The interaction between hydrolytic and oxidative pathways in macrophage-mediated polyurethane degradation

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Introduction: In response to polycarbonate-based polyurethane (PCNU) surfaces monocyte-derived macrophages (MDM) release reactive oxygen species (ROS) by means of protein kinase C (PKC) initiation of the respiratory burst[1]. Previous studies showed that activation of PKC by phorbol myristate acetate (PMA) resulted in reduced degradation of the aliphatic PCNUs by esterases (enzymes previously shown to be synthesized and released by MDM adherent to PCNUs), whereas the aromatic PCNU was unaffected[2]. However, the effect on degradation could not be correlated to the release of ROS. Few studies have been able to link the hydrolytic and oxidative pathways of MDM-mediated PCNU degradation.

Statement of Purpose: To investigate the oxidation of PCNUs and how this affects subsequent MDM-mediated degradation.

Methods: Human monocytes were isolated from whole blood and allowed to differentiate to MDM on polystyrene for 14d. MDM were then gently trypsinized, re-suspended in media and re-seeded onto three PCNU surfaces (synthesized with either 1,6 hexane diisocyanate (aliphatic) or 4,4'-methylene bisphenyl diisocyanate (aromatic), polycarbonate diol (PCN) and 1,4-butanediol (BD) in different stoichiometric ratios (HDI:PCN:BD, 4:3:1 (HDI431); HDI321; and MDI:PCN:BD, 3:2:1 (MDI321)) with a ^{14}C radiolabel incorporated into the structure with ^{14}C-BD. In a 72h experiment, PCNU coated slips were either pretreated with 20% H_2O_2 or deionized-distilled water (ddH_2O) as described previously[2]. In another 72h experiment, the samples were pre-treated with either H_2O_2 or ddH_2O for 24h and then incubated with MDM for 48h. The radiolabel release (RLR) was counted after the 24h pre-treatment and after incubating with MDM for 48h. Cell morphology and PCNU degradation were assessed by scanning electron microscopy (SEM). Esterase (EA) and acid phosphatase (AP) activities were measured spectrophotometrically[3]. For live cell ROS measurement experiments, after 1h the medium was replaced with or without PMA (10^{-7}M) and 5μM dihydroethidum (HET) for 15 minutes. The slips were then mounted in an aluminum chamber in media without HET and live images were taken[4]. Intracellular superoxide anion oxidizes the blue fluorescent HET to red fluorescent ethidium (at 547nm) allowing quantification of ROS produced.

Results/Discussion: SEMs of PCNU slips pretreated with 20% H_2O_2 showed that HDI431 had visible holes (Fig.1a, arrows) with more radiolabel release than from the other PCNUs whereas there were cracks in the MDI321 surface (Fig.1c, arrows) indicating a different pattern or site of oxidation. When MDM were seeded on H_2O_2-treated PCNUs, degradation of HDI321 and MDI321, but not HDI431 was decreased. EA was inhibited in MDM on all surfaces except MDI321, whereas inhibition of AP activity (related to the innate immune response) occurred on all surfaces (Fig.2). The material surface itself, induced H_2O_2 release from live MDM, with more H_2O_2 elicited by PMA treated MDM when cultured on HDI431 but not the other materials (data not shown).

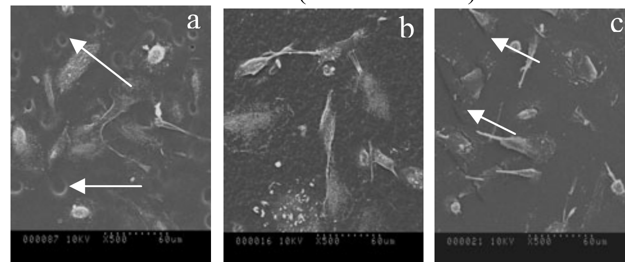

Figure 1: SEMs of MDM re-seeded on HDI431(a), HDI321(b), and MDI321(c) surfaces pretreated with 20% H_2O_2.

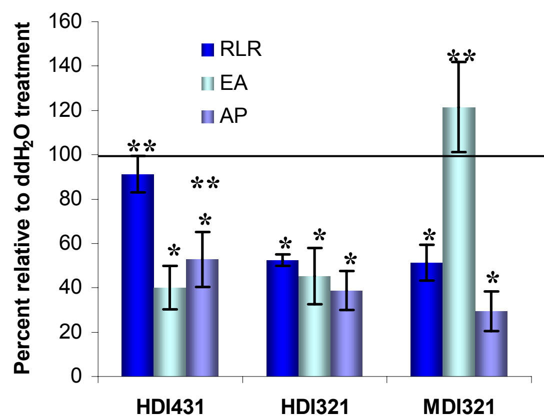

Figure 2: Effect of H_2O_2 pretreatment on MDM-mediated biodegradation, EA, AP activities following re-seeding on the PCNU surfaces. *significantly less than media control, $p<0.005$. **significantly greater than on the other two material surfaces, $p<0.005$. 100% is the no H_2O_2 treatment control.

Conclusions: For the first time, this study quantified the ROS produced by MDM in response to the PCNU surfaces with and without the PMA activation of PKC. The functional responses of MDM as measured by EA and AP activity showed that MDM react differently to the differentially oxidized surfaces. These findings highlight that the interaction of oxidative and hydrolytic degradative mechanisms may differ for each surface and therefore need to be taken into account when tailoring a material chemistry to produce desired cell responses for *in vivo* applications.

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