VEGF-A Has an Autocrine Role in Cell Response to Titanium Substrate Features

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Statement of Purpose: The initial interaction between cells and a biomaterial surface plays a significant role in determining the overall success of an implant. In orthopaedics and dentistry, success is typically determined by osseointegration of the implant with the surrounding bone tissue. It has been demonstrated that microrough Ti substrates support greater bone to implant contact and have higher removal torque values *in vivo* than do smooth surfaces[1]. Critical to this process is the establishment of a patent vasculature at the bone-implant interface. We have previously shown that a combination of microrough surface topography and high surface hydrophilicity of Ti substrates results in an increase in production of VEGF-A and FGF-2 by both an MG63 osteoblast-like cell line and primary human osteoblasts and that these increased levels cause the differentiation of endothelial cells in an *in vitro* tubule formation assay[2]. In addition to producing VEGF-A, osteoblasts are known to express the VEGF receptors, Flt-1 and Flk-1, and it has been shown that treatment of osteoblasts with VEGF-A stimulates their differentiation in a dose dependent manner[3]. In this study, we examined the effect of silencing of VEGF-A in MG63 cells on the response of cells to Ti substrate microtopography and wettability.

Methods: MG63 cells were transduced with shRNA specific for VEGF-A via lentiviral mediated transduction. Silencing of VEGF-A was verified by quantitative real-time PCR and ELISA. VEGF-A silenced cells and untransduced MG63 cells were plated in 24 well plates on Ti surfaces presenting two different surface topographies 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, Ang-1, OPG, and osteocalcin levels in the media were determined. In addition, the effect of VEGF-A silencing in MG63 cells on endothelial cell tubule formation was assessed using a fibrin gel assay and conditioned media from MG63 and VEGF-A silenced MG63 cell cultures.

Results: Consistent with previous results, we observed that MG63 cells cultured on microrough, hydrophilic Ti substrates increase production of osteoprotegerin and osteocalcin as well as VEGF-A and FGF-2 when compared to TCPS or smooth Ti substrates. In VEGF-A silenced cell cultures, secreted levels of these growth factors were significantly reduced on SLA and modSLA substrates when compared to MG63 (Fig. 1). When human aortic endothelial cells were cultured on fibrin gel, endothelial tube length was increased in the presence of conditioned media from MG63 cell cultures on both SLA and modSLA substrates while no differences were found in response to conditioned media from VEGF-A silenced cells cultures (Fig. 2).

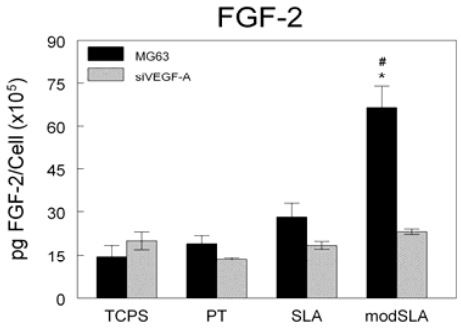

Fig 1: FGF-2 levels in VEGF-A silenced and MG63 cells on Ti substrates. *p<0.05 vs. TCPS; #p<0.05 vs. siVEGF-A

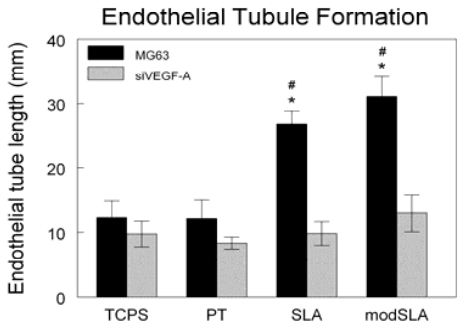

Fig 2: Endothelial cell tubule formation in response to conditioned media from either VEGF-A silenced or MG63 cell cultures. *p<0.05 vs. TCPS; #p<0.05 vs. siVEGF-A

Conclusions: This study suggests that VEGF-A has an autocrine role in the response of cells to Ti substrate features. When VEGF-A was silenced in an MG63 osteoblast like cell line, secreted levels of both osteogenic and angiogenic growth factors were reduced on Ti substrates with a microrough surface topography and high surface hydrophilicity. The reduced levels of angiogenic growth factors in VEGF-A silenced cells on SLA and modSLA substrates resulted in no significant differences in the differentiation of endothelial cells in response to conditioned media from VEGF-A silenced cell cultures. These results suggest that VEGF-A signaling plays a role in the response of osteoblasts to Ti surface microtopography and energy.

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