Functionalized Poly(ethylene glycol) Dimethacrylate Hydrogels with Engineered Surface Topography to Promote Re-Endothelialization of Small Diameter Vascular Grafts

Chelsea M. Magin, Anthony B. Brennan.
[1]The J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville Florida, USA,
[2]Department of Materials Engineering, University of Florida, Gainesville Florida, USA.

Statement of Purpose: The clinical application of synthetic small-diameter (d<6mm) vascular grafts has been limited due to high rates of occlusion from thrombosis and intimal hyperplasia. Intimal hyperplasia can be caused by compliance mismatch between the graft and the vessel wall and poor re-endothelialization of the luminal surface. Recently, it has been suggested that capturing endothelial progenitor cells (EPCs) and inducing their differentiation into the endothelial cell (EC) phenotype could be the ideal way to re-endothelialize a small-diameter vascular graft. It has been reported that substratum elasticity directs stem cell differentiation into specific lineages[1]. Therefore, poly(ethylene glycol) dimethacrylate (PEGDMA) hydrogels which have a highly adjustable shear modulus[2] (G=10kPa to 1Mpa) were chosen as a substratum material. Our group has demonstrated that patterning of fibronectin (Fn) on polydimethylsiloxane elastomer (PDMSe) could be used to grow an EC monolayer with density and morphology similar to that of the native artery[3]. We have also shown that topographical cues induced EC morphologies *in vitro* that were stable to the shear stresses that would be experienced *in vivo*. It was hypothesized that a functionalized PEGDMA hydrogel would provide good compliance, high fidelity of topographic features and sites for surface modification with biomolecules. These properties should ultimately provide physical and chemical cues to increase cell attachment and differentiation.

Methods: The hydrogel prepolymer solution was prepared by combining 20 to 100wt% PEGDMA (Mn≈ 1000g/mol) with 0.2wt% ammonium persulfate and ascorbic acid as chemical initiators and balancing the total with deionized water. To create a functionalized PEGDMA 5wt% of glycidyl methacrylate (GMA) was added to the solution. After stirring, the solution was centrifuged to remove air bubbles, pipetted into a mold, and cured for 45 minutes at 45°C. The equilibrium water content (EWC), solubility parameter and average molecular weight between crosslinks ($<M_c>$) of the hydrogels were determined by swelling experiments. Fidelity of topographic features was evaluated with white light optical profilometry and scanning electron microscopy. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was used to verify chemical composition of the hydrogels. Fibronectin was covalently grafted to the hydrogel by reacting the epoxide ring of GMA with the amine nucleophiles on the protein in an alkaline buffer. Immunofluorescence and ATR-FTIR were used to detect the presence of Fn graft. Porcine vascular endothelial cells (PVECs) from a primary culture provided by Dr. Edward Block's laboratory were seeded onto the hydrogels at 50,000cells/mL and grown for 24 hours. Cells were fixed with 10% formalin and stained with crystal violet. Three light micrographs were taken per sample. The number of cells per field of view was counted and the average number of cells/mm^2 was reported.

Results: Channels and Sharklet CE™ microtopographies were created in PEGDMA-co-GMA hydrogels without significant size variations due to swelling.

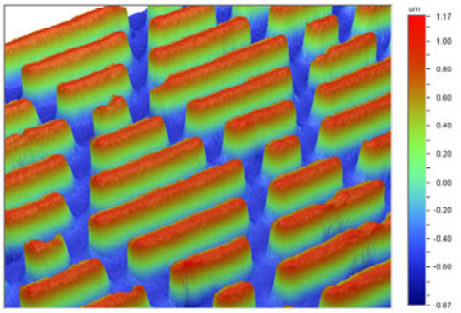

Figure 1. White light optical profilometry image of Sharklet CE™ topography cast in PEGDMA-co-GMA.

The EWC of PEGDMA-co-GMA hydrogels with 25wt% PEGDMA ranged from 50 to 60%. The $<M_c>$ for this hydrogel was measured to be 1480g/mol which is consistent with the predicted value. Fibronectin grafting was confirmed with ATR-FTIR and fluorescence intensity measurements (T-test α=0.05). A 1.6-fold increase in cells attached to the PEGDMA-co-GMA-graft-Fn surface versus the PEGDMA control that was exposed to the Fn grafting process was observed after 24h (T-test α=0.05). PVEC attachment assays also showed a 3.2-fold increase in the average number of cells attached to the Sharklet CE™ topography compared to smooth and channels (Tukey α=0.05).

Conclusions: PEGDMA-co-GMA hydrogels that have an adjustable modulus with a range of E=30kPa to 3Mpa which will allow for compliance matching to the native artery have been created and characterized. Fn grafting and topography both increased EC attachment after 24 hours. This combination of adjustable elasticity, surface chemistry and topography has the potential to promote the capture and differentiation of EPCs into a confluent EC monolayer that is non-thrombogenic and stable to shear stress making small diameter artificial vascular grafts feasible. Current work is focused on using these materials as substrata for EPC attachment and differentiation.

References:

1. Pfister PM. Biomaterials. 2007:28:567-575.
2. Engler AJ. Cell. 2006:126:677-689
3. Feinberg AW. Acta Biomater. 2009:5:2013-2024.