Monocyte-derived macrophage differentiation: Influence of polycarbonate-urethane chemistry on cellular function

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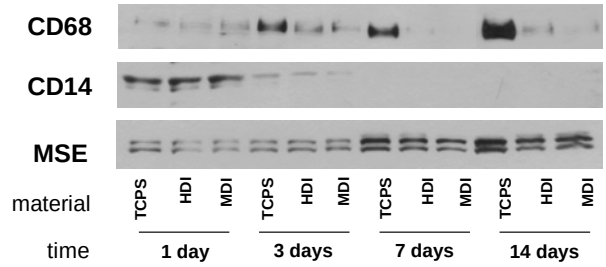
Statement of Purpose: Studies have demonstrated that monocyte-derived macrophages (MDM) possess degradative potential towards polycarbonate-urethane (PCNU) materials[1,2]. However, the degradation potential, morphology and function of these cells were investigated only once the MDM were fully differentiated and then reseeded to model PCNUs. The current study investigated the influence of non-degradable tissue culture polystyrene (TCPS) relative to two degradable model PCNUs on the progression of MDM differentiation. The function of monocytes differentiating towards MDM, particularly in terms of degradative capacity, was followed over a 14 day differentiation time course.

Methods: Model PCNUs were synthesized with either 1,6-hexane diisocyanate (HDI) or 4,4'-methylene bis-phenyl diisocyanate (MDI) reacted with poly(1,6-hexyl carbonate)diol (PCN) and 1,4-butanediol (BD) in stoichiometric ratios of HDI/PCN/BD 4:3:1 (referred to as HDI) or MDI/PCN/BD 3:2:1 (referred to as MDI). Monocytes were isolated from healthy human donors and differentiated for 14 days (on TCPS, HDI or MDI) towards MDM, in serum-supplemented RPMI 1640 without further cytokine supplementation. MDM were fixed at 14 days and prepared for scanning electron microscopy (SEM) or cell lysates were collected throughout the differentiation time course (1, 3, 7 and 14 days) for DNA analysis, enzyme activity and immunoblotting. MDM samples were prepared for DNA analysis and esterase activity (intracellular and secreted) as described previously[3]. Immunoblot analysis of cell lysates for CD14 (monocyte marker), CD68 (macrophage marker) and monocyte-specific esterase (MSE–candidate enzyme for degradation of PCNU)[4], was performed by loading lysates onto SDS-PAGE gels and transferring proteins to nitrocellulose membranes that were subsequently probed with the appropriate antibodies. Immunoblots were then exposed to film and bands were quantified (Quantity One® - Biorad) for relative banding intensity.

Results/Discussion: Quantification of DNA in cell lysates indicated that greater cell numbers were adherent by 14 days on both PCNUs relative to TCPS (MDI>HDI>TCPS)($p<0.05$ - data not shown). Since MDM are non-dividing cells the amount of DNA in cell lysates can be used as an indicator of cell number and also for loading equal cell numbers onto SDS-PAGE gels. When proteins from MDM lysates were probed for CD14 and CD68, increased expression of the macrophage marker CD68 was demonstrated with increased differentiation time point in TCPS-adherent MDM, whereas impairment of this expression in PCNU-adherent MDM occurred (Figure 1). Differentiation of MDM on all materials demonstrated appropriate down-regulation of the monocyte marker CD14 by day 3 (Figure 1). MSE is a candidate enzyme that has been shown to participate in MDM-mediated PCNU degradation[5,6]. Studies here have indicated that intracellular MSE protein expression increased over the MDM differentiation time course (Figure 1) suggesting that degradative potential increased with differentiation stage. Intracellular MSE protein at later time points, although increased at 7 and 14 days in HDI and MDI adherent cells relative to 1 and 3 days, was lower than MSE protein expression in MDM differentiated on TCPS. Correspondingly, esterase activity in combined cell lysate and conditioned media samples showed significantly greater activity for HDI samples by 14 days (data not shown) which potentially suggests that MDM differentiation on HDI stimulated the release of esterases in a response to degrade this material surface. These findings were further supported by the generation of "pitting" in the HDI material surface seen in SEM images from HDI-adherent MDM (Figure 2).



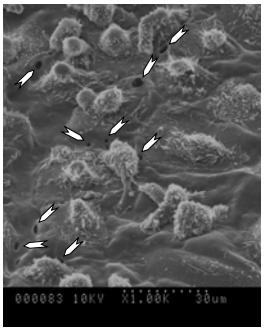


Figure 1 (above) Immunoblot analysis of MDM cell lysates for CD68, CD14 and MSE at day 1, 3, 7 and 14 of differentiation on TCPS, HDI or MDI.

Figure 2 (left) Human MDM differentiated for 14 days on HDI showing the generation of holes (white arrows) at material surface sites.

Conclusions: These studies have demonstrated that differences in the chemistry of material surfaces to which MDM are exposed during differentiation influences the phenotype and degradative potential of these MDM. This will modulate the foreign body response to PCNUs and enhance or accelerate the degradation of PCNU materials.

References: 1) Labow RS et al., *Biomater* (2001) 22:3025, 2) Labow RS et al., *Biomater* (2005) 26:7357, 3) Labow et al., *J Biomed Mater Res* (2001) 54:189, 4) Labow et al., *Biomater* (2002) 23:3969, 5) Labow et al., *Biomater* (2002) 23:3969, 6) Matheson et al., *J Cell Physiol* (2004) 199:8.

Acknowledgements: Donna Lee M. Dinnes is a CIHR Strategic Training Fellow in Cell Signaling in Mucosal Inflammation and Pain (STP-53877). Robert Chernecky performed imaging analysis of samples prepared for SEM (Faculty of Dentistry, University of Toronto).