Elastomer Crosslink Density Affects Protein Adsorption and Conformation

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Statement of Purpose: Cells are known to respond differently when grown on materials of varying stiffness[1]. However, the mechanism by which a cell senses its substrate is still unknown. Protein adsorption to a biomaterial surface is the precursor to cellular-biomaterial interactions. Cells, therefore, must receive information about biomaterial stiffness from the adsorbed protein layer.

An elastomer formed from acrylated *star*-poly(D,L lactide-*co*-ε-caprolactone) (ASCP) has previously been shown to support higher smooth muscle cell[2] and fibroblast proliferation on a lower crosslink density elastomer *in vitro* culture. ASCP elastomers are crosslinked by UV radiation and different ASCP crosslink densities are chemically similar and differ in only in bulk stiffness and polymer chain mobility. Because cell behavior is determined by the initial adsorbed protein layer on the elastomer surface, it was hypothesized that the crosslink density of the elastomer affects the composition and conformation of the adsorbed protein layer. The purpose of this research is to identify differences in the amounts and viscoelastic properties of adsorbed protein on different crosslink densities of ASCP elastomer.

Methods: ASCP pre-polymer (2000 g/mol and 5000 g/mol) was fabricated according to the procedure described in (3). Pre-polymers were mixed with a minimal amount of acetone and DMPA photoinitiator and crosslinked under UV radiation (30 mW/cm^2) to form the elastomer (ELAS 2000 and ELAS 5000, respectively). Protein adsorption mass was measured by radiolabelling. HSA (44 mg/mL), fibrinogen (2.5 mg/mL), immunoglobulin G (10.5 mg/mL), fibronectin (0.325 mg/mL) and vitronectin (0.225 mg/mL) were labeled with I^{125} using the iodine monochloride method[4] (HSA, Fg, IgG) or the Iodogen method[5] (Fn, Vn). ELAS 2000 and ELAS 5000 discs (n=4) were incubated in the radiolabelled protein for 12 hours. Radioactivity was converted to a protein mass using a standard curve.

Viscoelastic properties of adsorbed protein layers were quantified by quartz crystal microbalance with dissipation (QCM-D). Elastomer coated sensors (n=3) were inserted into the QCM-D module and conditioned in PBS overnight. Protein solution was then flowed over the sensor and the protein was allowed to adsorb for 12 hours. Protein layer viscoelastic properties were calculated by fitting the raw data to the Voigt viscoelastic model.

Results: Significantly more fibronectin adsorbed to the ELAS 5000 surface while significantly more IgG adsorbed to ELAS 2000 surface. Shear modulus of the adsorbed fibronectin and IgG layers were lower on the elastomer surfaces on which less protein was adsorbed.

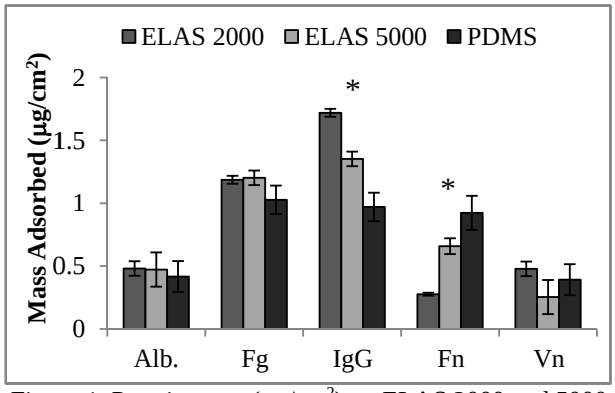

Figure 1. Protein mass (μg/cm^2) on ELAS 2000 and 5000

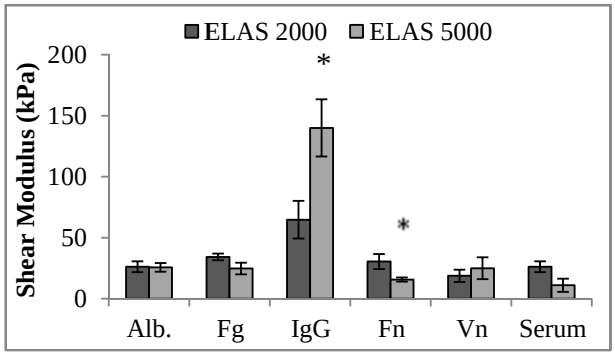

Figure 2. Protein modulus (kPa) on ELAS 2000 and 5000

Conclusions: Differences in fibronectin and IgG adsorption mass and viscoelastic properties were observed between the ELAS 2000 and ELAS 5000 surface, indicating that crosslink density does affect protein adsorption. Furthermore, higher Fn and IgG layer shear moduli were observed in layers with less adsorbed protein, suggesting that there is a difference in the conformation of the adsorbed protein rather than simply affinity. These differences in protein adsorption mass and conformation on the elastomers may be due to a difference in polymer chain mobility and ordered water at the material surface.

The results also emphasize the importance of examining protein adsorption from solutions of multiple proteins rather than individual protein adsorption. IgG layers have a much higher modulus compared to other proteins; however, despite the high concentration of IgG in serum, the adsorbed serum layer does not reflect the modulus of IgG, suggesting that IgG is not a large component of the competitively adsorbed serum layer.

References: 1. Discher DE. Science. 2005;310:1139-43. 2. Ilagan BG. Acta Biomater. 2009;5:2429-2440. 3. Amsden BG. Biomacromolecules. 2004; 5:2479-2486. 4. Weeks A. J Biomat Appl. 2012;0:1-11. 5. Sheardown H. Colloids Surf. B; 1997;10:29-33.