Micron/Nano Modified Titanium Alloy Induces MSCs Osteogenesis and Reduces Inflammatory Interleukin Production

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Statement of Purpose: Osseointegration, direct apposition of bone to a biomaterial surface, requires a well-orchestrated process that produces biological fixation of the implant, improving long-term stability and implant success. Bone formation around an implant can be influenced by the physical properties of the implant surface. Our group and others have demonstrated that increases in surface roughness yields increased bone formation *in vivo* and increased osteoblastic markers *in vitro*. Physical cues can also influence the microenvironment surrounding the implant and positively or negatively affect implant success in this fashion. In vitro, micro-rough titanium alloy (Ti6Al4V) surfaces induce osteogenic differentiation of mesenchymal stem cells (MSCs) and osteoblasts, an effect greater than that produced by smooth Ti6Al4V or poly-ether-ether-ketone (PEEK) surfaces. The aim of the current study was to examine if increasing the micron- and nano-scale roughness of Ti6Al4V surfaces could produce an osteogenic environment, and if these modifications can control the inflammatory microenvironment produced by cell culture on orthopaedic biomaterials.

Methods: Human MSCs were cultured on tissue culture polystyrene (TCPS), smooth Ti6Al4V, complex micron- and nano-rough Ti6Al4V (MMN), or PEEK for seven days in stem cell growth media. Cell number, alkaline phosphatase activity (ALP), and secreted osteocalcin (OCN) were measured as indicators of an osteoblastic phenotype. Levels of secreted cytokines IL1β, IL6, IL8, and IL10 were assayed in the conditioned medium using ELISA. Protein levels were normalized to total cell number. Data are presented as mean±SEM (n=6/condition), analyzed by ANOVA with Bonferroni's Student's t-test. Real-time qPCR arrays were performed to examine 84 mRNA related to inflammation, apoptosis, and necrosis. Data are presented as fold-change from TCPS, and changes greater than 2 considered significant.

Results: Surface modifications with multiple levels of roughened features, from micron- to nano-scale roughness, were achieved without modifying the chemical composition. Cell number was reduced on PEEK and the MMN Ti6Al4V surface compared to TCPS and the smooth Ti alloy surface. However, alkaline phosphatase activity and osteocalcin production were increased only on the Ti alloy surfaces, with the effect being greater on the MMN Ti6Al4V surface. Levels of the anti-inflammatory IL-10 were comparable in conditioned media of cultures grown on TCPS and the Ti6Al4V surfaces. Moreover, in cultures grown on the Ti alloy substrates, levels of IL10 were significantly greater than on PEEK. Production of pro-inflammatory proteins by the MSCs was greatest on PEEK compared to all other materials. Conversely, production was lowest on the complex, micron/nano-rough MMN Ti6Al4V surfaces and this was even lower than on TCPS. These were consistent observations, regardless of the protein analyzed: IL1β, IL6 or IL8.

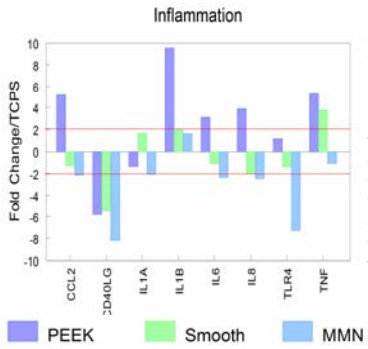

Figure 1. Real-time qPCR array analysis of inflammatory factor mRNA in MSCs cultured on titanium surfaces. Data are presented as fold change to TCPS.

Cells grown on PEEK up-regulated mRNAs for chemokine ligand 2 (CCL2), interleukin 1β, IL6, IL8, and tumor necrosis factor alpha (TNF). Cells grown on the MMN Ti6Al4V surface had an 8-fold reduction in mRNAs for toll-like receptor 4 (TLR4). MSCs on PEEK had reduced levels of the anti-inflammatory cytokine IL10 and increased levels of pro-inflammatory cytokines. Cells cultured on smooth Ti6Al4V had levels of these cytokines that were comparable to cells grown on TCPS. Cells grown on MMN surfaces had reduced levels of all three pro-inflammatory interleukins favoring osteogenesis versus fibrosis. mRNAs for proteins associated with cell death were also differentially expressed. Cells on PEEK had higher levels of factors strongly associated with cell death/ apoptosis. In contrast, cells cultured on MMN Ti6Al4V exhibited reduced cell-death cytokine factor levels.

Conclusions: Stem cells cultured on a complex micron/nano-rough Ti6Al4V surfaces increased osteoblastic markers and produced an osteogenic microenvironment. Additionally, MSCs reduced inflammatory interleukins, suggesting that surface topography modulates the inflammatory microenvironment. PEEK surfaces increased pro-inflammatory interleukin production and yielded lower levels of osteogenic differentiation in comparison to micro/nano modified Ti6Al4V. These surfaces also increased inflammatory cytokines that favor formation of fibrous tissue. Taken together our results may translate to more and faster peri-implant bone formation on MMN Ti6Al4V *in vivo*, leading to earlier stability of the bone-implant construct.