Osteoblast-Fibroblast Co-Culture on Anodized Titanium with Electrical Stimulation for Orthopedic Applications

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Statement of Purpose: The short average lifetime of orthopedic implants necessitates many revision surgeries following implant failures. A leading cause of these failures is fibrous tissue formation at the bone-implant interface due to poor initial osteoblast (bone-forming cell) adhesion, which results in decreased osseointegration. One strategy to improve initial osteoblast adhesion and proliferation and prevent chronic fibrous tissue formation is to modify the implant surface with nanometer features. Prior research indicates increased osteoblast adhesion and function on titanium with nanometer surface features similar to those found in natural bone, while the adhesion and function of fibroblasts (cells that contribute to fibrous tissue formation) on such surfaces has been shown to decrease. Another strategy that has been shown to increase osteoblast function is electrical stimulation. Little work has been completed concerning the competitive growth of fibrous tissue and bone tissue on electrically stimulated nanostructured implant surfaces. Therefore, osteoblast-fibroblast co-culture experiments were conducted on conventional and nanotubular titanium surfaces with varying levels of electrical stimulation such that the optimal combination of nanometer surface features and electrical stimulation parameters to promote osteoblast proliferation while minimizing that of fibroblasts could be determined.

Methods: 99.2% pure titanium foils (1cm^2) (Alfa Aesar) were anodized in a DC powered electrochemical cell using a platinum mesh cathode and a 1.5% HF electrolyte solution. A constant voltage of 20 V was applied for 6 minutes to generate a nanotubular topography.
Osteoblasts (ATCC CRL-11372) were cultured in DMEM supplemented with 10% FBS (Hyclone) and 1% P/S (Hyclone) under standard culture conditions (5%CO_2/ 95% air at 37^{o}C). Fibroblasts (ATCC CRL-1213) were cultured in EMEM supplemented with 10% FBS (ATCC) under standard culture conditions. 50% osteoblast media and 50% fibroblast media was used for co-culture experiments. The total seeding density in the co-culture experiments was 1500 cells/cm^2.
Fibroblasts were stained prior to seeding with Vybrant DiD (Invitrogen), and all cells were stained with DAPI (Sigma-Aldrich) following fixation. A multi channel electrical pulse generator (Ionoptix, MA) was used to stimulate samples for one hour each day. The stimulator featured two components: a voltage generator and a cell culture dish electrode assembly. The pulse duration and frequency were kept constant at 0.4ms and 20 Hz, respectively. The voltages used for these experiments were 10 and 15V, corresponding to current densities across the samples of 2.8 and 4.2 A/m^2 respectively. SEM and XPS analyses were performed on conventional and anodized titanium samples to characterize the material surface. Fluorescence microscopy was used to obtain cell density values.

Results: SEM analysis was used prior to cell culture experiments to confirm the formation of a homogenous nanotopography consisting of nanotubes 60-80nm in diameter on the anodized samples. XPS was used to confirm anodized samples maintained a surface chemistry comparable to that of conventional titanium samples.

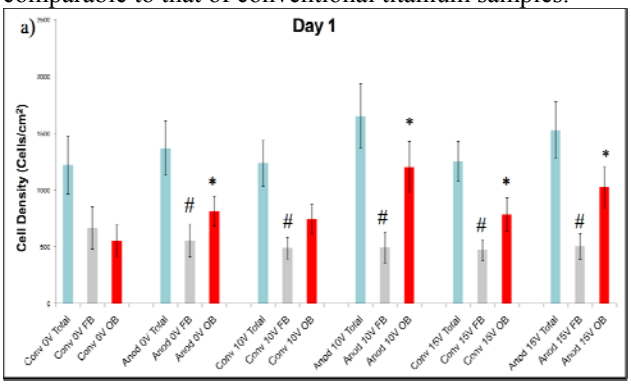

Figure 1. Day 1 Proliferation Results**:** Legend titles are in the format of material surface, voltage, cell type. Values are mean ±SEM, n=3, *p<0.05 with respect to conventional non-stimulated osteoblast, #p<0.01 with respect to osteoblast counterpart at the same voltage.

Osteoblast, fibroblast, and total cell densities were obtained and analyzed on days one, three and five. Statistical analysis shows that non-stimulated, conventional titanium surfaces exhibit equal fibroblast and osteoblast cell densities. However, conventional surfaces subject to stimulation have comparatively greater osteoblast densities, suggesting that electrical stimulation has a greater effect on osteoblast proliferation than fibroblast proliferation. There is also a statistical difference between osteoblast and fibroblast densities observed on anodized, stimulated samples, suggesting osteoblast proliferation is enhanced more by electric stimulation on anodized samples. The greatest total cell density is observed at day five on anodized titanium samples stimulated with 15V, but the greatest difference between osteoblast and fibroblast densities was observed on anodized samples stimulated with 10V at day five.

Conclusions: The combination of electrical stimulation and nanometer topography on anodized titanium may improve osteoblast responses necessary for enhanced orthopedic implant efficacy relative to the responses of fibroblasts. This may help reduce the competitive growth of fibrous tissue at the implant site and improve osseointegration, reducing the chances for premature implant failure. Additional work coupling nanotopography with applied electric stimuli needs to be performed to further elucidate why cellular responses were so different.

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