Novel Nanostructured Coatings Induce Osteogenic Differentiation of Human Mesenchymal Stem Cells

[1]Dimitrievska S; [2]Petit A; [1]Lima R S; [1]Marple B R; [2]Antoniou J; +[1]Bureau M N
+[1]Industial Materials Institute - National Research Council Canada; [2]Lady Davis Institute for Medical Research-SMBD Jewish General Hospital, and Division of Orthopedic Surgery, McGill University

Purpose: One of the design goals of ideal bone implant coating is osteoinductivity, the ability to induce osteogenic differentiation of human mesenchymal stem cells (hMSC). We evaluated the *in vitro* osteoinductive potential of high velocity oxy-fuel (HVOF) thermal sprayed TiO_2-10wt% HA (TiO_2-HA) nanocomposite coatings as alternatives to conventional FDA approved atmospheric plasma spray (APS) HA coatings. The TiO_2-HA nanocomposite coatings exhibit at least 2X higher bond strength values on Ti64 substrates than those of APS HA coatings, offering higher stability post-implantation. It is hypothesized that TiO_2-HA nanocomposites optimal nano and micro surface morphology and hybrid TiO_2/HA chemistry will enhance hMSC osteoblastic differentiation.

Methods: Human MSCs were isolated from multiple adult bone marrow samples as per our prior method [1] with Research Ethics Committee approval. The isolated hMSC were culture-expanded in basal (DMEM/10% FBS) and osteogenic medium (DMEM/10% FBS supplemented with 100nM dexamethasone, 10mM glycerolphosphate and 50 µM ascorbic acid-2-phosphate). Following proliferation and osteogenic differentiation (hMSC-ob), the hMSC and hMSC-ob were seeded on TiO_2-HA and reference HA and TiO_2 coatings. At predetermined culture time points, hMSC and hMSC-ob cultures were evaluated for (i) proliferation (Alamar Blue); (ii) cytoskeleton organization (F-actin/Vinculin); (iii) cell-coatings: interaction, growth and morphology (SEM); (iv) osteoblastic activity: alkaline phosphatase (ALP), osteocalcin (OC) expression; and (v) cellular mineralization: Alizarin Red (ARS) and fluorescent Calcein stains. ANOVA was used to determine statistical significance with a $p < 0.05$. Details concerning coatings preparation and processing were presented elsewhere [2].

Results: Both hMSCs and hMSCs-ob cultures revealed 1.5 times higher cellular metabolism (Alamar Bleu) when plated on TiO_2-HA nanocomposite coatings as compared with TiO_2 and HA reference coatings. Similarly after 14 days, the cytoskeletal organization of hMSCs and hMSC-ob cultures demonstrated more intense, dense and widespread F-actin deposition on the TiO_2-HA nanocomposites than on reference coatings (Fig. 1). Additionally, the cell growth kinetics, represented by red nuclei, were increased when plated on TiO_2-HA nanocomposites coatings and unaffected by culture conditions (basal unsupplemented *vs*. osteogenic medium), indicating substrate dependant behaviour. The level of expression of the osteoblastic markers ALP and OC was significantly higher in cells cultured on TiO_2-HA nanocomposites at day 7 and 21, the respective ALP and OC temporal peak times (Fig. 2).

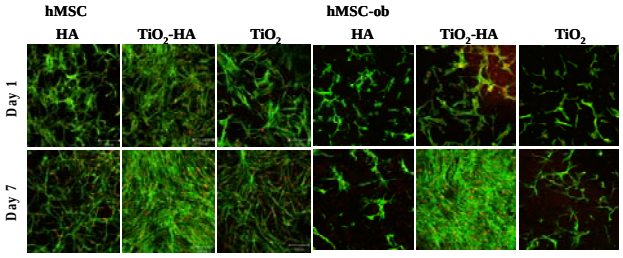

Figure 1. F-actin (green) organization and nuclei morphology (red) of hMSC and hMSC-ob at day 1 and 7 cultured on HA, TiO_2-HA nanocomposites and TiO_2 coatings. Original magnification was 200×.

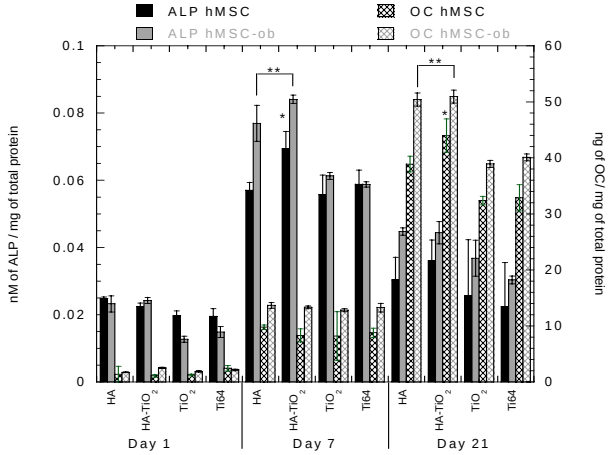

Figure 2: Normalized alkaline phosphatase activity (ALP), and osteocalcin expression (OC) in hMSC and hMSC-ob cells cultured on HA, TiO_2-HA nanocomposites and TiO_2 coatings and uncoated Ti64.

This trend of up-regulation of gene expression was also observed in the extracellular matrix mineral content as seen through Calcein and ARS histochemical staining. Indeed, the co-localization of ARS and Calcein stain in the absence of dexamethasone (hMSC cultures) was only observed when hMSCs were plated on TiO_2-HA. Control hMSC cultures on HA and TiO_2 were only found to mineralize when supplemented with dexamethasone. This suggests nodular cell aggregates are made up of cells committed to the osteoblastic lineage mainly driven by TiO_2-HA nanocomposite coating,

Conclusions: The present data demonstrated that the novel TiO_2-HA nanocomposite coatings support of the growth, proliferation, and differentiation of not only hMSCs, but also osteoblasts that derive from hMSCs. These findings collectively demonstrate the osteo-inductivity potential of the nanostructured TiO_2-HA nanocomposite coatings and their potential as novel mechanically (bond strength) and biologically improved load bearing implants coatings.

References:

[1] Mwale F. *et al*., Tissue Eng. 2006;9:2639-47.
[2] Lima R S *et al*., *J Thermal Spray Technol.*, 2007;16:40-63.