A degradable polar hydrophobic ionic polyurethane attenuates IgG-Fab site-induced monocyte activation

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Statement of Purpose: Monocytes and their derived macrophages (MDM) are an important component of the host response to an implanted biomaterial [1]. The monocyte/MDM's activation state will determine whether constructive tissue remodeling or fibrous tissue encapsulation occurs following implantation [2]. A degradable polar hydrophobic ionic polyurethane (D-PHI) has been shown to reduce pro-inflammatory monocyte activation relative to TCPS [3] and PLGA [4], as well as to support enhanced tissue integration and cell infiltration *in vivo* [5]. Since the adsorbed protein layer is known to be critical in directing the cellular response to biomaterials [1], adsorbed serum proteins were evaluated for their effect on activating monocytes adherent to D-PHI vs. TCPS. In particular, adsorbed IgG has been shown to be a potent substrate for MDM adhesion [6]. The objective of the present work was to evaluate the effects of adsorbed IgG on monocyte activation post-interaction with D-PHI vs. TCPS substrates, and to determine the mechanism through which D-PHI enables low monocyte activation.

Methods: D-PHI films were prepared by mixing a divinyl oligomer (DVO), methacrylic acid (MAA), and methyl methacrylate (MMA) in a 1:5:15 molar ratio along with the initiator benzoyl peroxide (0.032 mol/mol vinyl group) [7]. 50 µl of this mixture was cured in the wells of a 96-well polypropylene plate at 110°C for 24 hr. Monocytes were isolated from whole blood of healthy volunteers (University of Toronto ethics approval #22203) and cultured for up to 72 hr in RPMI-1640 medium supplemented with 10% autologous human serum (AHS) on D-PHI and TCPS surfaces either preadsorbed or non-preadsorbed with human IgG, IgG Fab fragment, or IgG Fc fragment (200 µg/ml). Three distinct donors were used for all experiments involving monocytes. Monocyte response was assessed by DNA mass quantification and scanning electron microscopy (SEM). Total protein quantification was performed using a micro BCA assay (Pierce) [8]. Exposed IgG Fab was quantified with an ELISA using an HRP-conjugated Fab-specific antibody (Sigma). Exposed Fab in monocyte experiments was blocked with a Fab-specific antibody (Sigma) supplemented with each medium change.

Results: Monocytes cultured on IgG-coated TCPS had 4.34 ± 0.68 times more DNA at day 3 vs. non-coated TCPS (550±185 ng) ($p<0.05$), while IgG-coated D-PHI had 1.03 ± 0.14 times as much DNA as its respective non-coated control (631±252 ng). SEM analysis indicated an enlarged, more spread cell morphology for monocytes adherent to IgG-coated TCPS vs. IgG-coated D-PHI (**Fig. 1B**). TCPS was shown to have significantly more exposed Fab fragment, as determined by ELISA (1.27 ± 0.01 A.U. vs. 0.14 ± 0.01 A.U. for TCPS vs. D-PHI, respectively), despite both materials adsorbing similar total amounts of IgG (1.11 ± 0.37 vs. 2.29 ± 0.66 µg/cm^2 for TCPS vs. D-PHI, respectively). IgG Fc fragment had no effect on monocyte retention or spreading, while IgG Fab fragment supported increased monocyte spreading similar to whole IgG (data not shown). Blocking exposed Fab reversed the effects of IgG on monocytes on TCPS, with no changes observed on D-PHI (**Fig. 1**).

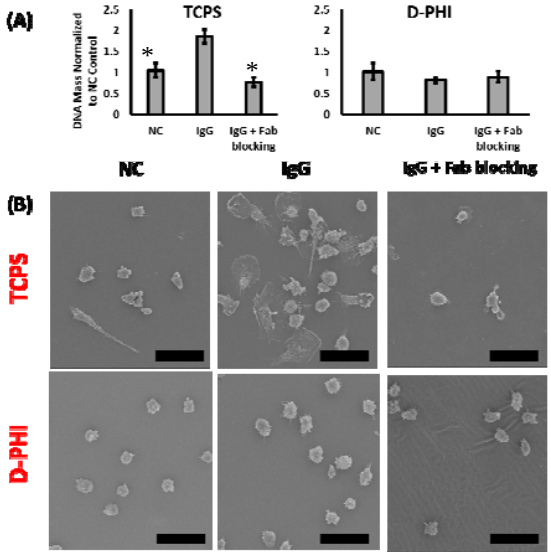

Figure 1 (A) DNA mass quantification and (B) representative SEM images for monocytes seeded on IgG-coated or non-coated (NC) TCPS or D-PHI with or without Fab blocking at day 3. Scale bars represent 30 µm. n=9 from three donors. Mean ± S.E. * $p<0.05$ vs. IgG coating.

Conclusions: IgG was shown to have a significant pro-monocyte activation effect when coated on TCPS, which was not seen on IgG-coated D-PHI. This was supported by the reduced exposure of the Fab region of IgG when adsorbed to D-PHI vs. TCPS, demonstrated through both Fab-specific ELISAs as well as by blocking exposed Fab in monocyte experiments with a Fab-specific antibody. Future work will examine the role of surface hydrophobic, ionic, and polar character in supporting a reduced monocyte activation through limiting Fab exposure, as surface chemistry, which is known to influence MDM activation, is a major differentiating factor between D-PHI and TCPS [8].

Acknowledgements: CIHR grant #230762, Cell Signals (Battiston), Ontario Graduate Scholarship (Battiston).

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