Decoupling PEG Hydrogel Mesh Size and Modulus with the Integration of 4-arm PEG

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Statement of Purpose: Bioactive hydrogels based on polyethylene glycol (PEG) coupled with collagen are of great interest in tissue engineering. PEG hydrogels intrinsically resist protein adsorption and cell adhesion. This provides a biological "blank slate" that can be used to carefully control cell-material interactions through the selective addition of bioactive molecules such as collagen. A range of hydrogel material properties are known to influence cell behavior in tissue engineered constructs, including modulus[1] and mesh size[2]. However, in order to increase the modulus of PEG-DA hydrogels, the mesh size is often sacrificed, which limits cell proliferation and viability. In order to determine the moduli and bioactivity levels that drive desired cell-material interactions, a hydrogel whose modulus and mesh size can be tuned in an uncoupled manner is needed. We hypothesized that the addition of an acrylated 4-arm PEG crosslinker would provide local increases in crosslink density which would increase gel modulus without significantly affecting overall permeability. In the current study, we report the effect of the 4-arm PEG crosslinker on modulus and mesh size of PEGDA-collagen hydrogels.

Methods: Linear and 4-arm poly(ethylene glycol) were acrylated using standard protocols. Briefly, the terminal hydroxyls were reacted with acryloyl chloride to form an ester linkage and acrylate end groups. The excess acryloyl chloride was neutralized, and the product was precipitated in cold diethyl ether. The structure was confirmed with NMR and FTIR spectroscopy.

Collagen Functionalization: Rat tail collagen type I (Sigma Aldrich) was functionalized with photoreactive crosslink sites to enable hydrogel formation.[3] Collagen was reacted with acrylate-PEG-N-Hydroxysuccinimide (ACRL-PEG-NHS, MW 3500, Jenkem Technology) in 50 mM sodium bicarbonate buffer (ph 8.5) at a molar ratio of 2:1 ACRL-PEG-NHS:NH_2 for 18 h. Excess ACRL-PEG-NHS and other reaction byproducts were removed via dialysis against 0.1 M HCl for 24 h and deionized water for 24 h (Slide-A-Lyzer, MWCO = 20,000, Pierce). Functionalization was confirmed with FTIR spectroscopy and SDS-Page.

Hydrogel Formation: A factorial design was used to determine the impact of PEG molecular weight (2000, 6000, and 10,000 g/mol), linear PEGDA concentration (10%, 20%, and 30%), and 4-arm PEG crosslinker concentration (0%, 10%, and 20%) on hydrogel modulus and mesh size. PEGDA hydrogel controls and PEGDA-collagen hydrogels were made with 1 mg/ml of functionalized collagen. A photoinitiator (Irgacure 2959, Sigma Aldrich) was added at a concentration of 10 mg/ml, and the solution was crosslinked into rings using a double walled cylindrical mold (ID = 3 mm, OD = 5 mm) via 10 min exposure to 365 nm UV light (Transilluminator, 9 mW/cm^2).

Hydrogel Characterization: After 24 hours of swelling in PBS, hydrogel rings were cut into slices (2-5 mm), and tensile testing to fracture was conducted at a uniaxial strain rate of 6 mm/min (Instron 3342). To determine the effect of hydrogel variables on mesh size, photoinitiated hydrogel plates (0.75 mm thick) were swollen in PBS for 24 hours. 8 mm diameter discs were soaked in 500µL of 50µg/ml dextran solutions for 24 hours. They were then transferred into a clean well plate with PBS for 24 hours. Two 150 µl aliquots were taken from each well and put into a 96 well plate. Fluorescence readings were taken at 480 nm of excitation and 520 nm of emission to determine the relationship between dextran hydrodynamic radius and diffusion into the hydrogel. This data was then used to provide a relative measure of hydrogel mesh size.

Results: *Synthesis:* An ester peak at 1704 cm^{-1} and loss of the hydroxyl peak at 3300 cm^{-1} in the FTIR spectra of PEGDA was indicative of successful acrylation. Proton NMR confirmed greater than 80% acrylation of all synthesized PEG diacrylates. FTIR spectroscopy also confirmed collagen functionalization as evidenced by an increase in the ether group peak at 1109 cm^{-1} with increased molar ratios of ACRL-PEG-NHS:NH2. SDS-Page displayed smearing of collagen bands in acrylated collagen due to decreased electrophoretic motility which further confirmed successful functionalization.

Hydrogel Characterization: A statistically significant increase in modulus was observed with increased concentration of 4-arm PEG crosslinker, **Figure 1**. Preliminary mesh size data also indicated a minimal mesh size decrease with the addition of the 4-arm PEG.

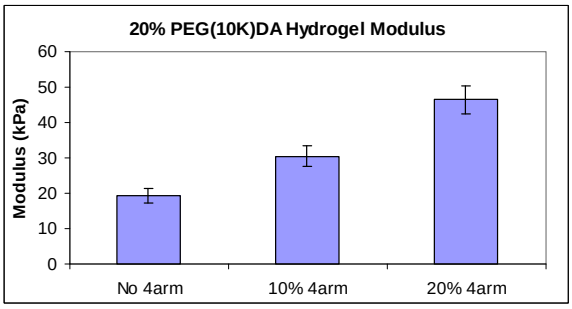

Figure 1. Effect of [4-Arm PEG Acrylate] on Hydrogel Modulus

Conclusions: We have successfully demonstrated that the modulus of PEG hydrogels can be significantly increased with the addition of a 4-arm PEG crosslinker. The ability to adjust modulus while maintaining mesh size and permeability has significant impact in scaffold design.

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