Elastomer Crosslink Density Affects Protein Adsorption and Conformation

+[1]Moira C. Vyner, [2]Lina Liu, [2]Heather D. Sheardown, [1]Brian G. Amsden
+[1]Queen's University, Kingston, Ontario, Canada, [2]McMaster University, Hamilton, Ontario, Canada

Introduction:

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 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.

Materials and Methods:

ASCP pre-polymer (2000 g/mol and 5000 g/mol) was fabricated according to the procedure described in Amsden, *et al.*[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. Human serum albumin (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. Mass of protein adsorbed from serum was measured by surface plasmon resonance (SPR). ELAS coated SPR sensors (n=3) were inserted into the module (Biacore 3000, Biacore, Sweden) and unsupplemented DMEM was flowed over the sensor until a baseline measurement was achieved (400 seconds). 330 μL of DMEM supplemented 10% fetal bovine serum was then injected into the flowcell (10 uL/min) for 1800 seconds. Following adsorption, unsupplemented DMEM was injected into the flowcell as a rinse step. Adsorbed protein mass was measured as the difference in signal between the stabilized post rinse data and the baseline. 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 phosphate buffered saline 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.

Since protein adsorption may be affected by the initial water layer bound to the elastomer surface, water adsorption was examined using ATR-FTIR analysis. ELAS 2000 and 5000 were conditioned in water for 0, 1, 4, and 24 hours. At each timepoint the elastomers were removed from the water, scanned and returned to the water. The O-H stretch (3350 cm^{-1}) peak was used as the indicator for surface water adsorption. Statistically significant differences in protein adsorption and viscoelastic properties were determined using a Student's t-test. Differences were considered significant at values of $p<0.05$.

Results:

Significantly more fibronectin adsorbed to the ELAS 5000 surface while significantly more IgG adsorbed to ELAS 2000 surface (Figure 1A). Shear moduli of the adsorbed fibronectin and IgG layers were lower on the elastomer surfaces on which less protein was adsorbed (Figure 1B). Serum from supplemented media adsorbed in greater amounts to the ELAS 5000 surface with a significantly lower shear modulus than the ELAS 2000 surface, suggesting that the composition or conformation of the adsorbed serum proteins are different between the two elastomer surfaces. The ELAS 5000 surface absorbed more water at all timepoints compared to the ELAS 2000 surface (Figure 2).

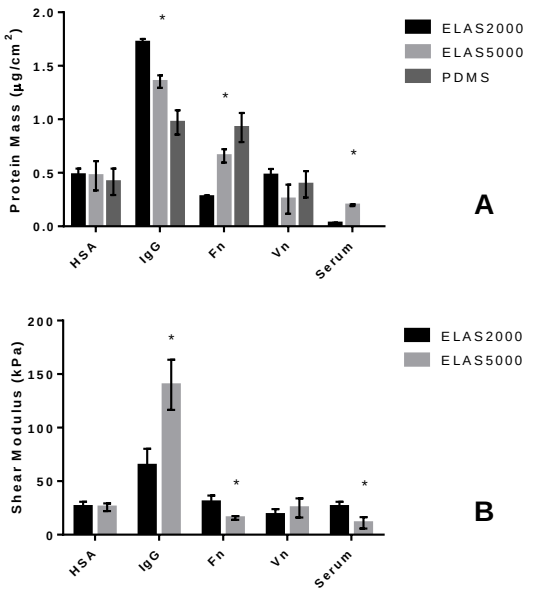

*Figure 1. A:Mass of protein adsorbed to ELAS surfaces; B: Shear modulus of protein layers adsorbed to ELAS surfaces. (n=3)*p<0.05 compared to ELAS2000*

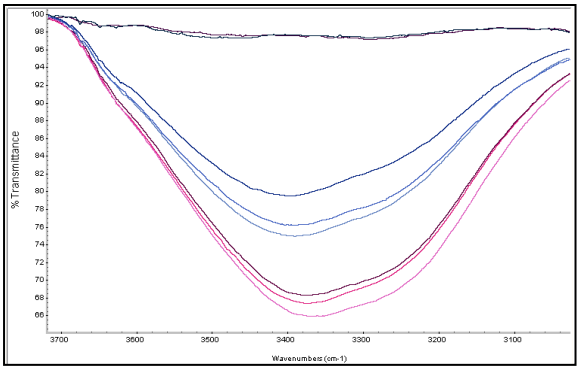

Figure 2. ATR-FTIR expansion of 3350 cm^{-1}(water stretching) peak. Scans of ELAS2000(top group) and ELAS5000(bottom group) incubated in water for 0, 1, 4, and 24 hours.

Discussion:

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 fibronectin 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. The results also emphasize the importance of examining protein adsorption from solutions of multiple proteins rather than individual protein adsorption alone. 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. The 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. ELAS 5000 absorbed more water to its surface than ELAS 2000, most likely because the ELAS 5000 material has a lower crosslink density and more mobile polymer chains, facilitating water absorption. A difference in water organization at the surface suggests that proteins may be experiencing different adsorption environments between the two elastomer surfaces. The surface that absorbs more water (ELAS 5000) may be adsorbing cell attachment proteins and/or inducing a more native protein conformation upon adsorption. In contrast, the ELAS 2000 surface absorbs less water and may appear more "hydrophobic", adsorbing immunoglobulins and/or causing some proteins to denature upon adsorption.

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