Determination and modulation of reactive oxygen species production and release by human peripheral blood-derived monocytes adhered to poly(ethylene glycol)-based materials

Heather Waldeck[1] & Weiyuan John Kao, Ph.D.[1,2]
[1] Department of Biomedical Engineering, College of Engineering, University of Wisconsin-Madison, WI USA
[2] School of Pharmacy, University of Wisconsin-Madison, WI USA

Statement of Purpose: Reactive oxygen species (ROS) play a vital role in mediating the cellular response to foreign materials by both acting as intercellular small signaling molecules and in the form of a respiratory burst performed by professional phagocytes. If ROS are produced in excess, however, it can lead to variety of consequences including degradation of the biomaterial, damage to the surrounding tissue, and increased tendency for fibrous tissue to form rather than the regeneration of native tissue. Previous work has shown that the composition of biomaterials can affect the extent of ROS production, but little investigation has been done into ROS production by cells, particularly primary monocytes, in the presence of poly(ethylene glycol) (PEG)-based hydrogel systems or into the affect of surface-associated proteins and relevant oligopeptide domains of these proteins. In this study, we examined the difference in ROS production and release by human peripheral blood derived monocytes adhered to varying compositions of PEG-based hydrogel systems, including an semi-interpenetrating network (sIPN) containing gelatin modified with the adhesion ligand, RGD.

Methods: Material construction: 13, 16, and 19 w/w% PEG hydrogels were constructed using 575 and 3400Da PEG diacrylate (PEGdA). sIPNs were composed of 575 PEGdA and unmodified, PEGylated peptide(RGD, PHSRN, or GDR)-modified gelatin at a 3:2 or 1:1 weight ratio. The method for the modification of gelatin with PEGylated-peptide follows previously established and characterized procedures. Characterization of intermediate and final products was done using HPLC, GPC, ^{1}H-NMR, and a method based on trinitrobenzenesulfonic acid and spectrophotometry. Prior to cell seeding, all surfaces were incubated for two hours with 10% serum containing, individual serum protein containing, or serum free media.

Monocyte isolation and culture conditions: Human peripheral blood monocytes were isolated from citrated whole blood of a healthy adult volunteer using a density-gradient, non-adhesion method.

ROS detection and cell enumeration: 3'-(p-aminophenyl) fluorescein (APF; ex/em: 490/515; Molecular Probes) was used to detect intracellular and extracellular $^{\bullet}$OH, $ONOO^{-}$, and HOCl. Hoechst 34580 dye (ex/em: 380/450; Molecular Probes) was used to estimate adhered cell number simultaneously. Monocytes were incubated at 37°C with 10µM APF and 2µg/ml Hoechst 34580 for 45 minutes prior to seeding at $1x10^{6}$cells/ml in serum-free, phenol-free HBSS onto sIPN, PEG hydrogel, and tissue culture polystyrene (TCPS) surfaces. As a control, 0.1µg/ml lipopolysaccharide (LPS) was added to stimulate ROS production. At 2, 24, and 48 hours, the fluorescent intensity of both probes was quantified using a fluorescence microplate detector. For quantification of extracellular release of ROS, supernatant containing 1µM APF was collected at each time point and analyzed fluorescently.

Results/Discussion: Despite decreased adherence to sIPN and PEG hydrogel surfaces compared to TCPS surfaces, monocytes adhered to PEG and sIPN surfaces produced greater amounts of ROS without the stimulation of LPS. Additionally, the addition of gelatin to the PEG hydrogel led to a general increase in the production of ROS with or without the stimulation of LPS or the presence of pre-adsorbed serum proteins.

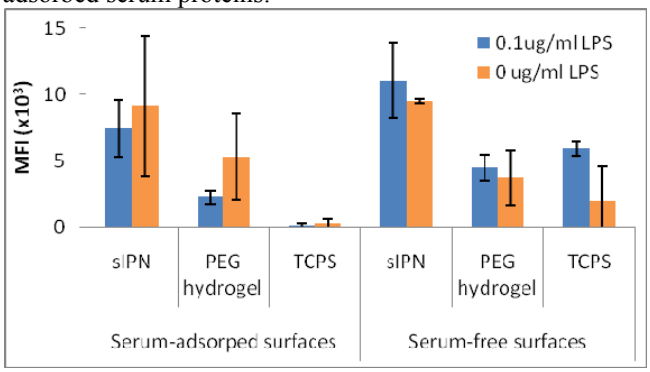

Figure 1. Intercellular production of ROS by primary monocytes adhered to sIPN, PEG hydrogel, and TCPS surfaces at 2hr with or without stimulation by LPS or serum pre-adsorption. Similar trends were seen at 24hr.

Extracellular production or release of ROS by adherent monocytes stimulated with LPS was also greater with the sIPN (14810±3395 MFI) as compared to the PEG hydrogel (7854±3847 MFI), while cells adhered to TCPS did not demonstrate any extracellular ROS. The addition of LPS led to increase in extracellular ROS by cells adherent to the sIPN (14810±3395 vs. 6197±42) but not the PEG hydrogel (7854±3847 vs. 7378±3002) suggesting that there may be a surface affect contributing to monocyte maturation.

Further work is needed to explore ROS production by monocytes adhered to additional surfaces, including RGD-modified sIPNs and surfaces adsorbed with individual serum proteins, as well as to further delineate the presence of intracellular and extracellular of ROS.

Conclusions: Adhesion of primary monocytes to PEG-based hydrogels stimulates intracellular and extracellular production or release of ROS. Alterations in the composition of the system may provide insight into the mechanisms of ROS production and thereby monocyte stimulation and offer methods by which to control cellular response through material formulation. Opportunities also exist for delivering signaling molecules using our sIPN system to further modulate ROS production which may be explored in the future. **Acknowledgements**: NIH Grant R01HL077825 and R01 EB006613