Polycarbonate-based polyurethanes Stimulate Reactive Oxygen Species Production in Macrophages

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Statement of Purpose: During the foreign body response to a biomaterial, monocytes are recruited to the surface where they differentiate to monocyte-derived macrophages (MDM). These MDM may be activated to release reactive oxygen species (ROS) by means of the protein kinase C (PKC) activation of the respiratory burst[1]. Previous studies showed that activation of PKC by phorbol myristate acetate (PMA) resulted in reduced degradation of aliphatic polycarbonate-based polyurethanes (PCNU)s by esterases, whereas the aromatic PCNU was unaffected[2]. However, the degradation could not be related to release of ROS. In this study it was possible to measure the ROS using dihydroethidium (HET), a blue fluorescent dye that passes into the cell membrane and becomes red when oxidized by superoxide anion, the precursor for H_2O_2 and the end product in the MDM-mediated respiratory burst.

Methods: Human monocytes were isolated from whole blood by differential centrifugation. The monocytes were allowed to differentiate to MDM on polystyrene for 14 days[3]. MDM were then gently trypsinized to remove the cells from the surface, resuspended in media and re-seeded onto two PCNU surfaces (synthesized with either 1,6-hexane diisocyanate or 4,4'-methylene bisphenyl diisocyanate, polycarbonate diol (PCN) and 1,4-butanediol (BD) in the following stoichiometric ratios: HDI:PCN:BD, 4:3:1 (HDI431) and MDI:PCN:BD, 3:2:1 (MDI321). After 1h, the medium was replaced with or without PMA (10^{-7}M, PKC activator) and 5μM HET (Molecular Probes). After 15 min the coated coverslips with the cells were mounted in an aluminum chamber in media without HET and live images (fluorescent and phase contrast) were taken with an Olympus IX70 inverted microscope using a Polychrome IV monochromoter and an IMAGO CCD camera from TillPhotonics at the start, 1 h and 2 h time points post seeding on the surface. The cells were maintained at 37°C and excited at 547 nM. Areas of interest were selected around each cell and the arbitrary pixel fluorescence intensities were summed as previously described[4].

Results and Discussion: Live cell imaging was employed to determine if the ROS production by an MDM seeded on a PCNU surface could be measured without another stimulator of the respiratory burst (PMA). There was a measurable ROS response for cells seeded onto both PCNU surfaces without PMA, with the ROS release by cells on HDI431 being higher than that for cells on MDI321 (Fig. 1, $p<0.05$). Adding PMA to the MDM at the same time as HET did not increase ROS production by MDMs on MDI321, and inhibited ROS production by MDM on HDI431 (Fig. 1, $p<0.05$). Figure 2 shows the different morphology of MDM re-seeded onto the two surfaces without PMA. The cells on the HDI431 surface were smaller than those on the MDI321 as previously found[3]. Although larger, the fluorescence of the MDI321 re-seeded cells was lower per pixel, resulting in a lower total sum of intensities (Fig. 1, $p<0.05$).

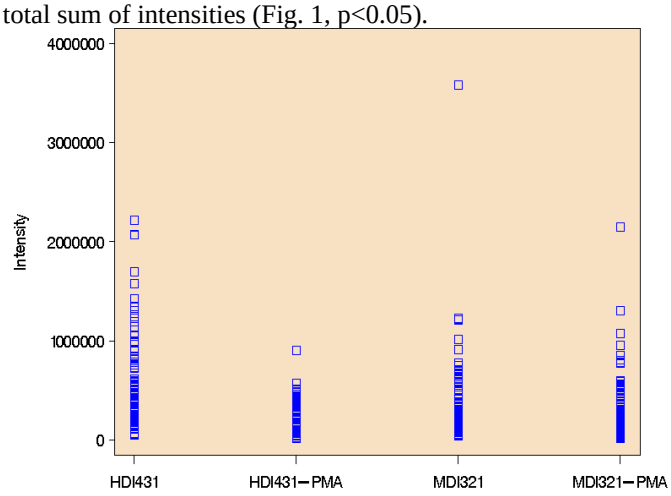

Figure 1: Live cell imaging of HET fluorescence (ROS) produced by MDM seeded on HDI431 or MDI321 in the presence or absence of PMA. Each box represents the sum of pixel intensities for one cell at one time point. Data represent one individual experiment.

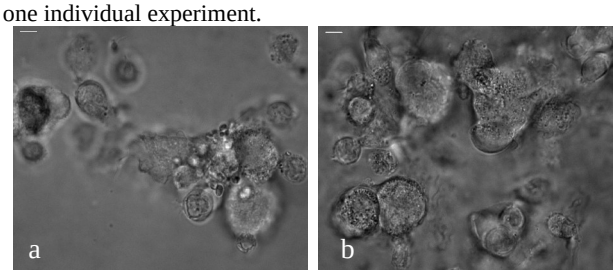

Figure 2: Phase contrast pictures of (a) HDI431 re-seeded MDM and (b) MDI321 re-seeded MDM. The white line (top left) represents 1 micron.

Conclusions: The ability to measure ROS production by MDM has allowed for a better quantification of the factors needed to trigger ROS production during the foreign body reaction to a biomaterial. Stimulation by the MDM being seeded onto the PCNU surface itself was sufficient to elicit ROS production in the cell. The inhibitory effect on ROS production by MDM on HDI431 supports previous data where MDM mediated PCNU degradation was reduced by PMA[2], whereas MDI321 degradation was unaffected. Therefore, as previously proposed[2], ROS production is differentially stimulated on the aromatic versus aliphatic surface, which alters surface chemistry and subsequent material biodegradation.

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