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ABSTRACT

Bone marrow stromal cells are regulated by the chemical and physical features of a biomaterial surface. When grown on titanium (Ti) and Ti alloy surfaces, such as titanium-aluminum-vanadium, with specific topographies that mimic the microscale, mesoscale, and nanoscale features of an osteoclast resorption pit, they undergo a rapid change in cell shape to assume a columnar morphology typical of a secretory osteoblast. These cells exhibit markers associated with an osteoblast phenotype, including osteocalcin and osteopontin, and they secrete factors associated with osteogenesis, including bone morphogenetic protein 2, vascular endothelial growth factor, and neurotrophic semaphorins. The pathway involves a shift in integrin expression from $\alpha5\beta1$ to $\alpha2\beta1$ and signaling by Wnt5a rather than Wnt3a. Conditioned media from these cultures can stimulate vasculogenesis by human endothelial cells and osteoblastic differentiation of marrow stromal cells not grown on the biomimetic substrate, suggesting that the surface could promote osteogenesis in vivo through similar mechanisms. In vivo studies using a variety of animal models confirm that implants with biomimetic surfaces result in improved osseointegration compared with Ti implants with smooth surfaces, as do meta-analyses comparing clinical performance of implant surface topographies.

Bone-facing nonresorbable implant components capable of bone ingrowth provide implant stability through the formation of a mechanical interlock. Over two decades, refinements have focused on pore size, pore configuration, modulus of elasticity at the interface with the bone, and degree of micromotion during initial bone ingrowth.[1] The advent of spine interbody fusion devices opened the door to examine the possibility that modifications to the surface at the microscale would improve bone formation and osseointegration of the implant. The dental implant industry provided a large literature on clinical success of a variety of surface designs, particularly on titanium implants.[2,3] The preclinical information underlying

the clinical studies indicated that surfaces that had a microstructure resembling an osteoclast resorption pit supported the more robust osteogenic response based on a number of outcomes, including osteoblast differentiation of bone marrow stromal cells (MSCs) and osteoprogenitor cells.[4] These studies, discussed later, also examined the mechanisms involved in the osteogenic response, enabling the application of this literature to orthopaedic implants manufactured using titanium and its alloys.[5,6]

Biology of Bone Healing and the Effect of Implant Surface Topography

Bone must be able to respond to a variety of loading conditions, requiring it to be metabolically active, continuously remodeling in response to mechanical stimulation, systemic factors such as hormones, and local factors produced by cells present within the tissue. Bone tissue consists of a mineralized type I collagen matrix; osteoblast lineage cells that synthesize, calcify, and maintain the matrix; osteoclasts that resorb the matrix and prepare it for subsequent rounds of formation; and osteocytes, the most abundant bone cell, that coordinate the activity of osteoblasts and osteoclasts and may also resorb the bone matrix.[7] Bone tissue also contains a complex vascular network together with its associated nerves and immune lineage cells, including monocytes, macrophages, and lymphocytes.[8,9]

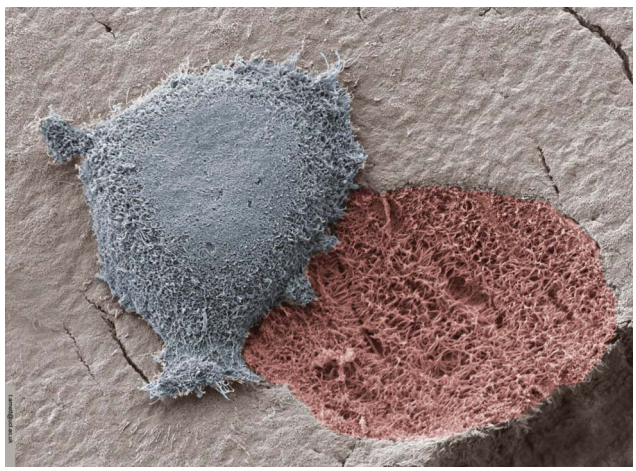
When an implant is placed in the bone, fluid at the surgical site adsorbs onto the surface. The affinity for and conformation of proteins on the surface are determined by its physical and chemical properties. One of the constituents in the wound fluid, fibronectin, adsorbs onto the surface and provides binding sites for the alpha 5 beta 1 ($\alpha5\beta1$) integrins present in MSCs. The clot that forms between the bone bed and the implant surface also contains a complex fibrillar network that enables MSCs to migrate to the site.[10] Monocytes and macrophages are also present at the site, and recent studies indicate that Ti substrates that have a complex microscale topography similar to an osteoclast resorption pit and have a hydrophilic surface chemistry support the prohealing macrophage M2 phenotype rather than the proinflammatory M1 phenotype.[9,11] The MSCs produce factors that modulate the response of immune cells within the environment, and the immune cells produce factors that recruit additional MSCs and immune cells.[12,13]

These surface properties also support osteoblastic differentiation of MSCs. When grown on such a surface, MSCs undergo a change in cell polarity, assuming a columnar morphology rather than being flattened and spread.[14] Their integrin profile changes from predominantly $\alpha5\beta1$ to $\alpha2\beta1$ and $\alpha1\beta1$, which bind RGD and GFOGR motifs in type 1 collagen.[10,15] In addition, they express markers associated with an osteoblast phenotype, including osteocalcin and osteopontin, and factors associated with modulation of osteoclast activity such as osteoprotegerin, which is a decoy receptor for RANK ligand, and transforming growth factor beta 1.[16,17] These changes occur rapidly and do not require the addition of any osteogenic media implants, such as dexamethasone or beta-glycerol phosphate.[18] The MSCs also express factors associated with vasculogenesis, such as vascular endothelial growth factor-165 and fibroblast growth factor-2, and factors associated with neurogenesis, such as semaphorins 3A, 3C, and 4A.[13,19]

Analysis of Ti, titanium-zirconium, and titanium-aluminum-vanadium (Ti6Al4V) surfaces that have been generated using various grit blasting/acid etching methods shows that osteoblastic differentiation of MSCs and osteoprogenitor cells is favored by topographies that mimic osteoclast resorption pits created during normal bone remodeling[5,6] (Figure 1). The osteoclast resorption pit has an average width of 30 to 100 μm, an average depth of 8 μm, and a nanotextured surface averaging 60 nm. Moreover, the pits are not isolated on the surface but are linked to each other by a scalloped border, created as the osteoclast migrates across the bone surface.[20]

The most effective biomimetic surfaces have irregular closely spaced microscale pits overlaid with microscale, mesoscale, and nanoscale textures that are shaped like

Figure 1



Photograph showing an osteoclast resorbing a bone surface and leaving the organic matrix exposed. Image courtesy of Prof Tim Arnett, University College London.

pointed isosceles triangles,[5] reminiscent of osteoclasts' resorption pits with the mesoscale and nanoscale structures on the resorption pit surface. When MSCs and osteoprogenitor cells are cultured on these surfaces, they produce high levels of BMP2[21] and express receptors for BMP2, indicating that they are inducing the osteoblast phenotype using autocrine and paracrine mechanisms.[22] This hypothesis is supported by the observation that addition of anti-BMP2[22] antibodies to the cultures blocks the effect of the surface on osteoblast differentiation. In addition, they produce Wnt11,[23] causing a shift from producing Wnt3a to Wnt5a (Figure 2).

Recent work has shown that semaphorins are also involved in mediating the effects of surface topography on osteoblastic differentiation of MSCs. Semaphorin 3A (sema3A) can work independently of Wnt3a, Wnt5a, and BMP2 to enhance osteoblast differentiation, with activation of sema3A occurring alongside of Wnt5A.[19] Addition of antisema3A antibodies to cultures of MSCs grown on microtextured Ti substrates blocks the effect of the surface on osteoblastic differentiation of MSCs, indicating the important role that these factors play in the process.

These results also imply that factors produced by MSCs on the surface could regulate the osteoblast differentiation of MSCs and osteoprogenitor cells not on the surface, and this is exactly what coculture studies show to be the case.[22] Addition of anti-BMP2 antibodies to MSC cultures blocks the stimulatory effect of the conditioned media on osteoblastic differentiation of MSCs not on the biomimetic surface. As noted above, growth on an osteoclast resorption pit biomimetic surface modulates factors produced by MSCs that regulate inflammation, vasculogenesis, bone remodeling, and neurogenesis.[24,25] This suggests that they might generate an osteoinductive milieu around the implant surface in vivo.

Effect of Surface Topography on Osteogenesis In Vivo

Cell culture provides a method for understanding the mechanisms involved in the response of cells and tissues to surface topography, but it does not provide definitive evidence that this affects osseointegration in vitro. Meta-analyses of clinical outcomes using various Ti dental implant topographies showed a strong correlation between clinical success and the expression of osteocalcin by cells grown on identical surfaces in vitro.[2,3] To begin to assess whether the implant surface design could affect osseointegration in the skeletal bone, we have conducted a number of studies including the use of grit-blasted Ti6Al4V pedicle screws in sheep spine, grit-blasted/acid-etched Ti and Ti6Al4V screws in rat and rabbit femurs, and grit-blasted/acid-etched Ti screws in osteoporotic rat femoral bone.[26-28] Animal models have also been used to assess the effectiveness of Ti implants with hydrophilic, microtextured surfaces in diseases, such

Figure 2

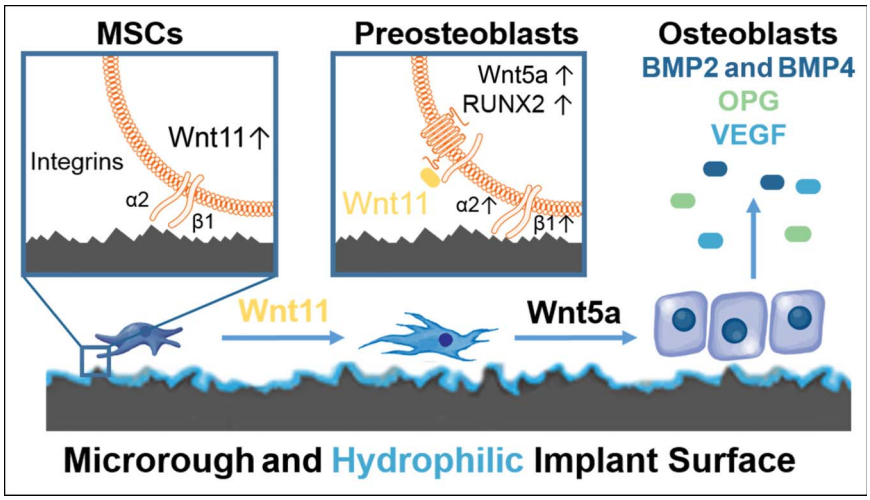


Diagram showing mechanisms involved in the regulation of osteoblastic differentiation of bone marrow stromal cells (MSCs) on microstructured Ti-based implant surfaces. MSCs migrate to the implant environment and attach by $\alpha 5\beta 1$ integrin binding to fibronectin. MSCs begin to sense the implant architecture shifting from production of $\alpha 2\beta 1$ integrin binding to collagen type 1 and upregulate noncanonical Wnt11 (left panel). Wnt11 acts internally and externally of the cell to increase the number of preosteoblasts in the implant environment. This method is achieved by increasing production Wnt5a and RUNX2 transcription in the nucleus (middle panel). These preosteoblasts mature into osteoblasts that produce robust concentrations of bone morphogenetic proteins 2 and 4, osteoprotegerin (OPG), and vascular endothelial growth factor (VEGF) necessary for bone apposition and mineralization (right panel).

as diabetes and osteoporosis, which compromise healing and bone quality.[27] These studies provided direct correlation between in vitro and in vivo outcomes and confirm the value of the biomimetic topography. We have also investigated the effectiveness of grit-blasted/acid-etched surfaces on three-dimensional printed Ti6Al4V devices in regenerating the alveolar bone sufficiently to support reconstruction of the mandible in humans and once again confirmed the importance of this biomimetic principle in achieving stable osseointegration.[26]

Role of Nanotextures in the Regulation of Osteogenesis

Most studies examining the role of surface topography in osteoblast differentiation have used polymeric constructs on tissue culture polystyrene surfaces to tease out the various contributions of stiffness and shape.[15,29] These studies have relied on the use of osteogenic culture media, which are high in Ca++ and have additives such as dexamethasone, which simulates alkaline phosphatase activity, together with a phosphate source such as beta-glycerol phosphate. Even with these additives, the MSCs or osteoprogenitor cells must form multicellular nodules before they begin to express an osteoblast phenotype, and the mineral that they deposit is due, at least in part, to the high calcium phosphate ion product that is generated by the action of alkaline phosphatase.[18] Numerous studies have shown that these effects are mediated by Wnt3a signaling. By contrast, when cells are cultured on osteoclast resorption pit biomimetic Ti surfaces, they shift from Wnt3a to Wnt5a signaling while still in monolayer.[14,19,23] This shift requires $\alpha 2\beta 1$ integrin signaling and is accompanied by a change in cell shape. Certainly, the polymer models provide valuable insights into MSC regulation, but without the microscale surface topography to underlie the nanofeatures, the results must be viewed with caution.

A wide variety of nanomodifications have been applied to implant surfaces to improve clinical outcomes. Some of these modifications are applied to machined surfaces, and their effectiveness in vitro is assessed using osteogenic media and only limited assessment of outcome measures.[30] Even when nanofeatures are generated on microstructured topographies resembling an osteoclast resorption pit, the specific shapes, sizes, and crystallinities of the nanostructures result in very different outcomes.[5,6,31] Although some of these do support osteoblastic differentiation of MSCs to some extent, the full panoply of outcomes is observed in only a limited subset of modifications.[31]

We have demonstrated that specific nanoscale topographies activate preosteoblastic cell differentiation[32] through a mechanism involving integrin-mediated focal adhesion kinase.[33] In addition, these biomimetic nanotopographies enhance bone graft osteointegration.[34] More recently, we have demonstrated that hydroxyapatite particle density regulates preosteoblastic cell differentiation.[35,36] Importantly, when specific nanofeatures are applied to microtextured Ti6Al4V surfaces with a biomimetic osteoclast resorption pit topography, MSCs and preosteoblasts display the full panoply of characteristics associated with well-differentiated osteoblasts and produce factors that support osteogenesis, vasculogenesis, neurogenesis, and prohealing immune response.[11,21]

Taken together, these studies suggest that specific nanofeatures, as well as their density and the underlying substrate topography, may positively affect osteointegration. Investigators have taken advantage of this observation by applying nanofeatures, such as Ti nanotubes or hydroxyapatite crystals, to the surface of polymeric materials, such as polyether-ether-ketone, to render them more osteogenic.[37-39] However, the underlying material lacks the microtopography that recapitulates the biomimetic topography that favors osteogenesis. Thus, even with bone ingrowth from the bone bed by creeping substitution, the interface with the implant is not bone outgrowth but fibrous connective tissue.[40]

Summary

Collectively, there is strong preclinical and clinical success supporting the use of implants that possess biomimetic surface topography. Using these surfaces, we have been able to elucidate the behavior of cells as they sense and respond to materials. Understanding these mechanisms is key to predict how the next generation of orthopaedic implants will need to be designed to improve implant longevity, reduce healing time, and reduce biofilm formation.

References

References printed in **bold type** are those published within the past 5 years.

1. Spector M: Historical review of porous-coated implants. *J Arthroplasty* 1987;2:163-177.

2. Buser D, Janner SFM, Wittneben J, Brägger U, Ramseier CA, Salvi GE: 10-Year survival and success rates of 511 titanium implants with a sandblasted and acid-etched surface: A retrospective study in 303 partially edentulous patients. *Clin Oral Implants Res* 2012;26:1121-1128.

3. Müller F, Al-nawas B, Storelli S, Quirynen M, Hicklin S, Castro-laza J: Small-diameter titanium grade IV and titanium-zirconium implants in edentulous mandibles: Five-year results from a double-blind, randomized controlled trial. *BMC Oral Health* 2015;15:123.

4. Albrektsson T, Johansson C: Osteoinduction, osteoconduction and osseointegration. *Eur Spine J* 2001;10:96-101.

5. Olivares-Navarrete R, Hyzy SL, Berg ME, et al: Osteoblast lineage cells can discriminate microscale topographic features on titanium-aluminum-vanadium surfaces. *Ann Biomed Eng* 2014;42:2551-2561.

6. Lotz EM, Olivares-Navarrete R, Hyzy SL, Berner S, Schwartz Z, Boyan BD: Comparable responses of osteoblast lineage cells to microstructured hydrophilic titanium-zirconium and microstructured hydrophilic titanium. *Clin Oral Implants Res* 2017;28:e51-e59.

7. Vahidi G, Rux C, Sherk VD, Heveran CM: Lacunar-canalicular bone remodeling: Impacts on bone quality and tools for assessment. *Bone* 2021;143:115663.

8. Salazar VS, Gamer LW, Rosen V: BMP signalling in skeletal development, disease and repair. *Nat Rev Endocrinol* 2016;12:203-221.

9. Hotchkiss KM, Reddy GB, Hyzy SL, Schwartz Z, Boyan BD, Olivares-Navarrete R: Titanium surface characteristics, including topography and wettability, alter macrophage activation. *Acta Biomater* 2016;31:425-434.

10. Keselowsky BG, Wang L, Schwartz Z, Garcia AJ, Boyan BD: Integrin a5 controls osteoblastic proliferation and differentiation responses to titanium substrates presenting different roughness characteristics in a roughness independent manner. *J Biomed Mater Res A* 2007;80:700-710.

11. Trindade R, Albrektsson T, Tengvall P, Wennerberg A: Foreign body reaction to biomaterials: On mechanisms for buildup and breakdown of osseointegration. *Clin Implant Dent Relat Res* 2016;18:192-203.

12. Terheyden H, Lang NP, Bierbaum S, Stadlinger B: Osseointegration—Communication of cells. *Clin Oral Implants Res* 2012;23:1127-1135.

13. Hu K, Olsen BR: The roles of vascular endothelial growth factor in bone repair and regeneration. *Bone* 2016;91:30-38.

14. Andre P, Wang Q, Wang N, et al: The Wnt coreceptor Ryk regulates Wnt/planar cell polarity by modulating the degradation of the core planar cell polarity component Vangl2. *J Biol Chem* 2012;287:44518-44525.

15. Lai M, Hermann CD, Cheng A, et al: Role of A2B1 integrins in mediating cell shape on microtextured titanium surfaces. *J Biomed Mater Res A* 2015;103:564-573.

16. Mustafa K, Rubinstein J, Lopez BS, Arvidson K: Production of transforming growth factor beta1 and prostaglandin E2 by osteoblast-like cells cultured on titanium surfaces blasted with TiO2 particles. *Clin Oral Implants Res* 2003;14:50-56.

17. Lotz EM, Berger MB, Schwartz Z, Boyan BD: Regulation of osteoclasts by osteoblast lineage cells depends on titanium implant surface properties. *Acta Biomater* 2018;68:296-307.

18. Langenbach F, Handschel J: Effects of dexamethasone, ascorbic acid and β-glycerophosphate on the osteogenic differentiation of stem cells in vitro. *Stem Cell Res Ther* 2013;4:117.

19. Lotz EM, Berger MB, Boyan BD, Schwartz Z: Regulation of mesenchymal stem cell differentiation on microstructured titanium surfaces by semaphorin 3A. *Bone* 2020;134:115260.

20. Henriksen K, Bollerslev J, Everts V, Karsdal MA: Osteoclast activity and subtypes as a function of physiology and pathology—Implications for future treatments of osteoporosis. *Endocr Rev* 2011;32:31-63.

21. Boyan BD, Lotz EM, Schwartz Z: (*) Roughness and hydrophilicity as osteogenic biomimetic surface properties. *Tissue Eng Part A* 2017;23:1479-1489.

22. Berger MB, Bosh KB, Jacobs TW, Joshua Cohen D, Schwartz Z, Boyan BD: Growth factors produced by bone marrow stromal cells on nanoroughened titanium-aluminum-vanadium surfaces program distal MSCs into osteoblasts via BMP2 signaling. *J Orthop Res* 2021;39:1908-1920.

23. Boyan BDBD, Olivares-navarrete R, Berger MB, Hyzy SLSL, Schwartz Z: Role of Wnt11 during osteogenic differentiation of human mesenchymal stem cells on microstructured titanium surfaces. *Sci Rep* 2018;8:8588.

24. Raines AL, Olivares-Navarrete R, Wieland M, Cochran DL, Schwartz Z, Boyan BD: Regulation of angiogenesis during osseointegration by titanium surface microstructure and energy. *Biomaterials* 2010;31:4909-4917.

25. Bang SM, Moon HJ, Kwon YD, Yoo JY, Pae A, Kwon IK: Osteoblastic and osteoclastic differentiation on SLA and hydrophilic modified SLA titanium surfaces. *Clin Oral Implants Res* 2014;25:831-837.

26. Cohen DJ, Cheng A, Kahn A, et al: Novel osteogenic Ti-6Al-4V device for restoration of dental function in patients with large bone deficiencies: Design, development and implementation. *Sci Rep* 2016;6:20493.

27. Lotz EM, Cohen DJ, Schwartz Z, Boyan BD: Titanium implant surface properties enhance osseointegration in ovariectomy induced osteoporotic rats without pharmacologic intervention. *Clin Oral Implants Res* 2020;31:374-387.

28. Deluiz D, Oliveira LS, Pires FR, et al: Incorporation and remodeling of bone block allografts in the maxillary reconstruction: A randomized clinical trial. *Clin Implant Dent Relat Res* 2017;19:180-194.

29. Olivares-Navarrete R, Lee EM, Smith K, et al: Substrate stiffness controls osteoblastic and chondrocytic differentiation of mesenchymal stem cells without exogenous stimuli. *PLoS One* 2017;12:e0170312.

30. Abraham CM: A brief historical perspective on dental implants, their surface coatings and treatments. *Open Dent J* 2014;8:50-55.

31. Cheng A, Goodwin WB, deGlee BM, et al: Surface modification of bulk titanium substrates for biomedical applications via low-temperature microwave hydrothermal oxidation. *J Biomed Mater Res A* 2018;106:782-796.

32. Lim JY, Hansen JC, Siedlecki CA, Runt J, Donahue HJ: Human foetal osteoblastic cell response to polymer-demixed nanotopographic interfaces. *J R Soc Interface* 2005;2:97-108.

33. Lim JY, Dreiss AD, Zhou Z, et al: The regulation of integrin-mediated osteoblast focal adhesion and focal adhesion kinase expression by nanoscale topography. *Biomaterials* 2007;28:1787-1797.

34. Loiselle AE, Wei L, Faryad M, et al: Specific biomimetic hydroxyapatite nanotopographies enhance osteoblastic differentiation and bone graft osteointegration. *Tissue Eng Part A* 2013;19:1704-1712.

35. Juhl OJ IV, Merife AB, Zhang Y, Lemmon CA, Donahue HJ: Hydroxyapatite particle density regulates osteoblastic differentiation through β-catenin translocation. *Front Bioeng Biotechnol* 2020;8:591084.

36. Juhl OJ IV, Latifi SM, Donahue HJ: Effect of carbonated hydroxyapatite submicron particles size on osteoblastic differentiation. *J Biomed Mater Res B Appl Biomater* 2021;109:1369-1379.

37. Girasole G, Muro G, Mintz A, Chertoff J: Transforaminal lumbar interbody fusion rates in patients using a novel titanium implant and demineralized cancellous allograft bone sponge. *Int J Spine Surg* 2013;7:e95-e100.

38. Jiya TU, Van Royen TSBJ, Resorbable PÁ: Posterior lumbar interbody fusion using non resorbable poly-ether-ether-ketone versus resorbable poly-L-lactide-co-D, L-lactide fusion devices. Clinical outcome at a minimum of 2-year follow-up. *Eur Spine J* 2011;20:618-622.

39. Najeeb S, Khurshid Z, Matinlinna JP, Siddiqui F, Nassani MZ, Baroudi K: Nanomodified peek dental implants: Bioactive composites and surface modification—A review. *Int J Dent* 2015;2015:381759.

40. Yang JJ, Yu CH, Chang B, Yoem JS, Lee JH, Lee CK: Subsidence and nonunion after anterior cervical interbody fusion using a stand-alone polyetheretherketone (PEEK) cage. *Clin Orthop Surg* 2011;3:16-23.