# PEG-based Hydrogels Modulate Reactive Oxygen Species Release and Cellular Death of Primary Phagocytes

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**Statement of Purpose:** The wound healing process consists of a complex interaction of degradation, regeneration, and remodeling involving a myriad of biological players. Initial events, mediated in part by the influx and subsequent response of monocytes and polymorphonuclear neutrophils (PMN), influence the progression of healing and the final composition of the healed tissue. The release of factors such as reactive oxygen species (ROS) by phagocytes mediated through controlled means ("respiratory burst") and/or mechanisms intertwined with cell death can drive pro- or anti-inflammatory behavior. ROS, in turn, play a vital role in mediating the cellular response to foreign materials by acting as both mediators of degradation and intercellular small signaling molecules influencing such processes as cell death or differentiation. ROS has been implicated in biomaterial degradation and device failure, however, little investigation has been published on this topic as well as on ROS production in the presence of poly(ethylene glycol) (PEG)-based hydrogel systems. Additionally, *in vitro* monocyte and PMN adhesion to PEG hydrogels as well as tissue culture polystyrene (TCPS) has been shown to decrease over time but the mechanisms behind this decrease is not fully understood. In this study, we characterized ROS release from and cell death of human peripheral blood derived monocytes and PMNs in the presence of PEG-based hydrogels.

**Methods:** ***Material construction:*** 13 w/w% PEG hydrogels were constructed using 3400Da PEG diacrylate (PEGdA). Semi-interpenetrating networks (sIPN) were composed of 8w/w% gelatin and 12w/w% 3400Da PEGdA TCPS and non-treated polystyrene (NTPS) were used as control surfaces. ***Monocyte isolation and culture conditions:*** Human peripheral blood monocytes and PMNs were isolated from citrated whole blood of a healthy adult volunteer using a density-gradient, non-adhesion method. Cells were seeded on surfaces at $1x10^6$ cells/ml in RPMI+10% autologous human serum. ***ROS detection:*** At 0, 2, 24, 96hr cells were incubated for 2hr in the presence of the extracellular ROS probe OxyBurst $H_2$HFF BSA (10µg/ml; Invitrogen). The addition of 100nM PMA was used as a positive control. ***Characterization of cell death/viability*:** The level of apoptosis, viability, and overall necrosis of adherent and non-adherent cells was determined by measuring caspase 3/7, intercellular protease, and extracellular LDH activity, respectively (ApoTox-Glo Triplex Assay, Promega). Levels of primary and secondary necrosis were differentiated by measuring HMGB1 and caspase-3 p20 subunit in the supernatant using an ELISA assay. TNF-α levels in the supernatant were also assayed by ELISA. MMP inhibitors GM6001 (10µM) and PD150606 (50µM) were added in order to asses MMP-dependence on active cell detachment.

**Results/Discussion:** For both cell types, ROS levels decreased from 2 to 96 hr and demonstrated increased ROS production in response to PMA (Figure 1). ROS production in response to PMA on PEG and sIPN surfaces, however, was not as high as that seen on NTPS surfaces despite similar levels of cell adhesion. At 0hr, ROS production by cells exposed to PEG (PMN only) and sIPN surfaces was significantly higher than NTPS surfaces without PMA stimulation. These results suggest a surface effect relating to ROS release as a result of either an active process and/or as a byproduct of cell necrosis. TNF-α production, a known inducer of both apoptosis and necroptosis, was significantly increased by monocytes in the presence of PEG hydrogels as compared to TCPS at 24 (2377±1305 vs. 221±128 pg/ml) and 168 hours (138±53 vs. 15±5 pg/ml). Apoptosis of monocytes exposed to both PEG and TCPS surfaces was significantly increased at 120 and 168 hr as compared to 2, 24 and 48 hours. Additionally, viability of detached monocytes increased from 2 to 24 hours suggesting a mechanism of active detachment. Work characterizing mechanisms of cell detachment including MMP-mediated pathways as well as various types of cell death are currently underway. Initial data suggests MMPs playing a critical role in the detachment of cells from TCPS but not PEG hydrogel surfaces.

**Conclusion:** Surface identity affects extracellular ROS production by primary monocytes and PMNs. Current work characterizing cell death on these surfaces may also provide insight in to the mechanism of ROS release and active cell detachment.

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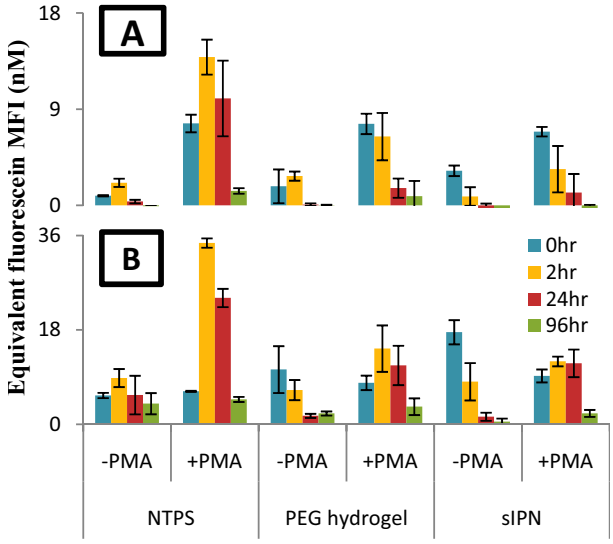


**Figure 1:** Extracellular ROS production by primary human monocytes (A) and PMNs (B) exposed to three different surfaces with or without PMA stimulation.