# Utilizing PEG Hydrogels to Investigate the Role of Macrophage Phenotype on Vascular Development

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**Statement of Purpose:** The immune system plays a crucial role in wound repair and tissue remodeling. Specifically, recent *in vivo* work has demonstrated the essential role of macrophages with respect to organogenesis, vascular anastomosis and tissue reorganization [1, 2]. As seen *in vivo*, macrophages possess the abilities to bridge endothelial sprouts as well as breakdown vascular plexuses [1]. Moreover, macrophages are known to adopt two main phenotypes depending on environmental stimuli- M1 macrophages which promote inflammation and M2 macrophages which are involved in tissue repair [3]. Thus the ability to control macrophage phenotype can directly influence the ability to regulate vasculature formation and even tissue regeneration. We propose to utilize a PEG-based hydrogel system in order to develop a 3D angiogenesis model investigating the role of macrophage phenotype on vessel formation. This system will illuminate the effects of immune modulation with respect to macrophage phenotype on vasculature development. Additionally, this work will provide insight into the juxtacrine and paracrine signaling effects that macrophages have on endothelial cells. Through the use of a biomimetic PEG hydrogel, we will be able to study macrophage phenotype interactions within a 3D co-culture environment.

**Methods:** Within these studies, C57BL/6 mouse primary bone marrow derived macrophages (BMDMs) and primary brain microvascular endothelial cells (ECs) were used. To establish M1 and M2 phenotypes, previously published cytokine levels were used [4]: 10 ng/ml IFN$\gamma$ and 100 ng/ml LPS to induce M1 and 20 ng/ml of Il-4 and IL-13 to induce M2. To assess polarization, flow cytometry was performed using the following markers: CD 11b, a universal macrophage marker, CD86 to indicate M1, CD206 to indicate M2.

The biomimetic hydrogel we use for our studies is prepared by integrating peptides into polyethylene glycol (PEG) diacrylate hydrogels via NHS ester amine substitution chemistry [5]. Specifically, a MMP-2/9 sensitive peptide, GGGPQGIWGQGK (referred to as PQ), and an adhesive peptide, RGDS, are PEGylated. The PEGlyated peptides, cells and a photoinitiator are mixed in a pre-gelation solution; the hydrogel is formed upon 30 seconds of white light exposure.

Immunocytochemisty was performed on the hydrogels to visualize network formation.

**Results:** BMDMs were capable of adopting both a M1 and M2 phenotype as ascertained by flow cytometry (Fig. 1A). The CD86 mean fluorescence intensity (MFI) of the BMDM when stimulated with the M1 cytokine profile was 639 versus an MFI of 141 for M2 stimulated BMDM and an MFI of 42.8 for unstimulated BMDM. Alternatively, BMDM stimulated with M2 cytokine profile expressed an MFI of 837 for CD206 versus 111 MFI for BMDM stimulated with M1 profile and a 167 MFI for unstimulated BMDMs.

To create a reference vasculature model, primary brain microvascular ECs were encapsulated within the hydrogel. These cells formed immature vasculature networks in the presence of mouse pericyte precursor cells (10T1/2 cells) (Fig. 1B).

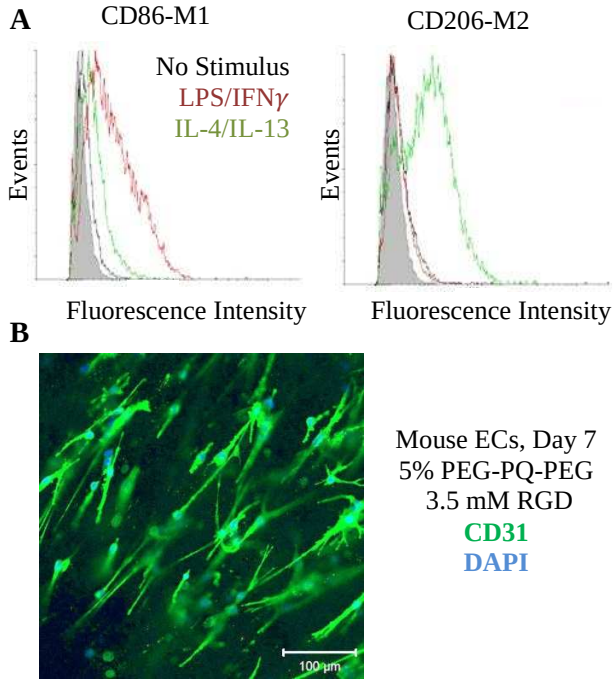


**Figure 1. A)** BMDMs are capable of M1 and M2 profiles via flow cytometry. **B)** Mouse ECs are capable to forming vessel networks in biomimetic PEG-based hydrogel.

**Conclusions:** The primary mouse ECs are capable of encapsulation within the biomimetic hydrogel; the ECs also degrade the hydrogel in order to create immature vessels within a co-culture system with pericyte precursor cells. Furthermore, BMDMs are capable of adopting M1 and M2 phenotypes in response to soluble stimuli as validated via flow cytometry.

Current work being investigated includes the encapsulation of ECs with M1/M2 stimulated BMDMs in order to elucidate the effects macrophage phenotype has on vessel formation within the hydrogel. Macrophage phenotype effects on vasculature have minimally been studied within 3D *in vitro* models; thus, this work provides crucial information concerning the role of macrophages in vascular development through the use of a functionalized PEG-based hydrogel. Future work will endeavor to uncover the paracrine effects of each macrophage phenotype on vessel formation as well as investigating any synergistic aspects of cell-cell contact between the macrophage and the ECs.

**References:** [1] DeFalco, T. *PNAS* (2014): 201400057. [2] Fantin, A. *Blood* 116.5 (2010): 829-840. [3] Martinez, FO. *F1000prime reports* 6 (2014). [4] McWhorter, FY. *PNAS* 110.43 (2013): 17253-17258. [5] Moon, JJ. *Biomaterials* 31.14 (2010): 3840-3847.