Osteopontin Expression by Osteogenic Cells Cultured on Nanoporous Titanium

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Statement of Purpose: The extracellular matrix found at the bone-biomaterial interface comprises various non-collagenous proteins (NCPs), including osteopontin (OPN).[1] Previous studies demonstrated that oxidative nanopatterning of titanium (Ti) enhances cytoplasmic and extracellular OPN immunoreactivity, and accelerates bone-like nodule formation in osteogenic cell cultures. The aim of the present study was to supplement immunolabeling data with quantification of OPN levels.
Methods: Oxidative nanopatterning with a mixture of H_2SO_4/H_2O_2 for 4 h was used to create a nanoporous network on the surface of machined Ti discs (Nano Ti).[2] Non-etched Ti discs were used as control (Machined Ti). Primary osteogenic cells derived from newborn rat calvaria were plated on Machined Ti and Nano Ti and grown under osteogenic conditions.[3] The OPN mRNA and protein levels were evaluated at days 4, 7 and 10 by real time PCR and ELISA, respectively. Immunofluorescence was also carried out at the corresponding time points.
Results: Results showed enhanced immunolabeling for OPN and higher mRNA and protein expression levels for cultures on Nano Ti surfaces at day 4 (Tukey test; $p<0,05$) but not at later time intervals (Figures 1-3). Statistical analysis showed no correlation (Pearson test, $r= -0.1724$) between protein and mRNA expression levels (Figure 4).

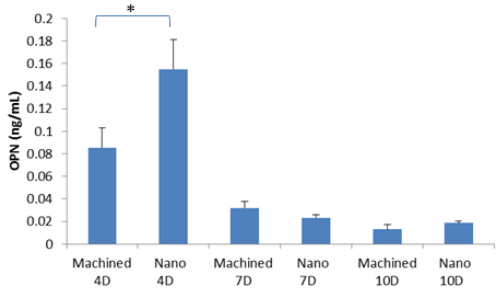

Figure 1- OPN level (ng/mL) in lysate of cell culture after 4, 7 and 10 days, grown on Machined Ti or Nano Ti. Data represent the mean of three replicates ± SD. * $p<0.05$.

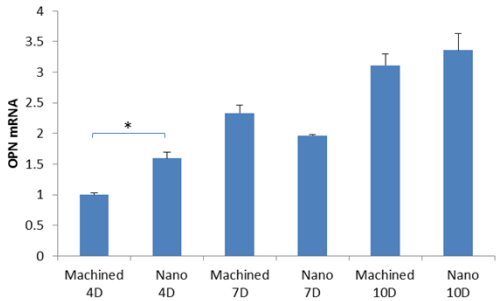

Figure 2- Relative expression of OPN after 4, 7 and 10 days of cell culture on Machined Ti or Nano Ti. Data represent the mean of four replicates ± SD. * $p<0.05$.

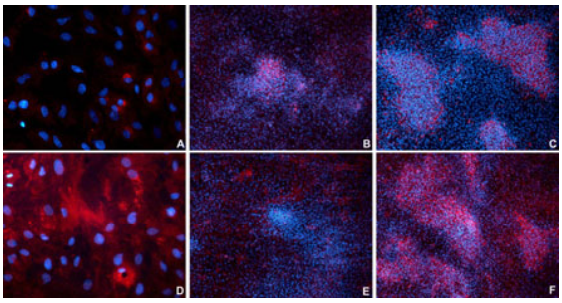

Figure 3- Immunofluorescence for OPN in osteogenic cell cultures at day 4 (A, D), day 7 (B, E) and day 10 (C, F), grown on Machined Ti (A, B, C) or Nano Ti (D, E, F).

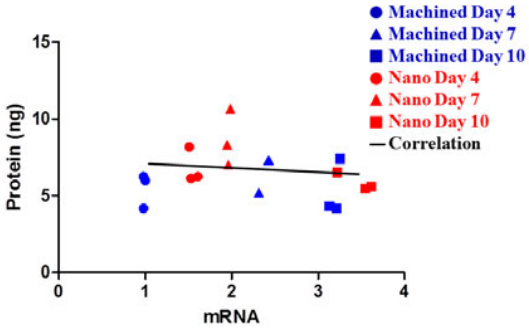

Figure 4- Pearson analysis of correlation between OPN mRNA and protein levels in osteogenic cell cultures grown on Machined Ti or Nano Ti.

Conclusions: Correlative immunolabeling, mRNA and protein analyses show that nanoTi enhances the initial production of OPN in osteogenic cell cultures. However, the data show that mRNA analysis does not necessarily predict protein output. Such nanoscale surface modification may contribute to the accumulation of NCPs observed on Ti implants and may improve regulation of cell dynamics at the tissue-implant interface to accelerate osteogenic processes.

References:
1. Nanci A. Microsc Res Tech 1996; 33:214-31.
2. De Oliveira PT. Biomaterials 2004; 25:403-13.
3. De Oliveira PT. J Biomed Mater Res A 2007; 80:554-64.

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