Development of Human Blood Outgrowth-Specific Terpolymer Biomaterials

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Statement of Purpose: Human blood outgrowth endothelial cells (HBOECs) are derived from the *ex vivo* culture of adult circulating stem cells, possess endothelial phenotype, and still retain stem cell characteristics such as robust proliferation capacity and rates. Although these cells possess great therapeutic potential challenges remain in their clinical utility such as difficult isolation and culture protocols and a poor understanding of cell surface markers. Therefore, we have created a material tuned to the specific adhesion of HBOECs for use in blood contacting biomedical device applications and as a substrate for improved HBOEC culture.

Methods: In previous work, we have developed a biostable methacrylic terpolymer copolymerized from hexyl-methacrylate (HMA), methyl methacrylate (MMA), and methacrylic acid (MAA). The physical properties of the biomaterial system can be tailored by controlling the molar ratio of HMA to MMA incorporated into the polymer backbone. The biological properties of the polymer system were modified through the incorporation of topography via electrospinning, covalent attachment of cell specific ligands, and the copolymerization of monomers bearing non-fouling pendant groups.

Results: Varying the ratio of HMA and MMA in the polymer backbone allows substrates of various moduli to be created and we discovered substrate stiffness has minimal impact on the behavior of adherent HUVECs. Electrospinning these materials resulted in scaffolds with drastically different topographies (Figure 1) and low porosity scaffolds (Panel A) resulted in increased HUVEC spreading, proliferation, and metabolic activity while aligned scaffolds (Panel D) resulted in cells with elongated morphology more similar to what is seen *in vivo*.

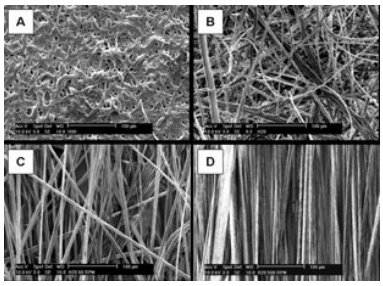

Figure 1. Morphology of electrospun polymer scaffolds: (a) slightly porous, (b) highly porous, (c) partially aligned, and (d) highly aligned.

To enhance specific biological interactions with the adult stem cell population, phage display was used to find peptide ligands which specifically bind HBOECs but not other commonly occurring circulatory cells (including mature ECs). Two candidate peptides (abbreviated "TPS" and "SYQ") were selected from the phage display work and incorporated into the polymer through chain transfer chemistry. From Figure 2 we can see that HBOECs responded favorably to the TPC containing materials as seen by increased cellular adhesion in serum free media.

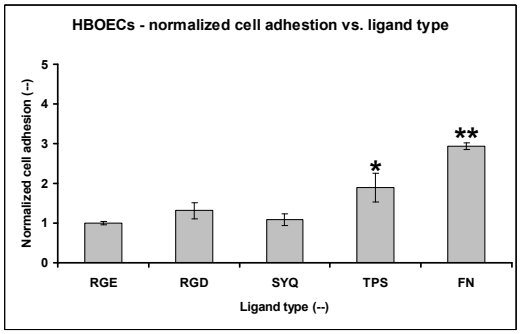

Figure 2. HBOEC adhesion to peptide modified materials.

Enhanced cellular attachment was not observed in experiments where media was supplemented with serum, most likely due to the non-specific adsorption of serum proteins onto the biomaterial surface. Therefore, the methacrylic acid co-monomer was replaced by one of two monomers which bares a non-fouling pendant group: poly(ethylene oxide) methacrylate or sulfobetaine methacrylate. Materials copolymerized from $\geq$ 15 mol % of either monomer resulted in polymer interfaces with excellent non-fouling interfacial properties as probed through fibrinogen adsorption and platelet adhesion. Fibrinogen adsorption data to PEGylated polymers are seen in Figure 3.

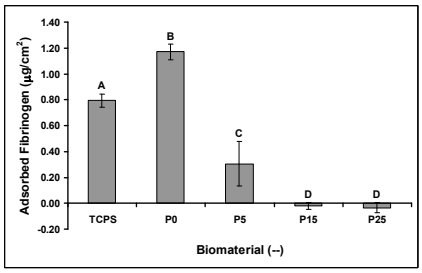

Figure 1. Fibrinogen adsorption to PEG-containing polymers.

Conclusions: Surface topography was determined to affect the proliferation, spreading, and metabolic activity of endothelial cells on biomaterial surfaces. Materials functionalized with the TPS-peptide enhanced adhesion of HBOEC adult stem cells in serum free media, and materials resistant to biofouling were produced.