Introduction: Biomaterial surface properties play a significant role in determining host cellular responses to implant materials for tissue engineering, surgical reconstruction and regenerative medicine therapies. Modifications to surface microarchitcture, chemistry, or energy can alter cell adhesion, proliferation, and gene expression. Titanium (Ti) is a widely used biomaterial in the orthopaedic and dental industries and modifications to Ti surface microtopography have been shown to affect the attachment and differentiation in vitro of osteoblasts, including MG63 osteoblast-like cells, normal human osteoblasts, MC-3T3-E1 cells, and fetal rat calvarial osteoblasts. When these cells are grown on surfaces with rough micron scale features and high surface energy, they exhibit a more differentiated phenotype and they produce factors that are associated with bone formation over bone resorption.

Osseointegration of implants in orthopaedic applications is not only dependent on bone apposition but also on the development of a vascular supply via angiogenesis. Angiogenesis is a critical process during embryonic development and in several physiological and pathological conditions, including the formation of new bone and bone fracture healing. Among the many identified growth factors that serve to control and induce angiogenesis are vascular endothelial growth factor (VEGF), basic fibroblast growth factor (FGF-2), and epidermal growth factor (EGF). VEGF and FGF-2 are both chemotactic for endothelial cells while EGF has been implicated in angiogenesis by stimulating the proliferation of endothelial cells. VEGF is produced by osteoblasts and hypertrophic chondrocytes, and affects vascular permeability in vivo. The interaction of VEGF with its receptors is one of the first signal transduction pathways activated during angiogenesis in endothelial cells.

In the absence of osteoblast differentiation, bone vascularization has been shown to be impaired suggesting that osteoblasts may play a role in directly stimulating endothelial cells. To test this hypothesis, we assessed the effects that Ti surface microarchitecture and energy have on the production of the angiogenic growth factors VEGF-A, FGF-2, and EGF.

Materials and Methods: Primary human osteoblasts and MG63 cells were cultured on traditional tissue culture polystyrene (TCPs), or on Ti surfaces with two different microtopographies as well as two different surface energies: PT (pretreatment smooth surface), SLA (sandblasted and acid etched rough surface), or modSLA (hydrophilic SLA surface). Conditioned media from cell cultures were examined for VEGF-A, FGF-2, and EGF levels using ELISAs. In addition, the effect of conditioned media from MG63 cell cultures on human aortic endothelial cell (HAEC) differentiation was determined using an in vitro endothelial tube formation assay. Data are means ± SEM, N=6 independent cultures, and are from one of two separate sets of experiments, both with comparable results.

Results: Examination of VEGF-A levels in the conditioned media of both primary human osteoblasts (data not shown) and MG63 cells (Figure 1) showed that the combination of rough surface microtopography and high surface energy on modSLA Ti surfaces significantly increased VEGF-A production over both smooth PT Ti surfaces and microrough Ti surfaces with a lower surface energy (SLA). The levels of FGF-2 and EGF were also significantly increased in MG63 cells cultured on modSLA Ti surfaces over smooth Ti surfaces whereas they were undetectable in primary human osteoblasts.

Endothelial tube formation occurred more rapidly in human endothelial cell cultures treated with conditioned media from MG63 cell cultures on PT, SLA, and modSLA Ti surfaces than on TCPs (Figure 2). The stimulatory effect was greatest in media from cultures grown on modSLA. At 4 hours after cell seeding, total endothelial tube length and the total number of branch points were significantly higher compared to PT Ti surfaces. By 12 hours after cell seeding, total tube length and the number of branch points were similar using media from all surfaces.

Discussion: In this study we show that a combination of microrough surface topography and high surface energy enhance the production of angiogenic growth factors by both primary human osteoblasts and an MG63 osteosarcoma cell line. The increased levels of VEGF-A, FGF-2, and EGF observed on cells cultured on modSLA Ti surfaces resulted in a more rapid response of endothelial cells using an in vitro endothelial cell differentiation assay. These data indicate that titanium surface properties may play a direct role in influencing angiogenesis surrounding an implant by upregulating angiogenic growth factor levels in osteoblasts.

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Figure 1. Extracellular VEGF-A, FGF-2, and EGF levels were examined in MG63 cells cultured on PT, SLA, and modSLA Ti surfaces.

Figure 2. The influence of conditioned media from MG63 cells cultured on PT, SLA and modSLA Ti surfaces on endothelial cell differentiation.