Electrical Stimulation Enhances Osteoblast Functions on Anodized Nanotubular Titanium

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Statement of Purpose: The use of nanostructured materials has been proposed to enhance the lifespan of currently used orthopedic implants. Specifically, Yao et al. [1] anodized the surfaces of titanium and created a nanotubular titania coating, which enhanced protein adsorption and osteoblast adhesion compared to unanodized titanium surfaces. Additionally, electrical stimulation has been used in orthopedics to heal bone non-unions and fractures in anatomically difficult to operate sites. In this study, these two approaches were coupled, as the efficacy of electrical stimulation to promote osteoblast density on anodized titanium was investigated in vitro under 4 different voltages up to 5 days of culture [2].

Methods: Titanium foils were anodized according to previous studies [1]. Osteoblasts (ATCC CRL -11372) were cultured in DMEM supplemented with 10% FBS and 1% P/S under standard culture conditions. For 5-day proliferation experiments, electrical stimulation was conducted for one hour each day and cell counts were performed at the 1st, 3rd and 5th days. The voltages used for these experiments were 1, 5, 10 and 15V, corresponding to 13, 65, 130 and 195 μA, respectively. For cell imaging, osteoblasts were stained with a Rhodamine fluorescent dye after 5 days of culture. Numerical data was analyzed using standard analysis of variance (ANOVA). All the cell experiments were repeated three times.

Results: SEM investigations of osteoblasts after a 4-hour adhesion test (figures 1a and 1b) revealed that the cells on anodized titanium formed more filipodia than on conventional titanium. Actin stained osteoblasts showed a more well-spread morphology on 15V stimulated anodized titanium than conventional non-stimulated titanium after 5 days of culture.

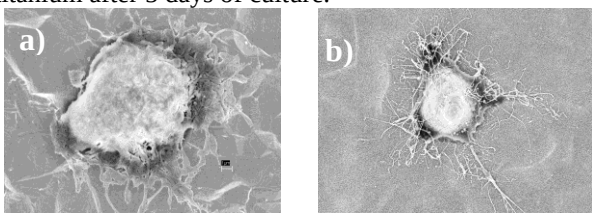

Figure 1: SEM pictures of osteoblasts cultured on a) conventional and b) anodized titanium for 4 hours.

The osteoblast densities from 1 to 5 days of culture are shown in figures 3a and 3b for conventional and anodized titanium samples, respectively. Results show that within the tested voltage range, the optimal voltage window for maximizing osteoblast densities was 10-15 V on both anodized and conventional titanium. It was also observed that osteoblasts responded to lower voltages on anodized titanium compared to conventional titanium. In addition, proliferation was enhanced 72% after 5 days of culture on anodized titanium at 15V of electrical stimulation compared to non-stimulated conventional titanium.

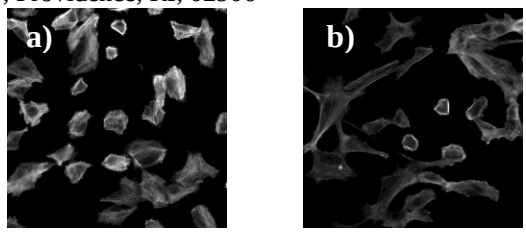

Figure 2: Fluorescent staining of osteoblasts on a) non-stimulated conventional and b) anodized 15V stimulated titanium

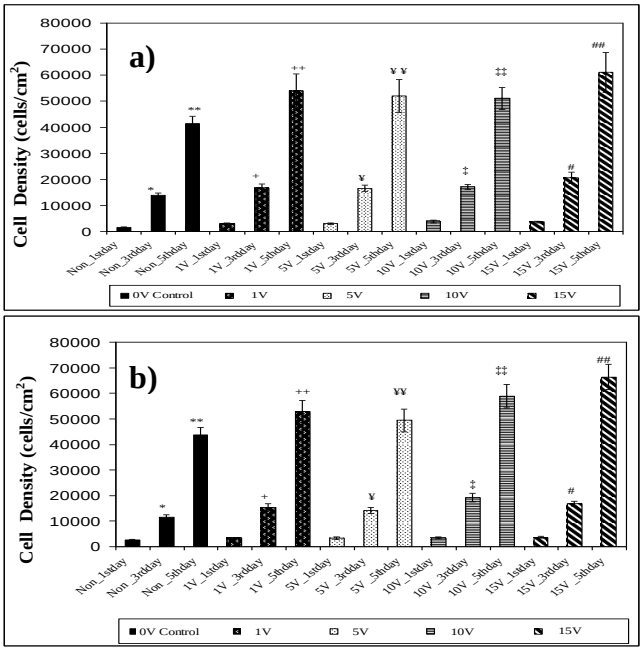

Figure 3: Osteoblast densities from 1 to 5 days on a) conventional and b) anodized titanium. Values are mean ± SEM, n=3, *p<0.05 compared to Non_1stday, **p<0.05 compared to 1V_1stday, +p<0.05 compared to 1V_1stday, ++p<0.05 compared to 1V_3rdday, ¥p<0.05 compared to 5V_1stday, ¥¥p<0.05 compared to 5V_3rdday, ‡p<0.05 compared to 10V_1stday, ‡‡p<0.05 compared to 10V_3rdday, #p<0.05 compared to 15V_1stday, and ##p<0.05 compared to 15V_3rdday. Non = non-electrical stimulated.

Conclusions: Osteoblasts cultured on anodized nanotubular titanium and stimulated with 15V electricity had higher cell densities than the ones cultured on conventional titanium. Thus, results of this study suggest that coupling the positive influences of electrical stimulation and nanotubular features on anodized titanium may improve osteoblast responses necessary for enhanced orthopedic implant efficacy.

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References:

[1] Yao C. et al. J Biomed Nanotech. 2005; 1, 1:68-73.
[2] Ercan B, Int J Nanomed. In Publication.