Poly(ethylene glycol)-Containing Hydrogels Promote the Release of Primary Granules from Human Blood-Derived Polymorphonuclear Leukocytes

Hannah Caitlin Cohen,[1] Tyler Jacob Lieberthal,[2] W. John Kao[1, 2]
[1]Pharmaceutical Sciences Division, School of Pharmacy, University of Wisconsin-Madison
[2]Department of Biomedical Engineering, University of Wisconsin-Madison

Statement of Purpose: The purpose of this study was to determine how selected biomaterials—poly(ethylene glycol) (PEG) hydrogels, polydimethylsiloxane (PDMS), tissue culture polystyrene (TCPS), and gelatin-PEG (GP) hydrogels—induce polymorphonuclear leukocyte (PMN) degranulation events. PMNs contain four subsets of granule proteins: primary granules, secondary granules, tertiary granules, and secretory vesicles. Upon activation, PMNs can exocytose their granule subsets to recruit monocytes (MCs) and mediate MC/macrophage activation and function,[1] thereby influencing subsequent inflammatory or wound healing events in the foreign body response. Secretory vesicles are generally released first, followed by tertiary granules, secondary granules, and lastly, primary granules. The release of primary granules is tightly controlled due to their abundance of microbicidal proteins and proteases which can injure bystander cells, degrade the extracellular matrix (ECM) and promote inflammation. We investigated the release of myeloperoxidase (MPO), a primary granule marker, and matrix metalloproteinase-9 (MMP-9), a tertiary granule marker, from human blood-derived PMNs cultured on the above-mentioned biomaterials.

Methods: Whole blood was collected from consenting, healthy human donors and PMNs were isolated using a gradient method followed by erythrocyte hypotonic lysis.[2] PEG hydrogels, PDMS and GP hydrogels were prepared as previously described, [3,4] cut into 8-mm disks using a biopsy punch and placed into 48-well plates. PMNs were statically seeded on the biomaterials and cultured for 2 or 4 hours in a humidified atmosphere at 37°C with 5% CO_2. At 2 and 4 hours, cell culture supernatants were removed and stored, and viable and necrotic cell adhesion was analyzed using LIVE/DEAD® Viability/Cytotoxicity Kit (Molecular Probes, Eugene, OR). MPO and MMP-9 concentrations in cell culture supernatants were measured using immunoassay kits (Hycult Biotech, Plymouth Meeting, PA and EMD Millipore, Billerica, MA) to determine the extent of PMN release of primary and tertiary granules in response to culture on the biomaterials. Experiments were repeated for each of four donors.

Results: There were no significant differences in adherent viable or necrotic cell densities among biomaterials. At 2 hours, supernatants from PMNs cultured on PEG hydrogels had significantly higher MPO concentrations than those from PMNs cultured on PDMS or TCPS (p = 0.001–0.05) (Figure 1). Similarly, at 2 hours, PMNs cultured on GP hydrogels released greater concentrations of MPO than PMNs cultured on PDMS or TCPS (Figure 1). MMP-9 release from PMNs was comparable among biomaterials (Figure 1), suggesting that PMNs cultured on PEG-containing hydrogels (i.e., PEG and GP hydrogels) have different mechanisms of release for primary and tertiary granules.

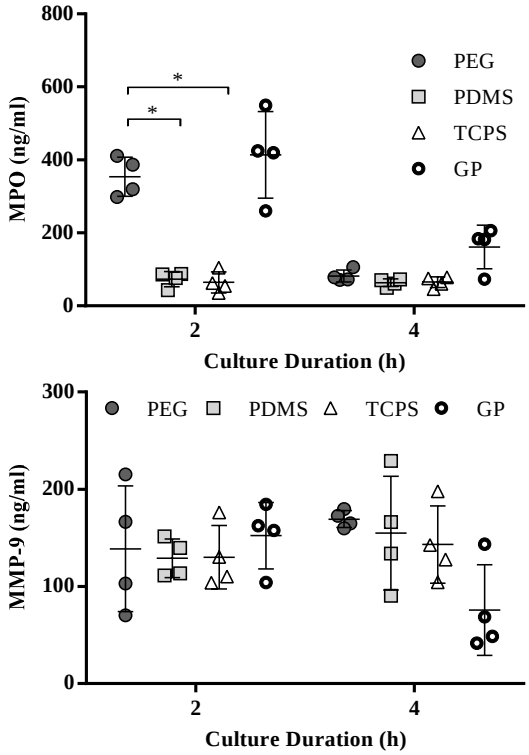

Figure 1. MPO (top) and MMP-9 (bottom) release from PMNs cultured on PEG hydrogels, PDMS, TCPS and GP hydrogels for 2 and 4 hours. Results are shown as mean ± standard deviation with each data point representing a different donor (n = 4); *: p = 0.01–0.05.

Conclusions: PEG-containing hydrogels promote primary granule release from PMNs, suggesting that PMNs may undergo an inflammatory response to PEG. Ongoing studies are investigating the signaling mechanisms involved in biomaterial-mediated PMN degranulation. By controlling biomaterial-mediated primary granule release from PMNs, we may be able to achieve a more favorable foreign body response that minimizes pro-inflammatory MC/macrophage polarization, limits injury to bystander cells and limits ECM degradation.

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References: 1. Soehnlein O et al. Trends Immunol. 2009;30:546-556. **2.** Waldeck H et al. Biomaterials 2012;33:29-37. **3.** Cohen HC et al. Am. J. Pathol. 2013;182:2180-2190. **4.** Xu KD et al. Acta Biomater. 2012;8:2504-2516.