Macrophage Interactions with Nanoporous Titanium Surfaces

Marianne B Ariganello[1] Sidney Omelon[2], Fabio Variola[2], Gaetano Isola[1,3] Antonio Nanci.[1]
[1]Université de Montréal, Quebec, Canada, [2]University of Ottawa, Ontario, Canada, [3]Messina University, Italy

Statement of Purpose: Upon the surgical implantation of any biomaterial, a host of inflammatory cells, including macrophages, will initially interact with its surface. It is now recognized that the function and/or phenotype of a macrophage can be dictated not only by the cytokine and cellular environment of the wound site, but also by the material itself. Depending on which phenotypic spectrum is stimulated, the macrophage can promote healing by initiating appropriate tissue repair or defend the body by degrading the material or creating a fibrous capsule around it. Studies on biomaterial biocompatibility often focus on the response of tissue specific cells (such as osteoblasts or fibroblasts); however because macrophages are one of the first cell types to interact with the biomaterial, they have the capacity to direct *downstream* cellular events. Therefore, understanding the influence of a biomaterial on macrophage signaling is essential to elucidate not only the immediate host response but ultimately also the long-term performance. It is becoming well established that cellular activity and function are influenced by the *surface* characteristics of the biomaterial. Since cell signaling occurs at the nanoscale level, surface modifications at the scale of the cell's sensing apparatus have a great potential to influence signaling events.

Objective: Investigate the response of macrophages to machine polished and surface modified titanium generated by oxidative nanopatterning.

Methods: Titanium disks were etched for 2 hrs with a mixture of H_2O_2 and H_2SO_4, forming a network of nanosized pores. Human U937 (macrophage-like) cells were chemically differentiated with phorbol myristate acetate for 72 hrs (D-U937) prior to seeding onto the material surfaces: Machine Polished Titanium (Ti_{POL}), Nanopatterned Titanium (Ti_{NANO}) and Glass coverslips. Forty-eight hours following seeding on the surfaces, samples were processed for light or scanning electron microscopy (SEM). Additionally, to better mimic the in vivo environment, U937s were chemically differentiated *directly* onto the three material surfaces for 72 hrs before fixation. D-U937s were analyzed for cell attachment and morphology (e.g. extent of cell spreading and degree of multinucleation). Functional capacity (phagocytosis of latex beads and generation of reactive oxygen species using CellRox™) and protein expression (SPARC and Osteopontin) were also determined using fluorescence microscopy.

Results: The data demonstrated that more D-U937s cells attached on the Ti_{POL} compared to Ti_{NANO} (see Figure 1), while similar numbers of D-U937 cells attached on Glass and Ti_{NANO}. Cells on Ti_{POL} had greater propensity for spreading, while cells on Ti_{NANO} had greater intercellular cell extensions. Immunofluorescence labeling showed high expression of SPARC by D-U937s on all surfaces. Cells on both Titanium surfaces also expressed high levels of Osteopontin (see Figure 2), however D-U937s on Ti_{POL} displayed a slightly higher Osteopontin signal. In addition, there was little detectable difference in oxidative stress on any surface and no differences in the formation of giant cells were found, indicative of the relative cytocompatibility of the surfaces. Interestingly, more D-U937s displayed punctate actin labeling, reminiscent of podosomes, when cultured on cultured on Ti_{NANO}.

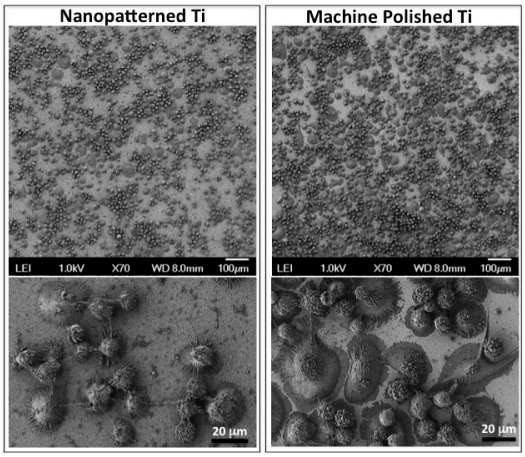

Figure 1. Scanning Electron Micrographs of D-U937s on Titanium Surfaces.

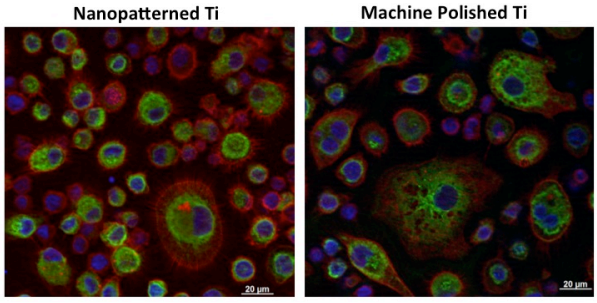

Figure 2. Expression of Osteopontin (Green) and F-actin (red) in D-U937s on Titanium Surfaces.

Conclusions: These results demonstrate that the physiochemical differences generated by the oxidative nanopatterning are sensed by macrophages, as evidenced most significantly by the variations in cell attachment between the surfaces as well as the finer morphological differences observed using SEM and light microscopy. These observations speak to the importance of understanding and controlling the surface of a biomaterial when designing a biocompatible implant, since differences at the nanoscale do impact cell behavior. Future studies will endeavor to look at the cytokine, chemokine and enzyme release profile of these macrophages as well as relating these in vitro observations to in vivo results.

Funding: This study was made possible by support from CIHR, NSERC, FRQS and RSBO.