Enhanced Mesenchymal Stem Cell Response on Ion Etched 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 a lack of bonding between the implant surface and the natural tissue, combined with the normal loading demands to which the joint is subjected. In some procedures, bone cement is used to fill the voids between the bone and the implant surface in efforts to better secure the implant. However, use of bone cement may result in formation of debris that might affect the healing process. More recently focus has shifted to cementless joint replacements which utilize a texturized or coated titanium surface to encourage osseointegration. The focus of texturization on these implants is to create more biomimetic interfaces on which the bone cells can adhere, proliferate and deposit new bone. Recent work has shown that manipulating the nanotopography of the implant surface can control osteoblast phenotype and morphology [1-4]. In this work, we present a simple ion-beam based etching method of altering the topography of titanium surface by creating hierarchal levels of uniform micro/nano-structures. Our preliminary cells studies suggest that changing the surface topography can regulate mesenchymal stem cell response.

Methods: Titanium alloy (Ti6Al4V) substrates (1cm x 1cm) were used for the studies presented here. The samples were sanded, polished with a 9µm diamond suspension, and finished with a colloidal silica/hydrogen peroxide mix, leaving a near mirror finish. The substrates were etched using an argon ion beam for 5 hours at energies of 300eV, 700eV, and 1100eV respectively. Scanning electron microscopy (SEM) was used to evaluate the micro- as well as nanostructure of the surface. For in vitro evaluation, mesenchymal stem cells were harvested from Wistar rats and cultured on texturized and untexturized surfaces. Short-term studies (7 days) were conducted to evaluate adhesion, proliferation, and viability of the cells. After 1 week, osteogenic media was supplied to differentiate the osteoprogenitor cells to osteoblastic phenotype. Long-term studies (up to 3 weeks after providing osteogenic media) were performed to assess the ability of these surfaces to influence the phenotypic behaviour in cells. Alkaline phosphatase activity and calcium deposition on the surfaces were evaluated using commercially available colorimetric assays.

Results: Each energy used in the etching process yielded a unique surface topography with micro and nano features. The microscale roughness of the surface was directly proportional to the beam energy used in etching. At 300eV, the surface remained relatively smooth but developed a slight roughness. The 1100eV etch produced a surface that was much rougher, with the formation of trenches and valleys. **Fig. 1** shows SEM images of the etched polished surfaces. The etching not only shaped the micro-surface but also yielded a difference in nanotopography. The higher energies produced evenly spaced nanogrooves, which were approximately 30nm apart (**Fig. 2**). Cell staining followed by fluorescence microscopy was used to evaluate the cell viability. After

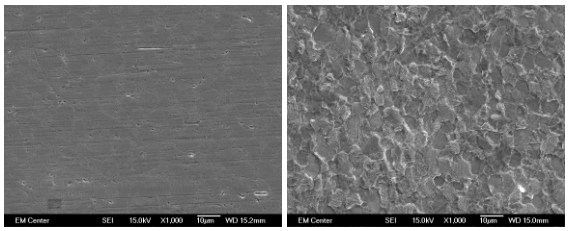

Fig. 1 *SEM images of a polished sample after 300eV etching (left); a polished sample after1100eV etching (right)*

1 day, cellular adhesion was consistent on all samples. However, cells on the smoother ion etched surfaces were more likely to form clusters indicated by cell spreading and migration, while the cells on rougher surfaces tended to stay separate. By day seven the affects of the surfaces was clear. The 300eV and 700eV ion etched surfaces displayed a higher degree of spreading and cell cluster formation, whereas the 1100eV ion etched surface appeared to have fewer cells and decreased cell spreading. **Fig. 3** shows the difference in cell density and cell spreading between the 700eV and 1100eV etch. It is believed that the rougher surface created in the 1100eV etch physically blocks cell to cell communication and limits the cells ability to spread.

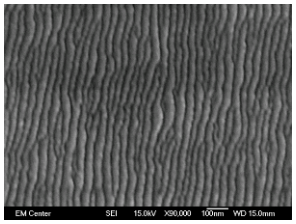

Fig 2. *SEM image of the nanotopography on a polished sample etched at 1100eV*

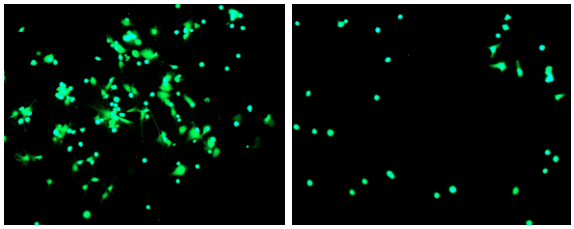

Fig. 3 *Day 7 fluorescent microscopy showing spreading of live cells on: a polished sample etched at 700eV (left); a polished sample etched at 1100eV (right)*

Conclusions: In this study, we have demonstrated that it is possible to use plasma etching to create uniform micro-nano architecture on titanium. Furthermore, this architecture can be controlled to increase cellular functions.

References:

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