Integrin α2 Signaling is Important in the Production of Angiogenic Growth Factors by Osteoblasts in Response to Titanium Surface Microstructure

Andrew L Raines[1], James Lee[1], Rene Olivares-Navarrete[1], Marco Wieland[2], Zvi Schwartz[1], Barbara D Boyan[1]
[1]Georgia Institute of Technology, Atlanta, GA, USA
[2]Institut Straumann, Basel, Switzerland

Statement of Purpose: The initial interaction between cells and a biomaterial surface play a significant role in determining the overall success of an implant. Titanium (Ti) is a widely used biomaterial in the orthopaedic and dental industries and it has been shown that modifications to Ti surface topology or chemistry can alter cell adhesion, proliferation, and differentiation on the surface[1]. Successful osseointegration of Ti implants is not only dependent on bone apposition but also on the development of a vascular supply via angiogenesis. We showed previously that a combination of microrough topography and high surface energy enhances the production of VEGF-A and FGF-2 by both an MG63 cell line and primary human osteoblasts (ORS, 2008), but the mechanisms involved are not known. Integrins are a family of heterodimeric cell adhesion proteins which mediate cell-matrix interactions. Integrin α2 expression is increased in osteoblasts grown on surfaces with rough microtopographies and silencing of α2 integrin results in a reduction in osteocalcin and local factor production, suggesting that α2β1 signaling might play a role in regulating expression of angiogenic factors as well. In this study, we assessed the effect that silencing of the α2 integrin has on the production of angiogenic growth factors by cells cultured on microrough Ti surfaces.

Methods: MG63 cells were transfected using a pSuppressor-Neo vector containing a U6 promoter and α2 integrin siRNA. α2 silencing was verified with Western blot analysis. Integrin α2 silenced cells and untransfected MG63 cells were plated in 24 well plates on Ti surfaces presenting two different surface structures and energies: smooth pretreated Ti (PT), sand blasted and acid etched Ti (SLA), and a hydrophilic SLA Ti surface (modSLA). Cells grown on tissue culture polystyrene (TCPS) were used as a control for all studies. Confluent cultures of cells were harvested and FGF-2, VEGF-A, and osteocalcin levels in the media were determined.

Results: Both MG63 cells and integrin α2 silenced cells displayed a reduction in cell number on microrough Ti surfaces though the reduction seen in α2 silenced cells was not as significant as that seen in untransfected MG63 cells. Osteocalcin levels were significantly reduced in α2 silenced cells when compared with untransfected cells. The levels of FGF-2 were increased on SLA and modSLA surfaces for both α2 silenced and untransfected cells when compared to TCPS controls. However, FGF-2 production by α2 silenced cells was significantly lower when compared to untransfected cells on modSLA surfaces (Fig 1). VEGF-A levels, however, were increased on modSLA surfaces in α2 silenced cells compared to untransfected cells (Fig 2).

Conclusions: These results suggest that signaling through the α2 integrin contributes to osteoblast differentiation. VEGF-A is a potent chemoattractant for endothelial cells and the interaction of VEGF with its receptors is one of the first signal transduction pathways activated in endothelial cells during angiogenesis while FGF-2 promotes proliferation and differentiation of endothelial cells[2,3]. By inhibiting the differentiation of MG63 cells on microrough Ti surfaces, these cells display high levels of VEGF-A production with low levels of FGF-2 production, suggesting that VEGF-A may be an indicator of early osteoblast differentiation while FGF-2 is produced by osteoblasts at later times to aid in the maturation of blood vessels.

References: (1) Martin JY et al., J Biomed Mat Res 29:389-401, 1995; (2) Mayer H, et al. J Cell Biochem. 2005. Jul 1;95(4):827-39; (3) Bandoh N et al. Cancer Lett. 2004. May 28;208(2): 215-25.

Acknowledgements: ITI Foundation, Institut Straumann, NIH AR-052102. Ti surfaces were provided by IS.

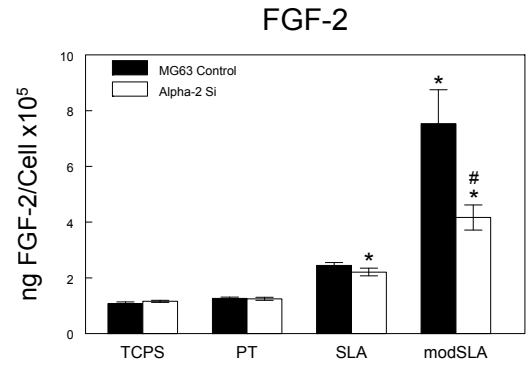

Fig 1: FGF-2 levels produced by α2 silenced and untransfected MG63 cells on Ti surfaces. *p<0.05 vs TCPS; #p<0.05 vs. MG63.

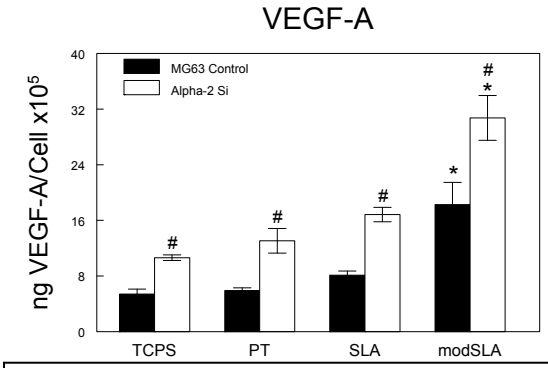

Fig 2: VEGF-A levels produced by α2 silenced and MG63 cells on Ti surfaces. *p<0.05 vs TCPS, #p<0.05 vs MG63.