The role of cell shape in macrophage polarization

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Statement of Purpose: The failure of many implanted medical devices can be attributed to the infiltration and activation of host immune cells in response to the biomaterial, or the foreign body response (FBR). Current strategies for mitigating FBR primarily focus on passivating material surfaces to minimize immune cell adhesion and activation[1]. However, recent work has shown that many immune cells, particularly macrophages, are versatile effectors that can assume a spectrum of functional phenotypes in response to their microenvironment[2]. In the presence of pathogenic microorganisms or dangerous substances, macrophage cells adopt a pro-inflammatory phenotype (M1) to recruit auxiliary immune cells and fight infections. Alternatively, they can polarize toward a pro-healing phenotype (M2) and assist in tissue repair. While M1 macrophages are generally associated with chronic inflammation and scarring, *in vivo* studies have found that M2 macrophages predominate in areas of constructive remodeling around implanted biomaterials[3]. Thus, modulation of macrophage phenotype presents a powerful strategy for designing biomaterials. Although biochemical regulation of macrophage phenotype has been well-characterized, little is known about the effect of physical cues on macrophage polarization. Several recent studies have begun to examine the effects of surface topography and geometry on macrophage phenotypes[4]. Nevertheless, a better understanding of the microenviromental cues that direct macrophage polarization is needed and can potentially lead to new biomaterials with improved remodeling outcome.

Methods: PDMS stamps were replica molded from the silicon wafers containing arrays of 20 to 50 µm micro-grooves, and coated with fibronectin. The fibronectin pattern was microcontact printed onto PDMS substrates, which were then blocked with Pluronic F127. Bone marrow-derived macrophages (BMDM) were seeded onto patterned and unpatterned (control) substrates for 24 h and stimulated with polarizing cytokines. Cell lysates were analyzed for arginase-I (M2 marker) and iNOS (M1 marker) by Western blots. Fixed cells were stained for the M2 marker arginase-I, and subsequently analyzed through flow cytometry. Cytometric data analysis and quantification were done using Cyflogic software v. 1.2.1 (www.cyflogic.com).

Results: In our initial work, we found that macrophage cells stimulated towards M1 and M2 phenotypes adopted markedly different cell morphologies: cells polarized towards M2 exhibited a significantly higher degree of cell elongation when compared to cells polarized towards M1 or control unpolarized cells. We use a micropatterning approach to directly control cell shape, in order to examine the effect of shape on macrophage polarization.

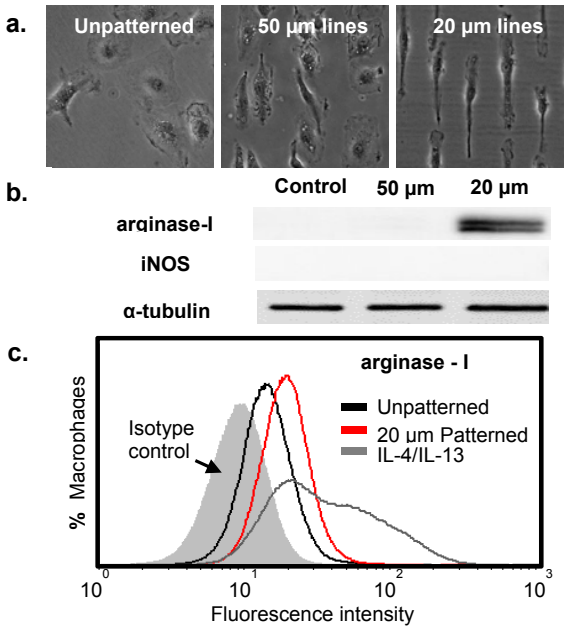

Figure 1. a) Phase contrast images of unpatterned and micropatterned macrophages b) Western blot of arginase-I and iNOS expressions in patterned and unpatterned cells c) Flow cytometry analysis of single cell arginase-I fluorescence intensities

Macrophage cells seeded on 20 µm-wide micropatterned lines of fibronectin spread along the patterned lines and adopted an elongated morphology, similar to those stimulated with pro-healing cytokines, Il-4 and Il-13 (Fig. 1a). Interestingly, we found these elongated micropatterned cells expressed more M2 markers arginase-I, CD206 and Ym-1, when compared to cells seeded on unpatterned surfaces (Fig 1b,c & data not shown). Upregulation of arginase-I occurred in cells cultured in basal media without the addition of exogenous cytokines, suggesting that cell shape, independent of soluble cues, provides an instructive signal to regulate macrophage phenotype.

Conclusions: Elongated cell shape, independent of cytokine stimulation, induces BMDM polarization towards a pro-healing (M2) phenotype. Current work is focused on examining the molecular mechanisms underlying shape-mediated M2 polarization. In addition, we are designing biomaterials that present physical cues to promote macrophage cell elongation and M2 polarization in vivo. These studies will begin to elucidate the role of the physical microenvironment on macrophage cell polarization.

References: 1. Brown BN. *Biomaterials* 2012; 33; 3792:3802 **2.** Mosser DM. *Nat Rev Immunol* 2008; 958:969 **3.** Brown BN. *Biomaterials* 2009;30;1482:1491 **4.** Saino E. *Biomacromolecules* 2011;12 (5); 1900:1911