Tuning Poly(Ethylene Glycol) Hydrogel Mechanics Independent of Density via Peptide Sequence

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Statement of Purpose: Photocrosslinked poly(ethylene glycol) (PEG) hydrogels have been extensively studied as biomaterials for tissue engineering due to their biocompatibility, tunable mechanical properties, and ease of modification. Yet this mechanical tunability relies on the ability to alter the total PEG content within the hydrogel, hindering the ability to create compliant materials below a critical PEG concentration to mimic soft tissue mechanics for many tissue engineering applications and vasculogenesis. Furthermore, altering the pre-polymer PEG content can affect the overall biochemical properties of the hydrogel environment. Here, we describe the synthesis and characterization of a photocrosslinkable matrixmetalloproteinase (MMP)-sensitive PEG-peptide macromer with internal vinyl groups introduced through the peptide sequence. We hypothesize that these vinyl groups can compete with the terminal acrylate groups during polymerization, creating defects that can decrease the overall stiffness of the hydrogel independent of the polymer matrix density. This capability should permit PEG hydrogels to be synthesized with highly compliant and tunable mechanical properties.

Methods: The MMP-sensitive peptides sequences GGGGGPQGIWGQGGGGK (PQ) and GGGGGPQGI WGQGG-Lys(alloc)-GK (PQ(alloc)) were synthesized using standard Fmoc chemistry. Heterobifunctional acrylate-PEG-succinimidyl valerate was reacted with the the peptide's amine-terminus and the carboxy-terminal lysine's amine side chain to create the PEG-PQ-PEG or PEG-PQ(alloc)-PEG macromers with terminal acrylate groups. Hydrogels were polymerized at 5% (wt/vol) PEG in the presence of the photoinitiator, eosin Y, and a 35s exposure to visible light. Compressive and rheological testing were performed using TA Instruments RSA III micro-strain analyzer and AR-G2, respectively. For 2D experiments, $2x10^5$ human umbilical vein endothelial cells (HUVECs)/mL were seeded onto hydrogels polymerized with 0.5 or 3.5 mM PEG-RGD to support cell attachment and fixed after 12 hours of culture. For 3D encapsulation experiments, the pre-polymer solution contained $3x10^7$ cells/mL (a 4:1 ratio, HUVEC:pericyte precursor) and 3.5 mM PEG-RGD. After polymerization, hydrogel co-cultures were fixed after 24 or 72 hrs. All cells experiments were labeled with DAPI and phalloidin (3D experiments were further stained for CD31 and α-smooth muscle actin (αSMA) expression) prior to visualization with confocal microscopy.

Results: The compressive moduli of the photopolymerized hydrogels were 15.21± 7.65 kPa (mean ± SD) for PEG-PQ-PEG and 1.74±0.43 kPa for PEG-PQ(alloc)-PEG. Similarly, rheological measurements of the storage and loss moduli (5.30±0.65 kPa and 6.1±5.0 Pa for PEG-PQ-PEG and 1.64±0.22 kPa and 5.8±0.6 Pa for PEG-PQ(alloc)-PEG) indicated that this reduction of the compressive modulus was purely elastic (Figure 1). In 2D cell studies, the more compliant PEG-PQ(alloc)-PEG hydrogels induced cellular aggregation into network-like assemblies with poorly defined cytoskeletal structures at high RGD concentrations and did not support attachment at low RGD concentrations. The stiffer PEG-PQ-PEG gels supported cell attachment and spreading in both conditions, but yielded more aggregation under low RGD concentrations (Figure 2A). In 3D studies of tubulogenic behavior, extensive tubule networks were observed after just 24 hrs with PQ(alloc)-containing gels, while minimal connections were observed after 72 hrs in PEG-PQ-PEG hydrogels (Figure 2B).

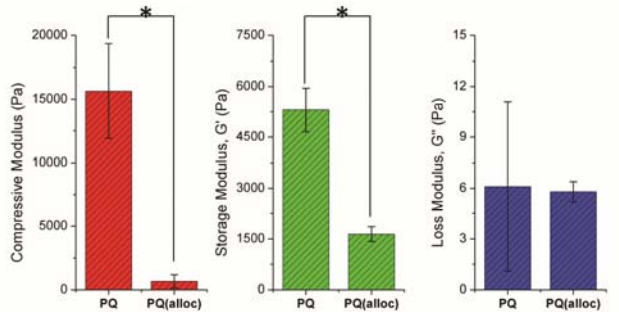

Figure 1. Measured compressive (red), storage (green), and loss (blue) moduli. *indicate significant differences as determined by a Tukey's HSD test ($p < 0.001$).

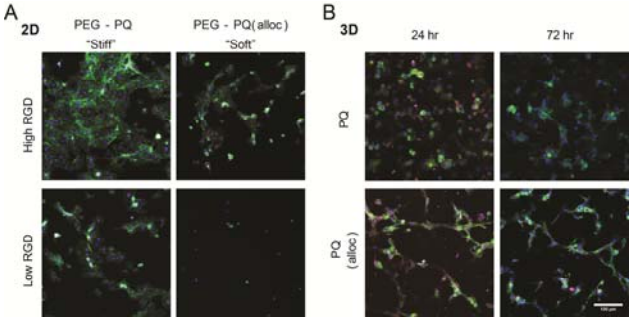

Figure 2. Cellular responses to PQ and PQ(alloc) containing hydrogels. A. 2D response of ECs seeded on top of high and low RGD hydrogels where spreading was favored on stiffer high RGD surfaces and aggregation was favored on softer high RGD or stiffer low RGD surfaces. B. In 3D, the softer gels permitted the formation of tubule networks in 24 hrs whereas stiffer gels required >72 hrs to form networks. (Blue – DAPI, Green – Phalloidin, Red - αSMA, Purple – CD31; scale bar: 100 μm)

Conclusions: We have described a new method to tune the mechanical properties of PEG hydrogels independent of the overall matrix density, viscoelastic properties, or protein/nutrient diffusivity by incorporating a competitive alloc crosslinking site in PEG-peptide-PEG hydrogels. Furthermore, the resulting hydrogels yielded cellular behaviors which indicate cells experience significantly different material compliances as assayed via 2D and 3D studies. This capability not only permits the creation of highly compliant PEG hydrogel environments, but also a general platform to decouple mechanical and biochemical responses within these materials.