Strength of Fibronectin Adsorption to Polymer Materials

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Statement of Purpose: Complete endothelialization of the lumen of synthetic vascular grafts (SVG) prior to implantation to mimic the internal composition of a native vessel may mitigate the inherent thrombotic nature of these materials[1]. We optimized a system previously on glass and subsequently tested this system in a rat femoral artery model. The results were not consistent with those seen on glass: the cell retention with fibronectin (FN) was significantly lower than with the dual ligand system[2]. Cells attached using protein mechanisms detach at the onset of flow and these proteins, adequate in tethering to the cell strongly, may not be attaching to the material with equal strength. The detachment of these cells from the material surface could occur through two pathways: cohesive failure where the cells detach due to membrane rupture; adhesive failure where the cells detach due to failure at the protein-material interface. Cells may detach cohesively from high surface energy materials like glass, but detachment from low surface energy substrates may be due to adhesive failure. This study will examine FN detachment from Glass, Mylar, a film version of polyethylene terephthalate, and Teflon-AF, an amorphous copolymer of 2,2-bistrifluoromethyl-4,5-difluoro-1,3-dioxole (PDD) and tetrafluoroethylene (TFE).

Methods: FN was labeled with ^{125}I using Iodo-gen® iodination reagent (Pierce, IL). FN was incubated with $Na^{125}I$ in tubes coated with Iodo-gen® dissolved in chloroform for 30 minutes. Non-labeled $Na^{125}I$ was removed with a PD-10 column. Glass, Mylar, and Teflon-AF samples were incubated with radiolabeled protein for 1Hr, washed with copious amounts of PBS, put into plastic 12 x 75 tubes, and counted in a scintillation counter for 5 min. Samples were sonicated for 5 minutes in PBS with a Bransonic Ultrasonic Cleaner, washed with PBS, put into different 12 x 75 tubes, and counted for 5 minutes in a scintillation counter. Samples were then put into tubes with 0.1% Triton X-100, sonicated for another 5 minutes, washed with PBS, transferred to different 12 x 75 plastic tubes, and counted for 5 minutes in a scintillation counter. In all cases the control was a tube with just PBS. Absolute and relative amounts of protein detached from surfaces were calculated in all cases.

Results / Discussion: Amount of protein attached to surfaces was greatest on Mylar, then Teflon-AF, and then Glass (Figure 1). Once the samples were sonicated for 5 minutes in PBS the percent retention was greatest on Mylar, then Glass, and then Teflon-AF and total protein amount on the surface after sonication followed a similar order. After sonication in a mild detergent the percent FN retention was greatest on Glass, then Mylar, and then Teflon-AF but total protein amount followed the same order as sonication in PBS alone (Figure 2).

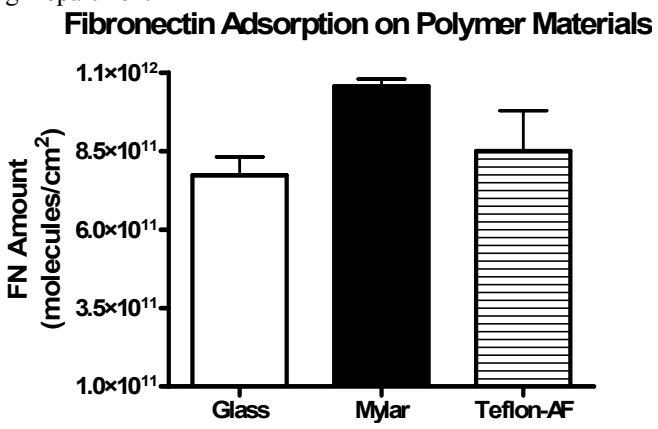

Figure 1. Total protein on Glass, Mylar, and Teflon-AF

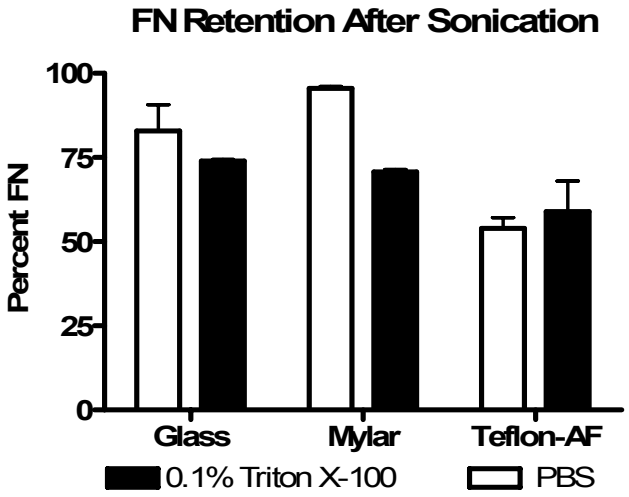

Figure 2. Percent FN retention on Glass, Mylar, and Teflon-AF after sonication

The protein retention on Mylar was significantly greater than Glass and Teflon-AF after sonication in PBS but this difference was only significantly greater than Teflon-AF after sonication in Triton.

Conclusions: Percent protein retention was lowest on Teflon-AF after sonication in PBS as well as in Triton X-100. This is an indication that, though proteins might bind in large amount to fluorinated surfaces, the strength of binding is weak. This would lead to adhesive failure of endothelial cells seeded onto SVGs. Future work will determine the "on and off" rates of FN on the SVG surfaces. A better understanding of these mechanisms will help design better experiments to achieve endothelialization of SVGs.

References: 1.Walluscheck KP. Eur J Vasc Endovasc Surg. 1996;12(3):321-30. 2. Chan BP. J. Biomed Mater Res. 2004 72B(1):52-63