Micro and Nano-patterned Surface Topographies for Control of Macrophage Form and Function

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Statement of Purpose: Macrophages are highly plastic cells that alter their phenotype in response to cues in the microenvironment.[1] In the presence of non-self pathogens and danger signals, macrophages polarize towards an inflammatory phenotype while in a wound healing microenvironment, they assume an alternatively activated phenotype.[1] Although many studies have elucidated how soluble cues influence macrophage phenotype, relatively few have focused on how insoluble cues in the microenvironment regulate their behavior. We recently demonstrated that macrophage cell shape or geometry of adhesion influences their phenotype.[2] More specifically, we found that forcing cells to elongate led to an increase in expression of markers associated with the alternatively activated pro-healing phenotype. In this study, we explore how surface topology might be leveraged to alter macrophage cell shape and phenotype. We fabricated micro and nano-patterned titanium surfaces using deep etch techniques to contain grooves, which have been previously shown to promote cell elongation.[3] We characterized the degree of macrophage elongation resulting from contact with a range of groove widths and examined the expression of phenotypic markers in macrophages on the bulk titanium. Ongoing work will examine whether elongation induced by grooves will in turn lead to changes in macrophage function by characterizing cell phenotypes in macrophages on different surface topographies. We believe that engineering the surface topography of materials may be a promising strategy to modulate the foreign body response to biomaterial implants.

Methods: Bone marrow derived macrophages (BMDM) were seeded onto deep etched titanium materials at a density of $7x10^5$ cells/mL. After 24 hours, samples were stained with CellTracker Green CMFDA. Cells were imaged using a fluorescent microscope and elongation was characterized in images captured by ImageJ. The long axis and short axis of each cell were manually traced and measured, and the ratio of the long axis to the short axis was calculated. In addition, the expression of arginase-I from samples was quantified via immunocytochemistry. Samples were incubated with primary and secondary antibodies, counterstained with Hoechst 33342 and then imaged using an Olympus inverted microscope. Mean fluorescent intensity was quantified by using CellProfiler software.

Results: BMDM were seeded on titanium surfaces with grooves of varied geometries, ranging from 150 nm to 50 μm. Cell tracker dye was used to examine their shape (Figure 1A). We found that macrophages elongated along the grooves of the titanium substrates in a biphasic manner, where the highest degree of elongation was observed on surfaces with grooves of approximately 450-500 nm wide (Figure 1A and 1B). Moreover, the production of arginase-I (Arg1), a marker of the pro-healing phenotype, was varied and dependent on groove size when most of M2 macrophages were assumed to express Arg1 marker. At very small groove widths, expression of Arg1 was low, but larger groove sizes an increase in Arg1 expression (Figure 1C). Thus, these data suggest that surface topographies might drive macrophages to polarize towards an anti-inflammatory phenotype.

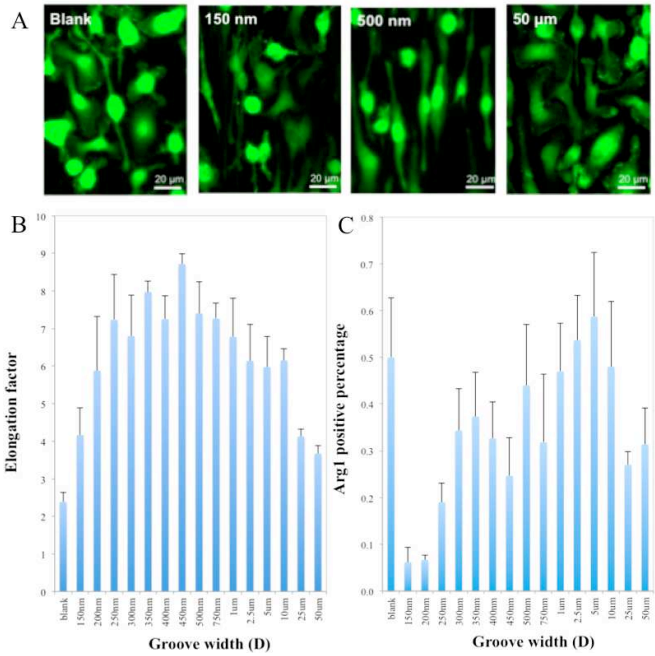

Figure 1. BMDM on patterned titanium substrate. (A) Fluorescence images of BMDM stained with CellTracker Green CMFDA. (B) Quantification of BMDM elongation factors corresponding to groove widths on patterned titanium substrate. (C) Quantification of Arg1 expression corresponding to the groove width. Mean ± SEM, $n = 3$.

Conclusions: In this work, we found that surface topographies on titanium influences macrophage elongation and polarization towards an anti-inflammatory phenotype. Current work is focused on examining macrophage function including cytokine secretion profiles by ELISA and expression of phenotypic markers by FACS and immunoblotting. A better understanding of how topographical cues influence macrophage function will lead to the development of biomaterials to encourage optimal wound healing and tissue repair.

References: [1]Mosser, D. M., Edwards, J. P. *Nat. Rev. Immunol.* 8, 958-69 (2008). [2]McWhorter, F. Y., el al. *Proc. Natl. Acad. Sci.* 110, 17253-172 (2013). [3]Vandrangi P., et al. *PLoS ONE* (2014) *(in press).*