Investigating the Migratory Behavior of Macrophage Subtypes

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Statement of Purpose: Macrophages are capable of activation along a spectrum of phenotypes between pro-inflammatory M1 and anti-inflammatory M2 states. Classically activated M1 macrophages are associated with tissue damage and secrete high levels of reactive species and inflammatory cytokines while M2 macrophages promote wound healing and facilitate tissue remodeling.[1] A fundamental understanding of how these macrophage phenotypes migrate to and behave at the implant site will help design better materials to specifically modulate the local immune response. Though migratory behaviors of other immune cell types have been examined, there has been little work done on macrophages aside from characterizations of macrophage chemotaxis. Here we use time lapse microscopy and introduce migration parameters to characterize different macrophage phenotype migration on flat surfaces *in vitro*. We examined the migratory behaviors of M0 (unstimulated macrophage), M1, and M2 phenotypes.

Methods: Bone marrow derived macrophages were seeded onto glass surfaces 24 hours prior to imaging; stimulation 6 hours post seeding with LPS and IFN-γ or IL-4 and IL-13 polarized the macrophages into M1 and M2 phenotypes respectively. Macrophages were imaged over a 24 hour period at 37°C using a 10X phase-contrast objective; images were acquired every 5 minutes. Centroid trajectories of each cells was manually tracked with ImageJ's built in MTrackJ plugin. An in-house developed Python script was used for data analysis. Means and medians for (i) pathlength, (ii) root mean square (rms) displacement, (iii) velocity, and (iv) maximum displacement were obtained.

(i) Pathlength $= \sum_{i=1}^{n} S_i$ (ii)RMS Displacement $= \sqrt{\frac{\sum_{i=1}^{n} d_i^2}{n}}$

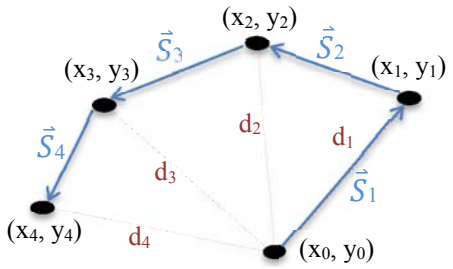

Figure 1. Schematic depiction of the motion of a macrophage over four time intervals. S_i represents the segmental distance traveled and d_i represents the displacement corresponding to the *i*th time interval.

Displacement plots were generated by evaluating each cell's new position in the *i*th time frame with respect to its initial position, tracing out the cell's trajectory over time.

Results: In our study, >15 cells from at least 3 independent experiments were evaluated for each macrophage phenotype. Macrophages moved freely on the glass surface, leading to isotropically distributed trajectories (**Figure 2a-c**). The displacement plots demonstrate that M1 macrophages are less motile than M0 and M2 macrophages. Quantification of median velocities indicated that the velocity speed of M0 and M2 macrophages were approximately three time higher than the M1 macrophages (**Figure 2d**).

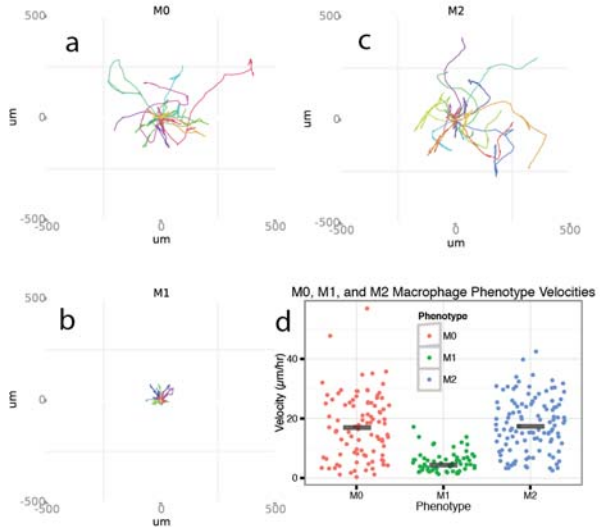

Figure 2. Displacement plots of (a) unactivated M0 (b) pro-inflammatory M1, and (c) pro-healing M2. (d) Representative velocity scatter plot across macrophage subtypes.

Conclusions: In this initial work, we find that macrophages polarized towards an M1, pro-inflammatory phenotype are relatively stationary, exhibiting very little migration over a 24 hour time period. In contrast, unpolarized macrophages and M2, pro-healing macrophages, exhibit significant migration. Although only flat glass surfaces were tested here, the established Python script can be used to quantify migration on other *in vitro* surfaces. Current work is focused on developing topographical surfaces of aligned collagen fibers. A better understanding of M1 and M2 macrophage migratory behaviors will elucidate the role of the microenvironment on migration.

References: [1] Murray PJ. Immunity 2014; 41(2): 14-20.