Osteoblast Responses to Titanium Surface Microstructure and Surface Energy are Donor Age Dependent

Schwartz, Zvi[1,2], Olivares-Navarrete, Rene[1]; Cochran, David L.[2]; Hyzy, Sharon[1]; Erdman, Christopher[1]; Wieland, Marco[3]; and Boyan, Barbara D.[1]

[1]Institute of Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, Georgia, USA; [2]University of Texas Health Science Center at San Antonio, San Antonio, TX; [3]Institut Straumann AG, Basel, Switzerland

Introduction: Titanium is widely used as an implant material due to its good biocompatibility and because of the ability to modify its properties chemically and physically. Several surface modifications have been made in order to increase bone formation around Ti implants. Surfaces with combination micron-scale and submicron scale roughness support greater bone to implant contact and increased pullout strength than smooth surfaces. Sandblasting produces micron-scale roughness, whereas acid etching produces submicron scale roughness. Their combination produces a complicated three-dimensional microtopography that resembles osteoclast pits on bone wafers. In vitro studies using osteoblast-like cell lines as well as normal human and rat osteoblasts show that differentiation is increased on these surfaces. When osteoblasts are grown on surfaces with the same complex microtopography that have been modified to retain high surface energy, differentiation is further enhanced. While studies using cell lines can provide a general view assessment of the potential of a material, clinical results may differ, in part due to donor variability. Patient age is one factor that contributes to this, but it is not known if donor age affects the responsiveness of osteoblasts to surface microstructure or surface energy.

Methods: Ti disks were prepared from 1 mm thick sheets of grade 2 unalloyed Ti (ASTM F67 "Unalloyed titanium for surgical implant applications") with a 15 mm in diameter. The disks were washed in acetone, processing through 2% ammonium fluoride/2% hydrofluoric acid/10% nitric acid solution at 55°C for 30 s to produce pretreatment Ti disks (PT) with an Ra <0.2 μm. PT disks were coarse grit-blasted with 0.25-0.50 mm corundum grit at 5 bars until the surface reached a uniform gray tone, followed by acid etching (SLA), with an Ra of 4 μm. SLA disks were modified to retain high surface energy (modSLA) and had an identical morphology to the SLA disks. Osteoblasts were isolated enzymically from frontal and parietal bones of 1, 3 and 10 month old male immunocompromised rNu/rNu rats (8 rats/batch). Validated rat osteoblasts were plated at 20,000 cells/cm2 density on tissue culture polystyrene (plastic), and this same number of cells was plated on Ti surfaces. Confluent cultures were treated with media containing vehicle, 10^{-9} or 10^{-8}M $1\alpha,25(OH)_2D_3$. Data were calculated as means±SEM for N=6 independent cultures for each variable. Statistical significance was determined using ANOVA followed by Bonferroni's modification of Student's t-test.

Results: The figure below shows results for alkaline phosphatase specific activity of the cell layer and reflects the results obtained for osteocalcin as well as PGE2, TGF-β1 and osteoprotegerin content of the conditioned media. Donor age did not impact cell response to plastic. All cells exhibited a surface effect in the absence of hormone, but cells from 10-month donors were least sensitive. Response to $1\alpha,25(OH)_2D_3$ was greatest on the SLA and modSLA substrates. Cells from 1-month and 3-month rats exhibited more robust response to $1\alpha,25(OH)_2D_3$ than cells from 10-month old donors.

Discussion: These results confirm earlier findings showing that osteoblasts exhibit a more mature phenotype on rougher Ti surfaces and that response to $1\alpha,25(OH)_2D_3$ is enhanced. Osteoblasts from immunocompromised rats behave like normal rat and human osteoblasts and to various osteoblast-like cell lines. Importantly, these results provide the first evidence that donor age may impact the response of osteoblasts to surface morphology and chemistry, and to systemic hormones that regulate osteoblast activity, but these differences may not be observed under standard culture conditions.

Acknowledgements:

This research was supported by the ITI Foundation, NSF, NIH, and Children's Healthcare of Atlanta.