genes	In co-culture conditions, hydrophobic acid-etched surface promoted both proliferation and expression of angiogenesis associated genes in human umbilical vein cell, this is in contrast to previous mono-culture studies The behavior of endothelial cells could be influenced by both titanium surfaces and interaction with osteoblasts Co-culture could show more information than mono-culture
Cell adhesion and proliferation Osteogenic differentiation	No significant difference of cell proliferation and extracellular calcium deposition was observed for titanium dioxide/zirconia coating compared with titanium dioxide coating Significantly higher alkaline phosphatase activity, Runx2 expression levels and osterix was observed for the titanium dioxide/zirconia coating compared with the titanium dioxide coating
Cell adhesion	Cell density could be enhanced on the titanium coated with nanostructured poly-lactic-co-glycolic acid Applied nanotopography to titanium (via anodization) and porous poly-lactic-co-glycolic acid (via sodium hydroxide chemical etching) can enhance osteoblast cell proliferation
Cell proliferation Alkaline phosphatase activity	The kinetics of cell adhesion affected the rate of subsequent proliferation The cells grown on rough surfaces showed polygonal morphology, and elongated morphology on smooth surfaces The cell colonization was promoted by surface with soft-SLA more than hard-SLA Cells proliferated to a greater extent on S-SLA and machined smooth surface than on H-SLA
Cell attachment Cellular proliferation Wettability Gene expression profiling of human mesenchymal stem cell populations Early induction of mineralized matrix deposition by human mesenchymal stem cell populations	Cell number decreased in testing samples, and expressed higher levels of the osteogenic markers SPP1, RUNX2 and BSP early in culture. Deposits of calcified matrix and increased expression of the osteogenic promoter WNT5A were observed in testing samples at earlier time Osteogenic differentiation of human mesenchymal stem cell populations on rough titanium surfaces enhance early mineralization of the matrix, and improved the wettability of the hydrophilic surface
Cell adhesion Cell growth	A 2-step anodization treatment can produce a multi-level surface-porous anodic layers consisting of interlaced macroscopic grooves overlaid with submicron pores, this alters the surface topography and surface chemistry The macroscopic grooves promote osteoblast adhesion and growth, while the submicron scale pores might be beneficial to osteoblast adhesion

TABLE 3
Continued

Study	Evaluated Surfaces	Cell Type Used
Yang et al[98]	Hydroxyapatite coating on titanium Hydroxyapatite and heparin-bone morphogenetic protein-2 complex on titanium Heparin-bone morphogenetic protein-2 complex on titanium	MG-63 cells
Yeung et al[109]	Plasma-sprayed hydroxyapatite coating Titanium dioxide-plasma electrolytic oxidation coating Calcium phosphate-plasma electrolytic oxidation coating	Human osteosarcoma cells (MG-63)
Zhao et al[47]	Novel hydroxylated/hydrated titanium surfaces	Osteoblast-like osteoscarcoma cell MG-63

composition. Present surface modification techniques can increase surface area and hydrophilicity.[45] Electrochemical functionalization can modify both the surface chemistry and the wettability. Electrochemical anodization of the grit-blasted and acid-etched titanium produces the most hydrophilic material compared with polished titanium and grit-blasted and acid-etched titanium without electrochemical anodization.[46] The changes of surface compositions show positive effects by incorporating hydroxide ions[47], fluoride ions,[48] calcium ion,[49,50] phosphate ions,[51] magnesium ions,[52] and bioactive substances.[53] Generally, a combination of surface modifications are used to create a titanium surface with a combination of surface properties. However, there is a lack of optimized quantitative data to support any single or combined implant surface modification.

Biological Responses to Various Surfaces for Dental Implants

Laboratory human cell–based studies

The adhesion, differentiation, and proliferation of human osteoblastic cells on the titanium surface are crucial for successful osseointegration. This osteoblastic cell interaction with the titanium surface can be modulated by the chemical composition, the surface energy, the roughness, and the topography of the respective surfaces (Table 3).

Osteoblastic cells adhere in several different phases.[54] In the attachment phase, the titanium surface links to the osteoblast via physiochemical forces like ionic forces and van der Waals forces. In the adhesion phase, the osteoblast binds further via different biomolecules like integrins, cell surface receptors, extracellular matrix protein, cell membrane proteins, and cytoskeleton proteins.[4,54] Integrins transmit a signal from the extracellular matrix to regulate osteoblast adhesion, motility, shape, growth, and differentiation.[54,55] Integrins form focal contacts when extracellular matrix-adsorbed biomolecules interact between the bone cells and the titanium surface.[4,55]

The following titanium surface modifications can promote stronger cell adhesion. There is greater cell adhesion for aluminium oxide (Al_2O_3)-blasted and Al_2O_3-blasted and hydrofluoric acid–etched surfaces compared with polished titanium.[56,57] Niobium vapor deposition on titanium alloy promoted stronger osteoblast attachment and spreading compared with tin vapor deposition, chromium vapor deposition, and controls.[58] Niobium oxide has a significant effect favoring cell adhesion and differentiation.[59] Coating of titanium implants with titanium carbide[60,61] or with recombinant human dentin matrix protein 1[62] increases the proliferation, adhesion, and differentiation of the osteoblast. In addition, electrical deposition of calcium phosphate can also improve adhesion and proliferation.[44] Similarly, calcium ion–implanted titanium can enhance expression of the cell proliferation–associated nuclear Ki-67 antigen and increase the number of mitotic cells.[63] Osteoblast viability, adhesion, and gene expression were enhanced by the addition of 3D nanostructure to the magnesium-loaded mesoporous titanium oxide coating[52] or by magnesium implantation of the sandblasted and acid-etched titanium surface.[64] Quercitrin-nanocoated surfaces also promote faster stem cell adhesion and mineralization than control surfaces.[65] Compared with unmodified surfaces, cell adhesion can also be significantly enhanced by grafting peptides[50,66,67] or bioactive polymer to titanium.[68] Titanium implants modified by acrylic acid surface grafting–collagen I coupling can enhance cell adhesion compared with galvanostatic anodization.[53] In contrast, multilevel surface-porous anodic layers with macroscopic grooves produced by a 2-step anodization treatment can promote osteoblast adhesion and growth.[43] Anodic spark deposition with alkali etching,[69] ionic plasma deposition, and direct nitrogen ion immersion plasma

Table 3

Extended Continued

Evaluated Parameters	Conclusion
Cell proliferation Alkaline phosphatase activity Calcium deposition Osteocalcin mRNA gene expression	MG-63 human osteosarcoma cell lines grown on hydroxyapatite and heparin-bone morphogenetic protein-2 complex on titanium increased the amounts of alkaline phosphatase activity, calcium deposition and the levels of osteocalcin mRNA gene expression compared with those grown on hydroxyapatite coating, heparin-bone morphogenetic protein-2 complex on titanium or pristine titanium At 3 and 7 days, cell proliferation on both surfaces with heparin-bone morphogenetic protein-2 complex on titanium with or without hydroxyapatite was significantly higher
Cell viability Collagen synthesis	Plasma electrolytic oxidation coating are significantly more hydrophilic and have lower surface roughness than the plasma-sprayed coatings MG-63 viability was lower in titanium dioxide-plasma electrolytic oxidation coatings Collagen synthesis was significantly higher in the calcium phosphateand titanium dioxide-plasma electrolytic oxidation coatings than in plasma-sprayed hydroxyapatite coatings
Cell differentiation Bone-related protein: Transforming growth factor-beta1 (TGF-β1) Prostaglandin E2	Osteoblasts on hydroxylated/hydrated titanium surfaces exhibited more cell differentiation, osteocalcin, increased alkaline phosphatase activity, and higher production of prostaglandin E2 and TGF-β1

deposition[70] of polymeric and metallic coatings can also enhance osteoblast adhesion.

However, commercially available dental implant surfaces are comparable in terms of bone cell adhesion. Baldi et al[71] compared 5 different implants (Tapered Internal [BioHorizons Implant Systems], Nanotite [3i Implant Innovations],[72] Osseotite [3i Implant Innovations],[73] Straumann SLActive Standard Implant [Institut Straumann],[73] and SwissPlus [Zimmer Dental]) and found no significant difference in cell adhesion.

The following titanium surface modifications can promote greater cell differentiation. The osteogenic differentiation was greater on rough surfaces compared with smooth surfaces and is unaffected by the surface chemistry.[45] The expression of integrins and cell differentiation was significantly modulated by titanium nanopore size. Nanotopographic surfaces can increase cell differentiation[74,75] and was more potent in 30- and 150-nm nanopore surfaces than the 300-nm nanopore surface.[76] The surface topography may influence the phenotypic expression of osteoblast-like cells.[77] Similarly, Mendonça et al[78] reported that nanoscale alumina surface promoted greater cell differentiation and osteoblastic gene expression than machined and acid-etched surfaces. The nanostructured layer can induce overexpression of many bone turnover proteins and increase the number of osteoblast surface receptors.[61] Optimum nanospacing of titania–zirconia–zirconium titanate nanotubular surfaces with inner diameter of 40 ± 12 nm induces the highest density of bone cells.[79]

In addition, Knabe et al[80] observed that HA-coated titanium has the most effect on cell differentiation compared with the other surfaces tested. On a hydroxylated titanium surface, osteoblasts were more differentiated and exhibited increased alkaline phosphatase activity.[47] The calcium phosphate–impregnated surface induced significantly higher cell differentiation levels and alkaline phosphatase activity than the controls.[51] Titanium surfaces preincubated with blood also exhibited increased alkaline phosphatase and collagen type I compared with control surfaces.[81] Sandblasted and laser-pitted titanium surfaces produced higher early production of alkaline phosphatase compared with control.[82,83] Lipid-functionalized solid titanium[84] and laminin-derived functional peptide coating on pure titanium[50] support osteoblast maturation via synergistic increases in total alkaline phosphatase activity. Plasma-sprayed titanium dioxide/zirconia coating can significantly increase alkaline phosphatase activity, Runx2 expression levels, and osterix compared with titanium dioxide coating.[85] Titania-HA nanocomposite coatings promote significantly higher alkaline phosphatase activity and osteocalcin than the pure hydroxyapatite- and titanium-coated substrates.[86] Osteocalcin is a late osteoblastic differentiation marker; an increase in osteocalcin would indicate that more undifferentiated cells have switched to the osteogenic lineage.[87] Osteocalcin was higher on the sandblasted and acid etched surfaces compared with controls and was significantly higher when nanostructures are superimposed via a simple oxidation process on these surfaces.[88]

Osteogenic differentiation on rough titanium surfaces is enhanced by early mineralization of the osteogenic matrix, and can be further enhanced by the wettability of hydrophilic surface.[89] Osteoblastic differentiation and mineralization can also be enhanced by ultraviolet radiation and alendronate sodium trihydrate treatment of the titanium surfaces.[90] Gene expression for osteoblast differentiation were up-regulated in human bone cells on carbide-coated titanium compared with uncoated titanium.[60] Cell differentiation to osteoblasts can be further induced by α2β1 integrin–mediated osteoblast response to microstructured titanium.[91]

Turned titanium surface exhibited the lowest cell proliferation.[83] Other smooth pretreated titanium surfaces reported a high proliferation rate, but with an immature osteoblastic phenotype that is low in integrin levels and cell differentiation.[92] The following are some of the titanium surface modifications that can promote greater cell proliferation. The

rate of cell proliferation was significantly higher on the poly-lactic-co-glycolic acid/nano-HA nanofiber–coated and the poly-lactic-co-glycolic acid/collagen/nano-HA–coated scaffolds compared with the poly-lactic-co-glycolic acid and poly-lactic-co-glycolic acid/collagen fibers for both pure titanium and the titanium alloy.[93] Heo et al[94] reported that cell proliferation was higher on rough 75 μm Al_2O_3-blasted surfaces compared with machined and anodized surfaces. This sandblasted surface subjected to additional plastic deformation with a punching process can further increase the cell proliferation.[95] Laser-engineered porous titanium promotes cell viability and cell proliferation, with a greater cell response at a hemispherical porosity of 20 μm.[96] Similarly, Rosales-Leal et al[56] reported improved cell proliferation in hydrofluoric acid–etched surfaces and Al_2O_3 blasted. These acid-etched and sandblasted surfaces induced higher cell proliferation compared with plasma-sprayed surfaces.[80] Cell proliferation can be further enhanced by magnesium implantation of the sandblasted and acid-etched titanium surface[64] and by zinc-implanted pure titanium.[97] The titanium surface immobilized with bone morphogenetic proteins with[98] or without heparin modification can also increase cell proliferation compared with unmodified titanium.[99] Porous titanium implanted with adipose tissue–derived mesenchymal stem cells can also enhance cell proliferation and extracellular matrix production.[100]

However, variations in methods or materials used in titanium surface modifications do not always promote cell proliferation. Klinger et al[101] observed that cell proliferation was not statistically different between machined surfaces and rough sandblasted surfaces that were acid etched with or without hydrofluoric treatment. Furthermore, hot acid etching of sandblasted surfaces resulted in decreased cell proliferation compared with the polished surface.[102] Uggeri et al[103] reported that cell proliferation is also greater in machined smooth titanium and in zirconium oxide–sandbasted and acid-etched titanium compared with aluminium oxide–sandbasted and acid-etched titanium. In addition, Kohal et al[104] observed that cell proliferation was retarded in anodized surfaces for the first 7 days; however, at 28 days, cell proliferation was at the same level for all surfaces.

Rausch-Fan et al[105] compared hydrophobic and hydrophilic surfaces and found increased cell proliferation in hydrophobic acid–etched, followed by hydrophilic acid–etched, hydrophobic coarse grit–blasted and acid-etched, and hydrophilic coarse grit–blasted and acid-etched surfaces. Hydrophilic surfaces have a higher surface energy in comparison to hydrophobic surfaces. These hydrophilic-modified titanium surfaces support homogeneous spatial osteoblast cell growth and mineral deposition compared with hydrophobic titanium surfaces.[106,107] The chemically modified hydrophilic SLActive surfaces up-regulated more osteogenic transcription genes than the hydrophobic SLA surfaces.[108]

Hydrophilicity can increase the expression of alkaline phosphatase, osteoprotegerin, and osteocalcin and can significantly decrease VEGF-A and TGF-β1.[91] The plasma electrolytic oxidation coating is significantly more hydrophilic and induces significantly higher collagen synthesis compared with the plasma-sprayed hydroxyapatite coatings.[109] In contrast to previous mono-culture studies, the hydrophobic acid–etched surface promoted both proliferation and expression of angiogenesis-associated genes in human umbilical vein cells under coculture conditions.[110]

Conclusion

The surface chemistry, the surface topography, and the surface energy of the titanium surface have a crucial effect on osteoblast and osteocyte function. However, this relationship between patterns of gene expression and adhesion/ differentiation/ proliferation of bone cells on various titanium surfaces is not clear. This interaction is the basis for successful bone-to-implant contact during osseointegration. These in vitro studies can be used to postulate how these individual cells would react to different dental implant surface characteristics in the living body. Thus, selected titanium surface characteristics that induce favorable osteoblast function in vitro can then be further evaluated via animal and human studies. This will provide further information on how these surfaces would perform in relation to the longevity of these surface-treated implants when subjected to the oral environment and to functional loading. Animal and human studies are beyond the scope of this review and will be discussed in future reviews.

Abbreviations

AES: Auger electron spectroscopy
Ag: silver
Al_2O_3: aluminium oxide
BMPs: bone morphogenetic proteins
$Ca(H_2PO_4)_2$: calcium dihydrogen phosphate
CaP: calcium phosphate
Cr: chromium
EDS: energy dispersive X-ray spectroscopy
HA: hydroxyapatite
HCL: hydrochloric acid
HF: hydrofluoric acid
HRTEM: high-resolution transmission electron microscopy
H_2SO_4: sulphuric acid
IGFs: insulin-like growth factors
Mg: magnesium
NaOH: sodium hydroxide
Nb: niobium
Nb_2O_5: niobium oxide
PDGFs: platelet-derived growth factors
RGD: arginylglycylaspartic acid
S_a: surface area roughness
SEM: scanning electron microscope
SIMS: secondary ion mass spectroscopy
Sn: tin
TGF-β1: transforming growth factor β1
Ti-N: titanium-nitrogen
TiO_2: titanium oxide
UV: ultraviolet
VEGFs: vascular endothelial growth factors
XPS: X-ray photoelectron spectroscopy
ZrO_2: zirconium oxide

Note

M.T., W.X., H.E., and J.B.S. declare that they have no conflict of interest as to the content of the manuscript. S.R.J. holds common stock in the company DENTSPLY International (York, Pa), which is a company that markets various dental implant systems. Nonetheless, S.R.J. does not believe that the above-mentioned disclosure presents any conflict of interest with respect to the subject matter of this review.

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