Controlled chemical oxidation of titanium creates a nanotopography that enhances in vitro osteogenesis

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Introduction: Although it is generally agreed that the implant surface influences osseointegration, there is still no agreement on which topographic features optimize cellular reactions. In bone, microtextured surfaces generate a three–dimensional environment that favors tissue regeneration. However, cells grow and thrive on nanostructured extracellular matrices and the various cell/matrix/substrate interactions that regulate gene expression take place on the nanoscale. It has been shown that various cell types, including osteoblasts, respond to nanotopography, however, the effects of nanotopography on bone cell biology remains to be defined.
Chemical deoxidation and reoxidation of titanium surfaces with a mixture of H_2SO_4/H_2O_2 produces a unique nanoporous topography [1,2]. The aim of the present study was to evaluate the effect of this nanostructure on various parameters of *in vitro* osteogenesis.
Methods: Polished titanium discs (cpTi) were treated with a mixture of H_2SO_4 (37N) and H_2O_2 (30%) for 4 h at room temperature [1,2]. Surfaces were examined using a field emission scanning electron microscope (SEM) and atomic force microscope (AFM). Osteogenic cells were plated on treated and untreated discs at a cell density of $2x10^4$ cells/well. They were cultured for periods up to 14 days under standard osteogenic conditions. Cell growth was quantified at 4 days of culture time using the UMR-106 rat osteogenic cell line. Cell morphology was observed by SEM and fluorescence labeling of actin cytoskeleton (Alexa Fluor-conjugated phalloidin) and nuclear stain (DAPI). Alkaline phosphatase (ALP) activity and bone-like nodule formation (Alizarin red S (AR-S)) staining were also evaluated.
Results: The chemical treatment produced a nanotextured surface consisting of nanopits (24±5 nm in diameter) that resulted in a significant increase in hydrophilicity (immediate contact angle was 74.1±2.7° for controls and 8.7±0.8° for etched surfaces; Mann-Whitney, p=0.05, n=3). AFM characterization indicated that surface roughness increased about four times (RMS 15.2±3.2 nm) after the chemical treatment. No major changes in cell shapes of calvaria-derived osteogenic cultures were observed between treated and control Ti surfaces at 6 and 24 hours. In both cases, the cells had polyhedral outlines but seemed to possess more filipodia on nanotopography. At 6 hours, nanotextured surfaces exhibited a 3-4-fold increase in the proportion of immunoreactive cells exhibiting a peripheral OPN labeling. Extracellularly, OPN labeling was only observed on treated surfaces and resembled the tracks of matrix molecules left behind by migrating cells. At 3 and 4 days, BSP and OPN immunoreactivity dramatically increased both intracellularly and extracellularly on nanotextured surfaces and extracellular FN assembly was slightly more abundant on treated than on control surfaces. UMR-106 cell density was significantly higher on the nanotextured surface. ALP activity peaked earlier on treated surfaces (statistically significant higher levels at Day 10), and was lower than on control at Day 14. AR-S staining showed significantly more bone-like nodule formation on nanotopography than on control surface both at days 10 (1.2±0.8% and 0.01±0.02%) and 14 (9.8±1.6% and 2.2±1.6%) (Mann-Whitney, p<0.01).

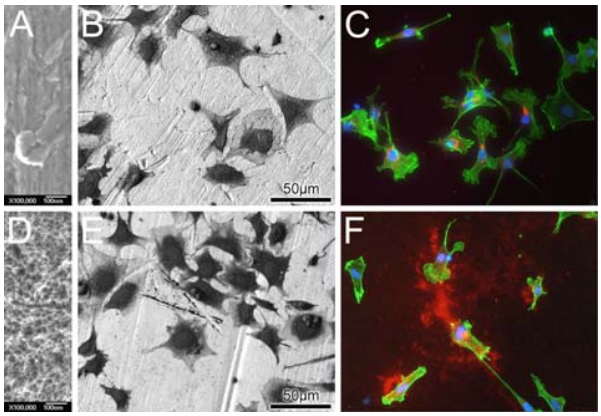

Figure 1. SEM images illustrating the surface features of control (A) and nanotextured (D) cpTi, .and the corresponding (B, E) appearance of calvarial osteogenic cells at 6 h of culture. (C, F) Triple-labeled immunolabeling preparation with OPN (red)-actin (green)-DNA (blue) showing OPN tracks on nanotopography at Day 1 (F).

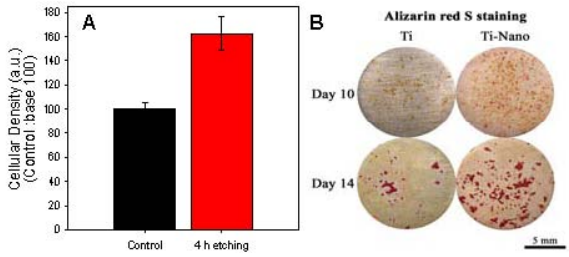

Figure 2. (A) UMR-106 cell density at 4 days on control (black) and nanotextured (red) cpTi. (B) AR-S stained nodule formation at 10 and 14 days.

Discussion: Simple chemical treatment of cpTi with a mixture of H_2SO_4/H_2O_2 produces a bioactive nanotexture that influences in vitro osteogenesis early on, ultimately leading to an increase in bone nodule formation. The ability to control nanoscale features is likely to foster the development of a new generation of biomaterials with "intelligent surfaces" that will selectively influence the fate of cells at the tissue-biomaterial interface to promote tissue repair and regeneration.

References: [1] Nanci A, Wuest JD, Peru L, Brunet P, Sharma V, Zalzal S, McKee MD. J Biomed Mater Res 40:324-335, 1998. [2] De Oliveira PT & Nanci A. Biomaterials 25:403-413, 2004.

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