A Poloxamine-Polylysine Acrylate Scaffold for Modular Tissue Engineering

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Statement of Purpose: A major obstacle in creating clinically relevant tissue constructs is the lack of an internal vascular network. We use a modular approach to generate vascularized tissues: the functional cells of interest are embedded in submillimetre sized gel cylinders (modules) and endothelial cells (EC) are seeded on the surface of the modules. When several modules are randomly packed together, the spaces between them create a network of interconnected channels lined with EC, mimicking a vascular network[1]. Collagen type I or poloxamine methacrylate have been used to fabricate the modules. Collagen modules support cell attachment, but have limited resistance to compression under flow. Poloxamine, a synthetic four arm block copolymer of polyethylene oxide and polypropylene oxide, is cell compatible, but it does not allow cell attachment[2]. The goal of this project is to develop the chemical methods required to synthesize a new poloxamine acrylate based biomaterial that is photocrosslinkable and can be used to make stiff, cell adhesive scaffolds. Polylysine was used here to confer cell attachment properties to the polymer, while the acrylate groups make it photocrosslinkable. Moreover, due to the relative ease of chemical modification of the polylysine side chain amino groups, polylysine is a good model molecule for future polymer synthesis aimed at introducing other desired functionalities (through other peptides) into the scaffold biomaterial.

Methods: A poloxamine-polylysine acrylate polymer was synthesized in three reaction steps, adapting a published methodology[3]. (1) Polylysine (average MW = 4200 Da) was acrylated using N-hydroxysuccinimide acrylic acid ester and the reaction product was characterised by ^{1}H-NMR. (2) The hydroxyl groups on poloxamine (average MW = 15 000 g/mol) were activated by tresylation with tresyl chloride and the reaction product (tresylated poloxamine) was characterised by ICP-AES for sulphur content and by thin layer chromatography (TLC) (3) The acrylated polylysine was reacted with the activated poloxamine to obtain the final product, poloxamine-polylysine acrylate (PPA). The final product was characterised by CHN elemental analysis and SDS-PAGE, followed by Coomassie staining. Aqueous solutions containing a mixture of PPA and poloxamine methacrylate were photocrosslinked by exposure to a 365nm UV light source in the presence of a photoinitiator (Irgacure 2959, Ciba, NY) to obtain hydrogels in a 96 well-plate or cylindrical modules. Calcein AM and ethidium homodimer-1 (EthD-1) staining (Live/Dead™ assay) were used to visualize live (green fluorescence) and dead cells (red fluorescence), respectively, and assess Human Microvascular Endothelial Cell (HMEC-1, a cell line) attachment and viability at different time points during cell culture.

Results: A poloxamine-polylysine acrylate polymer was synthesized using a three step chemical synthesis strategy. Typical coupling efficiencies and reaction conditions are shown in Table 1.

Table 1. Synthesis of Poloxamine-Polylysine Acrylate. Typical Reaction Conditions and Coupling Efficiencies

Reaction Step	*Typical Reaction Conditions*	*Coupling Efficiency (Analysis Method)*
1. Acrylation of Polylysine	pH 8.5, RT, 2 hours	3-4 acrylate groups / polylysine molecule (^{1}H-NMR)
2. Tresylation of Poloxamine	THF, on ice, 3 hours	90% or higher (ICP-AES for sulphur and TLC)
3. Poloxamine-Polylysine Acrylate Synthesis	DMF, TEA, RT, overnight	Up to 70% (CHN elemental analysis and SDS-PAGE)

As shown in Figure 1, HMEC-1 attach and survive for at least 7 days after seeding on photocrosslinked hydrogel scaffolds containing PPA.

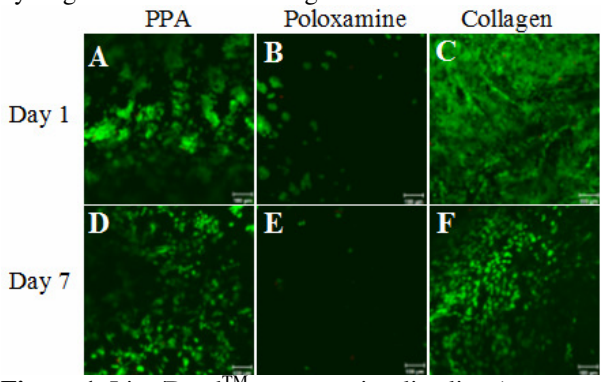

Figure 1. Live/Dead™ assay to visualize live (green fluorescence) and dead (red fluorescence) cells. HMEC-1 seeded on top of different hydrogels (200 µL gels in a 96 well-plate). 200 000 cells/gel. Poloxamine Methacrylate 5wt% mixed with PPA 6wt% (A,D). Poloxamine Methacrylate 5wt% (B,E,). Collagen 0.3wt% (C,F). Day 1 (A-C) and day 7 after seeding (D-F). Scale bar = 100 µm.

Conclusions: The synthesized PPA polymer enhances endothelial cell adhesion on poloxamine based, photocrosslinked hydrogel scaffolds. Future work will focus on introducing other desired functionalities into the poloxamine based scaffold using the chemical synthesis methods developed in this study.

References: **1.** McGuigan A, Sefton MV. Tissue Eng. 2007; 13: 1069-1078. **2.** Sosnik A, Sefton MV. Biomat. 2005; 26 : 7425–7435. **3.** Banerjee P, Griffith LG et al. J Biomed Mater Res. 2000; 50: 331-339