**Proteomic analysis of protein adsorption onto a degradable-polar/hydrophobic/ionic polyurethane**

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**Statement of Purpose:** Protein adsorption is the first event that occurs following biomaterial implantation [1]. It is known to be influenced by various surface properties, including specific functional groups [2]. A degradable polar/hydrophobic/ionic polyurethane (D-PHI) has previously been shown to support the growth of vascular smooth muscle cells (VSMCs) [3] and to induce a more anti-inflammatory monocyte phenotype relative to tissue culture polystyrene (TCPS) [4]. Monocyte phenotype is known to be influenced by the composition of the adsorbed protein layer [5], which in turn is influenced by various properties of the biomaterial surface. The objective of this study was to determine the unique surface properties of D-PHI relative to TCPS and poly(lactide-*co*-glycolide) (PLGA), as well as their different protein adsorption characteristics in order to gain understanding of D-PHI's unique cell-material interactions.

**Methods:** D-PHI films were prepared by previously established methods [3]. A degradable divinyl oligomer (DVO), methyl methacrylate (MMA, Sigma) and methacrylic acid (MAA, Sigma) were mixed in a 1:15:5 molar ratio. Films were cured for 24hrs at 110°C using the initiator benzoyl peroxide (BPO, Sigma). PLGA (75:25 lactide:glycolide, Sigma) films were prepared by dissolution in chloroform at 5wt% and casting on 5mm diameter glass cover slips. Films were left overnight to allow for solvent evaporation. Surfaces were characterized by x-ray photoelectron spectroscopy (XPS) and water contact angle analysis. Proteins were adsorbed to surfaces by incubation in a solution of RPMI-1640 medium with 10% fetal bovine serum (FBS) overnight (37°C, 5% $CO_2$). FBS was chosen as it will be used for future tissue culture work with VSMCs and monocytes. The surfaces were then rinsed three times with PBS and the adsorbed protein layer was eluted with a 2% sodium dodecyl sulphate (SDS) solution and protein content was quantified with a bicinchoninic acid (BCA) protein assay. Proteins were eluted from the different materials and were separated by 2D electrophoresis, silver stained (Bio-Rad) and the spots of interest were identified by mass spectroscopy (MS). Spots of interest were those showing differences in staining intensity between the 3 surfaces, with differences repeated a minimum of 2 times.

**Results:** D-PHI has been shown to have differential surface chemistry when compared to PLGA and TCPS, with differing hydroxyl (TCPS), carboxyl (PLGA>D-PHI>TCPS), carbonate (D-PHI), hydrocarbon (TCPS> D-PHI> PLGA) and urethane (D-PHI) functionality (data not shown). Contact angles varied in decreasing order of PLGA (89.8±2.1°), D-PHI (80.5±0.5°) and TCPS (48.2±0.8°). Protein adsorption measurements indicated a significantly greater amount of protein ($p<0.05$) adsorbed to the D-PHI (5.84±0.23 μg/cm$^2$) films compared to PLGA (1.65±0.16 μg/cm$^2$) and TCPS (1.58±0.07μg/cm$^2$). Differences in the make-up of the adsorbed protein layer are reported in Table 1.

Table 1. Protein staining intensity on biomaterial surfaces (☒ = absent, ☐ = low, 🟨 = medium, 🟥 = high)

| Identified Protein | Staining Intensity | | |
|---|---|---|---|
| | D-PHI | PLGA | TCPS |
| Anti-thrombin III (ATIII) | 🟥 | 🟨 | 🟨 |
| Apolipoprotein E (ApoE) | 🟥 | 🟥 | ☐ |
| Hemoglobin | ☐ | 🟨 | 🟨 |
| $\alpha_2$-macroglobulin ($\alpha_2$M) | 🟨 | ☐ | ☐ |
| $\alpha_1$-antitrypsin (AAT) | 🟨 | ☒ | ☒ |
| Transferrin | 🟥 | 🟨 | 🟥 |
| Tetranectin (TN) | 🟨 | 🟨 | ☐ |
| $\alpha_2$-antiplasmin | ☐ | ☐ | ☒ |
| Vitronectin (VN) | ☐ | ☐ | ☒ |
| Transthyretin | 🟨 | 🟥 | 🟥 |

Several of the proteins identified by MS have previously been shown to influence monocyte/macrophages, including ATIII [6], ApoE [7], $\alpha_2$M [8], AAT [9], TN [10] and VN[5]. These results demonstrate that in addition to adsorbing different quantities of protein from serum, there is also a difference in the specific proteins being adsorbed to D-PHI as compared to PLGA and TCPS. The diverse chemistry of D-PHI, with hydrophobic, polar and ionic character, may be an important mediator of this unique protein adsorption profile as well as being a factor in rendering D-PHI more conducive to supporting protein adsorption in general.

**Conclusions:** D-PHI films had greater protein adsorption (~3x greater) as compared to PLGA and TCPS surfaces. Several of the proteins identified by 2-DE and MS analysis may have important implications in understanding the previously observed response (anti-inflammatory) of monocytes to D-PHI when compared to other biomaterial surfaces [4]. Proteins identified that are known to influence monocyte phenotype will be assessed for their protein adsorption to D-PHI, PLGA and TCPS surfaces as well as for their influence on monocyte phenotype in order to better understand why D-PHI exhibits a unique non-inflammatory state when interacting with monocytes, in comparison to PLGA and TCPS.

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