Mesenchymal Stem Cell Response on Ion Beam Sputter-coated Hydroxyapatite Surfaces

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Statement of Purpose: One of the leading causes of failures in total joint replacements is loosening of the joint stem. A loose implant can result in pain, osteolysis, or complete loss of joint function. This phenomenon is usually initiated by the combination of a lack of bonding at the implant/tissue interface and normal loading demands placed on the joint. In some procedures, bone cement is used to fill the voids between the bone and the implant surface. However, the use of bone cement may increase the chances of infection or result in the formation of harmful wear debris as the implant ages. For these reasons, focus has shifted to cementless joint replacements that utilize a texturized or coated titanium surface to encourage osseointegration. The focus of coatings on these implants is to create more biomimetic interfaces on which the bone cells can adhere, proliferate and deposit new bone. Previous work has shown that hydroxyapatite coatings can improve the performance of these implants in both *in-vitro* (1) and in clinical applications (2). In this work, we present an ion beam processing method to create a uniformly texturized hydroxyapatite coating. We also compare the rat mesenchymal stem cell (MSC) response of this surface to amorphous and annealed HAp coatings produced by ion beam sputter coating and furnace annealing. Our results suggest that changing the surface topography on a HAp coating does affect the MSC response.

Methods: Titanium alloy (Ti6Al4V) substrates (1cm x 1cm x 3mm) and hydroxyapatite powder (Alfa Aesar) were used to construct the samples. Four substrates were tested: titanium etched at 700eV, titanium etched at 700eV with sputter coated HAp, titanium etched at 700eV with an annealed HAp coating, and titanium etched at 700eV with an annealed HAp coating and a post annealing etch. An untreated titanium alloy sample was used as the control. All ion beam processing was performed with argon plasma. Annealed samples were heated to 600^0C for 2 hours and left in the furnace overnight to cool to room temperature. Scanning electron microscopy (SEM) was used to evaluate the micro- as well as nanostructure of the surface. X-ray Diffraction (XRD) was used to confirm the presence of a crystalline HAp phase after annealing. For in vitro evaluation, MSCs were harvested from a wistar rat and cultured on the samples for up to four weeks. Short-term studies (MTT assay and Calcein-AM staining) were conducted to evaluate adhesion, proliferation, and viability of the cells for first 7 days of initial culture. After 7 days, osseogenic media was supplied to differentiate the osteoprogenitor cells to osteoblastic phenotype. Long-term studies (Alkaline phosphatase (ALP) activity, and total protein content measurement for up to 3 weeks post differentiation; and Osteocalcin immunofluroescence after 3 weeks of post differentiation) were performed to assess the ability of these surfaces to influence the phenotypic behaviour in cells. SEM images were used to examine the morphology and degradation of the HAp film.

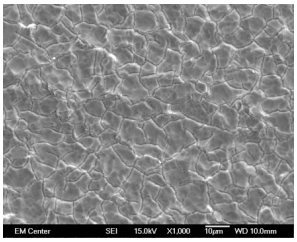

Fig 1. SEM image of the texturized surface created by a post anneal etch

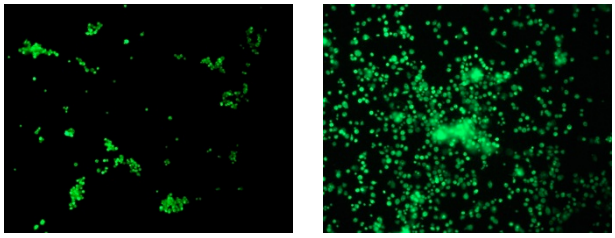

Fig 1. Cell distribution on as-sputtered HAp surface (left) compared to an annealed-etched HAp surface (right)

Results: Each processing variation yielded a surface topography with unique micro and nano features. The 700eV etch produced a uniform roughness over the surface of the titanium. This roughness is attributed to uneven etching caused by crystal orientation variations in the grains of titanium. The as-sputtered coating of HAp retained the micro-features of this texturization although there was no indication of crystallinity in the coating. After annealing, a crystalline phase of HAp was detected, but the coating sustained mild damage in the form of cracking. The final etching of the samples deepened these cracks and created a unique texturization of the HAp surface. Preliminary degradation studies have shown that the cracked surfaces caused by annealing are no more prone to delamination than the as-deposited HAp. During the *in-vitro* cell study, MTT results demonstrated all surfaces were able to sustain healthy cells. It was noted that at both assessment time points, the etched-annealed substrates performed slightly better than the other substrates. Calcein-AM staining indicated that although HAp coated substrates supported lower cell adhesion than the uncoated titanium, it appeared that these cells had higher degree of spreading with formation of colonies. ALP and osteocalcin staining results confirmed that there were varied cellular responses to the sample preparations.

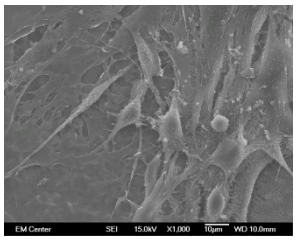

Fig 2. SEM of cell spreading one week after the addition of differentiation media

Conclusions: This study confirms that annealing and post anneal etching of an ion beam sputter coated hydroxyapatite film have the ability to alter cell behavior and cell growth. Future work will focus on the introduction of antibacterial elements into these coatings.

References:

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2. Hydroxyapatite Coated Prostheses in Total Hip and Knee Arthroplasty. Dumbleton J., Manley M.T. 11, 2004, The Journal of Bone and Joint Surgery , Vols. 86-A, pp. 2526-2540.