Material-Modulated Polymorphonuclear Leukocyte Degranulation Affects Monocyte Adhesion on Endothelial Cells Under Shear Stress

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Statement of Purpose: The purpose of this study was to determine how biomaterial-mediated polymorphonuclear leukocyte (PMN) degranulation affected subsequent acute inflammation events in the foreign body response. We previously showed that poly(ethylene glycol) (PEG)-containing hydrogels mediated a several-fold increase in PMN primary granule exocytosis in comparison to PMNs cultured on tissue culture polystyrene (TCPS) and polydimethylsiloxane (PDMS).[1,2] The primary granule subset is important to the progression of the inflammatory response because it contains several proteins like cathepsin G and azurocidin, which can recruit monocytes to the site of degranulation.[3] Monocytes activated by PMN granule proteins can arrest on the endothelium and extravasate into the tissue. Once recruited, monocytes affect further downstream wound healing events. Thus, we investigated whether media conditioned with PMNs cultured on selected biomaterials—PEG, PDMS, TCPS, and an interpenetrating network (IPN) composed of PEG diacrylate and cysteine-PEG conjugated gelatin—could influence monocyte transendothelial migration and adhesion to endothelial cells.

Methods: PMNs and monocytes were isolated via gradient separation from human peripheral whole blood collected from healthy volunteers. PEG hydrogels, PDMS, and IPN hydrogels were prepared as previously described,[1,4] cut into 8-mm discs, and placed in a 48-well plate. PMNs (5.3×10^3 PMNs/mm^2) were cultured on each biomaterial condition for 2 hrs in 37ºC with 5% CO_2 in RPMI-1640 with 10% autologous human serum (AHS). Collected PMN-conditioned media (PCM) was used in static modified Boyden microchemotaxis chambers (Neuro Probe, Gaithersburg, MD) and in flow chambers (Ibidi, Martinsried, Germany). For static experiments, conditioned media was placed below a microchemotaxis chamber filter with or without a human umbilical vein endothelial cell (HUVEC) monolayer. For adhesion under shear stress experiments, conditioned media was incubated with HUVECs grown to confluence in gelatin-coated µ-Slide IV$^{0.4}$ linear channel flow chambers and stimulated with TNF-α. Monocytes were perfused across HUVECs at a shear stress of 5 dyn/cm^2. Transmigrated monocytes and firmly adhered monocytes were counted in a 10x field for both static and shear conditions.

Results: PMNs cultured on PEG released 633 ng/mL of primary granules HNP1-3 in comparison to 27 ng/mL and 44 ng/mL HNP1-3 for PMNs cultured on PDMS and TCPS, respectively.[1] Figure 1A shows the fold increase in monocyte transmigration in response to PCM compared to transmigration in response to RPMI-1640 with 10% AHS (represented by the dotted line). No significant differences were observed between biomaterials with or without a HUVEC monolayer. However, with the addition of shear stress, PCM from PMNs cultured on PDMS resulted in a significant increase in monocytes adherent to TNF-α-activated HUVECs compared to monocyte adhesion to TNF-α-activated HUVECs incubated with a vehicle control (RPMI) (Figure 1B). Data represents the fold-increase in adherent monocytes normalized by monocyte adhesion to unstimulated HUVECs (represented by the dotted line) after 15 minutes of monocyte exposure to shear stress.

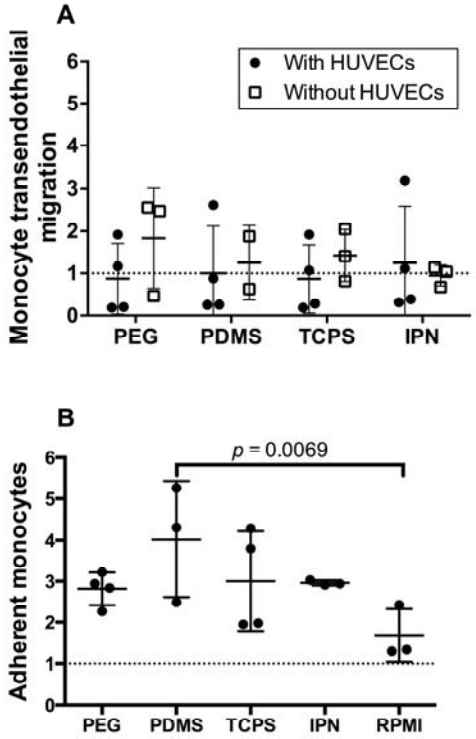

Figure 1. (A) Transmigrated monocytes and (B) monocytes adherent to HUVECs in response to PMN-conditioned media.

Conclusions: The release of primary granule proteins for PMNs adherent on PEG is likely above the optimal range of inducing monocyte transmigration whereas the concentration for that of adherent PMN on PDMS resulted in an observable elevation of monocyte adhesion on endothelial cells.

References: **1.** Cohen HC et al. Am. J. Pathol. 2013;182:2180-2190. **2.** Cohen HC et al. J Biomed Mater Res A 2014;102:4252-4261. **3.** Soehnlein O et al. Trends Immunol. 2009;30:538-46. **4.** Xu KD et al. Acta Biomater. 2012;8:2504-2516.