

# OSTEOBLASTS GENERATE AN OSTEOGENIC MICROENVIRONMENT WHEN GROWN ON SURFACES WITH ROUGH MICROTOPOGRAPHIES

B.D. Boyan<sup>1,2</sup>, S. Lossdorfer<sup>3</sup>, L. Wang<sup>1</sup>, G. Zhao<sup>1</sup>,  
C.H. Lohmann<sup>4</sup>, D.L. Cochran<sup>2</sup>, & Z. Schwartz<sup>1,2,5</sup>

<sup>1</sup>*Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA, USA*

<sup>2</sup>*Department of Periodontics, University of Texas Health Science Center, San Antonio, TX, USA*

<sup>3</sup>*Department of Orthodontics, University of Bonn, Bonn, Germany*

<sup>4</sup>*Department of Orthopaedics, University of Hamburg-Eppendorf, Hamburg, Germany*

<sup>5</sup>*Department of Periodontics, Hebrew University Hadassah, Jerusalem, Israel*

**INTRODUCTION:** Osteoblast attachment to materials, proliferation, and differentiation are sensitive to the microtopography of the surface (1). On rougher surfaces, osteoblasts assume a more differentiated morphology. Attachment is reduced and those cells that attach exhibit reduced proliferation and increased expression of markers of osteoblastic differentiation. Moreover, they exhibit responses to hormones and growth factors in a manner typical of a differentiated osteoblast. Osteoblast-like cells cultured on osteoclast-resorbed bone surfaces showed similar behavior (2) demonstrating the importance of microtopographical features to bone remodeling in vivo. These studies suggest that microrugosity may also modulate the ability of osteoblasts to synthesize and secrete factors that enhance osteogenesis and at the same time, control osteoclast formation and activity.

**METHODS:** To test this hypothesis, we examined the response of several cell models at various states in the osteoblast lineage to titanium surfaces with smooth and rough microtopographies. Cells were cultured on Ti disks fabricated by Institut Straumann AG (Waldenburg, Switzerland) to have one of three different surfaces: pretreatment (PT) surfaces were smooth ( $Ra < 0.02 \mu\text{m}$ ); grit blasted/acid etched surfaces (SLA) had a mixed morphology with larger pits 30 to 100  $\mu\text{m}$  in diameter overlaid with small pits 1 to 3  $\mu\text{m}$  in diameter, resulting in an average  $Ra$  of 4  $\mu\text{m}$ ; and titanium plasma sprayed (TPS) surfaces had surfaces covered with irregular projections, resulting in an average  $Ra$  of 5  $\mu\text{m}$ . Cell models included osteoblast-like human osteosarcoma cells (MG63), fetal rat calvarial cells (FRC), mouse osteocyte-like cells (MLO-Y4), and normal human osteoblasts (NHOst) isolated from a female patient. In all experiments cells were cultured on the three surfaces as well as on tissue culture plastic. There were 6 separate cultures per variable and all

experiments were repeated a minimum of two times to ensure validity of the observations.

**RESULTS:** All cells examined exhibited increased levels of local factors associated with osteoblast differentiation in their conditioned media when cultured on SLA and TPS, including TGF- $\beta$ 1, PGE<sub>2</sub>, and NO. In addition, responsiveness to steroid hormones associated with osteogenesis and matrix calcification,  $1\alpha,25(\text{OH})_2\text{D}_3$  and  $17\beta$ -estradiol, was also increased, resulting in synergistic enhancement in local factor production. Inhibition of prostaglandin production reduced the effects of surface microtopography, indicating that the mechanism involved prostanoid synthesis. Both constitutive cyclooxygenase-1 and inducible Cox-2 played roles based on specific inhibition of each enzyme. Integrin signaling was also important since anti-beta-1 integrin antibody enhanced osteoblastic differentiation. This observation supported other studies in our lab showing that reduced access to RGD-binding sites increased osteocalcin production (collaboration with M. Textor and S. Tosatti, ETHZ). Cells on SLA and TPS also exhibited elevated levels of osteoprotegerin (OPG) in their conditioned media, which was correlated with increased OPG mRNA. In contrast, RANK ligand mRNAs were present in very low levels and no soluble RANKL was detected in the media. OPG was upregulated by  $1\alpha,25(\text{OH})_2\text{D}_3$  and MG63 cells produced higher levels of  $1\alpha,25(\text{OH})_2\text{D}_3$  on these surfaces.

**DISCUSSION AND CONCLUSIONS:** These studies show that the microtopography of the surface modulates growth and differentiation of osteoblast-like cells at various states of maturation in the osteoblast lineage. Part of the effect is due to integrin signaling, suggesting that changes in cell morphology imposed by surface architecture may be a contributing factor. Altered surface energy may also influence the conformation of adsorbed proteins, which may affect integrin binding to the substrate. Once the cells attach, they produce

autocrine and paracrine factors, including steroid hormones that can act back on the osteoblasts further affecting phenotypic expression. Both  $1\alpha,25(\text{OH})_2\text{D}_3$  and  $17\beta$ -estradiol enhance osteoblastic differentiation and  $1\alpha,25(\text{OH})_2\text{D}_3$  is needed for mineral deposition in the extracellular matrix. TGF- $\beta$ 1 and PGE<sub>2</sub> increase differentiation by increasing alkaline phosphatase specific activity. Although TGF- $\beta$ 1 retards terminal differentiation, PGE<sub>2</sub> promotes this process. Indeed, prostaglandin is needed for the increase in TGF- $\beta$ 1 (3). NO increases osteoblast growth and is anti-apoptotic. Together, these factors provide an environment that is osteogenic.

Bone remodeling depends on osteoblast initiated activation of osteoclasts via presentation of RANKL to its receptor, RANK, on the osteoclasts. By increasing OPG levels, the osteoblasts provide a decoy receptor for RANKL, preventing its binding to RANK. In addition, by not releasing soluble RANKL into the media, OPG is not occupied and can bind to RANKL on the osteoblast membrane. Thus, growth on microrough Ti resulted in cells that exhibited a more osteoblastic phenotype and produced local factors and hormones that maintained and enhanced that phenotype, while at the same time producing factors that decreased osteoclast formation and activity.

**ACKNOWLEDGEMENTS:** This research was funded by a grant from the ITI Foundation, the Georgia Research Alliance, and the Georgia Tech/Emory Center for the Engineering of Living Tissues. Culture disks were a generous gift of Institut Straumann AG. Dr. Lossdorfer is recipient of a postdoctoral fellowship from the German government. The authors thank their collaborators Drs. Adele Boskey (Hospital for Special Surgery, NYC, NY), Marcus Textor (ETHZ, Zurich, Switzerland), Lynda Bonewald (University of Missouri at Kansas City, Kansas City, MO), and J. Edward Puzas (University of Rochester, Rochester, NY).

#### Reference List

1. Martin JY, Schwartz Z, Hummert TW, Schraub DM, Simpson J, Lankford J, Dean DD, Cochran DL, Boyan BD 1995 Effect of titanium surface roughness on proliferation, differentiation, and protein synthesis of human osteoblast-like cells (MG63). *J Biomed Mater Res* 29:389-401

2. Boyan BD, Schwartz Z, Lohmann CH, Sylvia VL, Cochran DL, Dean DD, Puzas JE 2003 Pretreatment of bone with osteoclasts affects osteoblast phenotypic expression. *J Ortho Res* In Press:

3. Sisk M, Lohmann CH, Cochran DL, Sylvia VL, Simpson JP, Dean DD, Boyan BD, Schwartz Z 2001 Inhibition of cyclooxygenase by indomethacin modulates osteoblast response to titanium surface roughness in a time-dependent manner. *Clin Oral Implants Res* 12:52-61