CHONDROCYTE MATURATION STATE DETERMINES CELL RESPONSE TO IMPLANT COMPOSITION AND SURFACE ROUGHNESS

*Dean, D.D., Lohmann, C.H., **Lincks, J., Sylvia, V.L., +Boyan, B.D., ***Schwartz, Z.,
*+The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284
(210)567-6326, FAX (210)567-6295, messier@uthscsa.edu

Relevance to Musculoskeletal Conditions: Endochondral ossification is important for the successful osseointegration of an implant. By understanding the role that surface roughness and composition play in this process, better devices can be developed.

Introduction: Implant composition and surface roughness are two factors that directly affect the interaction of an implant with surrounding tissues. We have previously reported that commercially pure titanium (cpTi) surfaces prepared by electropolishing, grit-blasting, plasma spraying, or sputter-coating affected chondrocyte proliferation and differentiation in both a surface roughness- and a cell maturation-dependent manner (1,2). The aim of the present study was to determine if surface topography and implant composition affect chondrocyte differentiation and local factor production. Cells at two distinct states of chondrogenic maturation were cultured on the surfaces of cpTi or Ti6Al4V (Ti-alloy) disks that had been machined to produce a rough surface or polished to produce a smooth surface, that had comparable average roughness (Ra) values to cpTi surfaces produced by electropolishing and grit-blasting, but differed in surface topography.

Materials and Methods: cpTi (Ti) and Ti alloy (Ti-A) disks were machine-polished to have an Ra of 0.22-0.24 μm for smooth (S) surfaces or machined to have an Ra of 3.4-4.2 μm for rough (R) surfaces. The disks were cut to fit into the well of a 24-well culture dish. Characterization of the surfaces was performed by profilometry, Auger and FT-IR spectroscopy, and scanning electron microscopy (SEM). Confluent, third passage rat costochondral resting zone (RC) and growth zone (GC) cartilage cells (3) were subpassaged onto the disks and on tissue culture polystyrene (plastic) and cultured in DMEM containing 10% FBS, 50 μg/ml sodium ascorbate, and 1% antibiotics until they reached visual confluence on plastic. Cell morphology was assessed by SEM. Cell proliferation was measured by cell number and [^{3}H]-thymidine incorporation; cell differentiation by alkaline phosphatase specific activity (ALP), collagen production by [^{3}H]-proline incorporation and proteoglycan sulfation by [^{35}S]-sulfate incorporation. TGF-β release into the media was measured using an ELISA kit (Promega Corp.), while PGE_2 release was assessed using a RIA kit (NEN Research Products). Statistical differences were determined by ANOVA, post-hoc testing was performed by use of the Student's t-test with Bonferroni's modification.

Results: Surface roughness and disk composition had an effect on cell number and proliferation of both RC and GC cultures. Compared with the plastic controls, cell number and proliferation were decreased on the rougher surfaces, but the effect was greater on Ti-R. Moreover, proliferation of GC on Ti-R was significantly less than on Ti-A-R. Cellular ALP was reduced on rough implant surfaces for both RC and GC. In contrast, cell layer ALP for the RC cultures was reduced on the rough surfaces but enzyme activity for the GC cultures was increased on the Ti-R surface. % Collagen synthesis by the RC cultures was less on Ti-R, while that of the GC cultures was increased. Proteoglycan sulfation was inhibited on all surfaces compared to plastic, but both RC and GC cultures produced less proteoglycan on Ti-R than on Ti-A-R. PGE_2 production by both cell types was enhanced on both types of rough surfaces. Moreover, RC cultures produced more TGF-β1 on the rough surfaces than on the smooth surfaces and more on Ti-R than on Ti-A-R (Fig. 1). In contrast, TGF-β release by GC cells was only enhanced on Ti-R.

Discussion: This study shows that machined surface roughness affects chondrocyte proliferation, differentiation, matrix synthesis, and local factor production, and that these parameters are additionally affected by chemical composition of the material. Moreover, the nature and extent of the cell response is dependent on the stage of cell maturation. Enhanced differentiation of GC grown on rough vs. smooth and on cpTi vs. Ti-alloy was indicated by decreased proliferation and increased ALP and matrix synthesis. For RC, the effect of surface roughness was similar whether the surfaces were machined (this study) or grit-blasted (1). In contrast, GC exhibited an increase in cell layer ALP on machined rough cpTi, whereas on grit-blasted surfaces of equivalent Ra cell layer ALP was not affected. Local factor production supports the hypothesis that surface morphology and chemistry modulates RC and GC differentiation through autocrine and paracrine mechanisms. This was particularly evident in GC cultures; TGF-β1 production was increased only on Ti-R. The results of this study suggest that during endochondral bone formation adjacent to implant surfaces, average roughness, but not necessarily topography, may promote chondrocyte differentiation, and at a more mature state of chondrogenesis, chemical composition of the material may play an additional important role.

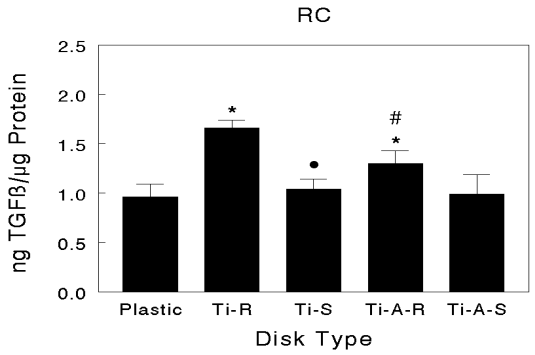

Figure 1: TGF-β1 production by RC cells during culture on Ti and Ti-alloy disks. RCs were cultured on plastic, cpTi (Ti-S, Ti-R), or Ti alloy (Ti-A-S, Ti-A-R) disks for 24 hours after they had reached confluence on plastic. At harvest, the media were collected, and TGF-β1 content measured by ELISA. Values are the mean ± SEM of six cultures. *P<0.05, plastic vs. disk surfaces; #P<0.05, Ti-A-R vs. Ti-R; •P<0.05, S vs. R surface.

Acknowledgments: Supported by the Center for the Enhancement of the Biology/Biomaterials Interface at UTHSCSA.

Affiliations: **Lackland AFB, San Antonio, TX; ***Hebrew U, Jerusalem.

References: (1) Schwartz et al.: J Biomed Mat Res 30:145-155, 1996; (2) Hambleton et al.: J Orthop Res 12:542-552, 1994; (3) Boyan et al.: Bone 9:185-194, 1988

☒ One or more of the authors have received something of value from a commercial or other party related directly or indirectly to the subject of my presentation.

☐ The authors have not received anything of value from a commercial or other party related directly or indirectly to the subject of my presentation.