Effects of Adhesive Cues on Macrophage Cytokine Secretion: a Single Cell Analysis

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Statement of Purpose: Macrophages are tissue-resident immune cells that are indespensible during wound healing. To orchestrate this complex process, macrophages must communicate and coordinate with both immune and non-immune cells, largely through their secretion of vast array of cytokines and chemokines. As macrophage secretome is critical to the outcome of wound healing, understanding what regulates it is of key interest. Recent studies suggest that surface topography[1], ECM composition[2,3] and adhesion-induced changes in macrophage cell shape[4] can all affect macrophage activation and secretion. However, further delineating these factors using traditional cell culture and molecular biology techniques is difficult, especially considering that macrophage activation and secretion can be remarkably hetergeneous. Therefore, to better understand how physical and adhesive cues can regulated macrophage cytokine secretion, a single cell technique that allows for controlled soluble and physical microenvironment is required. Here, we develop a microwell system to precisely control macrophage adhesion on a single cell level. Combining this with an immunofluorescence-based detection strategy, we examine the effects of adhesive cues on cytokine secretion by single adherent macrophages.

Methods: PDMS microwell arrays were created from silicon wafers containing microposts using soft lithographic techniques. The bottoms of the wells were coated with various ECM proteins to support cell adhesion, while the remainder surface area was blocked with pluronics. Murine macrophages were seeded sparsely into the wells to isolate single cells. Macrophages were allowed to adhere and spread for 18h prior to cytokine induction. Once stimulated, detection substrates that had been conjugated with capture antibodies were inverted over the wells. After cytokine interrogation, cells were stained with a dead cell dye, fixed in the microwells and counterstained with Hoechst. Detection substrates were incubated with a fluorescent detection antibody and imaged. (Fig. 1A) The fluorescence intensity of the detection substrate corresponding to wells containing single macrophages was analyzed.

Results: PDMS microwells of the same area but different shapes were used to isolate single macrophages. After 18h, cells were able to spread and conform to the shape of the wells. (Fig. 1B) By inverting a detection substrate conjugated with capture antibodies over the wells, single macrophages were interrogated for secretion of inflammatory cytokines TNFα and MCP-1 and anti-inflammatory cytokine IL-10. The effects of ECM proteins, such as collagen and fibronectin, on single macrophage secretion were examined. (Fig. 1C & D)

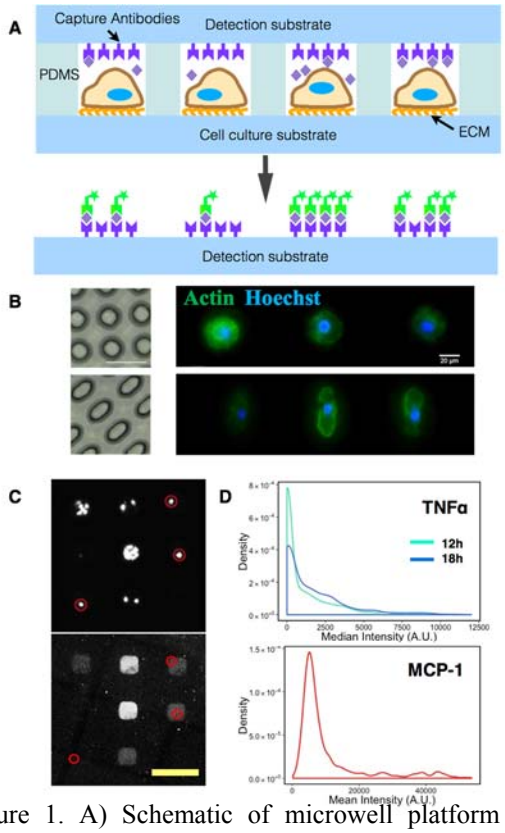

Figure 1. A) Schematic of microwell platform for isolation of single macrophages and detection of their cytokine secretion via immunofluorescence. B) Phase contrast images of PDMS microwells of the same area but different aspect ratio. Scale bar = 50 μm. (left) Immunofluorescence images of single murine bone marrow-derived macrophages in microwells after 18h. Scale bar = 20 μm. (right) C) Single RAW264.7 macrophages identified by red circles (top), and their corresponding MCP-1 secretion (bottom). Scale bar = 100 μm. D) Quantification of single macrophage secretion of TNFα over 12h and 18h (top) and MCP-1 over 24h (bottom). All macrophages were stimulated with 10 ng/ml LPS and IFNγ prior to cytokine interrogation.

Conclusions: We developed PDMS microwells that promote selective cell adhesion and thus control cell shape and area on a single cells level. Using an immunofluorescence-based detection strategy, we can detect cytokine secretion by single cells in the wells. We use this platform to study the effects of physical and adhesive factors on macrophage secretion on a single cell level. This technique is amenable to studying other cell-surface interactions.

References: [1] Chen, S et al. *Biomaterials* 31.13 (2010) [2] Adair-Kirk TL et al. *Int J Biochem Cell B* 40.6-7 (2008) [3] Kao, W et al. *J Biomed Mater Res* 55.1 (2001) [4] McWhorter, FY et al. *PNAS* 110.43 (2013)