Regulating Endothelial Cell Dynamics through Peptide-Encoded Material Compliance

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Statement of Purpose: Endothelial cells (ECs) respond to a variety of environmental stimuli to undergo complex morphological and biological changes during angiogenesis and vasculogenesis. The ability to regulate these behaviors offers great promise in the development of engineering tissues and therapeutic materials. Photopolymerizable poly(ethylene glycol) (PEG) hydrogels have become an important tool to study EC behaviors due to their "blank slate" nature, allowing the custom tailoring of hydrogel mechanics and biochemistry. However, changes in hydrogel mechanics often require changes to the polymer density, affecting other physical properties of the hydrogel such as the crosslink density, degradation kinetics, and diffusion of biomolecules in the hydrogel. Here, we present a method of controlling the PEG hydrogel mechanical properties through peptide sequence. By building upon past biomimetic materials approaches, the mechanical properties of PEG-based hydrogels can be tuned independently of polymer concentration, degradation, and diffusion. ECs can then sense and respond to these changes, exhibiting compliance-dependent morphological behaviors.

Methods: Matrix metalloproteinase-sensitive peptides GGGGGPQGIWGQGGGGK, PQ, and allyloxycarbonyl (alloc) protected lysine containing analogue GGGGGPQ GIWGQGG-Lys(alloc)-GK, PQ(alloc), were synthesized and verified via MALDI-MS. Acrylate-PEG-succinimidyl valerate was coupled to the peptide's N- and C-termini, creating diacrylate PEG-peptide-PEG macromers, PEG-PQ or PEG-PQ(alloc). For encapsulations, the pre-polymer solutions (5% w/v) were photo-polymerized with 3×10^4 cells/μL (4:1 EC:pericyte) and 3.5 mM PEG-RGDS to support cell adhesion. Gels were fixed after 1, 3, or 6 day, stained for CD31 and α-smooth muscle actin (αSMA), and visualized with confocal microscopy. Networks were quantified by tubule length, branch points, and network to total cell volume ratio. Significance was assessed using ANOVA ($p < 0.05$).

Results: At 5% (w/v), Compression testing revealed moduli of 15.21 ± 7.65 kPa for PEG-PQ and 1.74 ± 0.43 kPa for PEG-PQ(alloc) with a similar ~10 fold reduction in modulus observed at 7.5% and 10% (Figure 1A). Mixing of the two PEG macromers indicated changes in compressive moduli were alloc concentration dependent (Figure 1C), suggesting the alloc acts as a competitive crosslinking site. However, the modification does not alter the MMP-dependent degradation or diffusive behaviors of the hydrogel. To investigate network formation, ECs and pericytes were co-cultured in PEG-PQ or PEG-PQ(alloc) hydrogels. Networks were observed within 24 hours in PEG-PQ(alloc), while networks only begin after 3 days in PEG-PQ hydrogels (Figure 1D). Here, the compliance of PEG-PQ(alloc) gels led to enhanced network formation kinetics as assessed by tubule length, branching, and network volume to cell volume ratios (Figure 1E-G), despite identical polymer densities and RGDS content. These EC network structures become lumenized after 3 days of culture with the compliant PEG-PQ(alloc) hydrogels exhibiting significantly more and larger lumens at the 3 day time point. Furthermore, the ability to encode mechanical properties without altering the overall polymer density allows for long-term material persistence and, therefore, long-term culture. We have shown stable EC networks through at least 4 weeks of culture and the depositions of Collagen IV and Laminin, perivascularly.

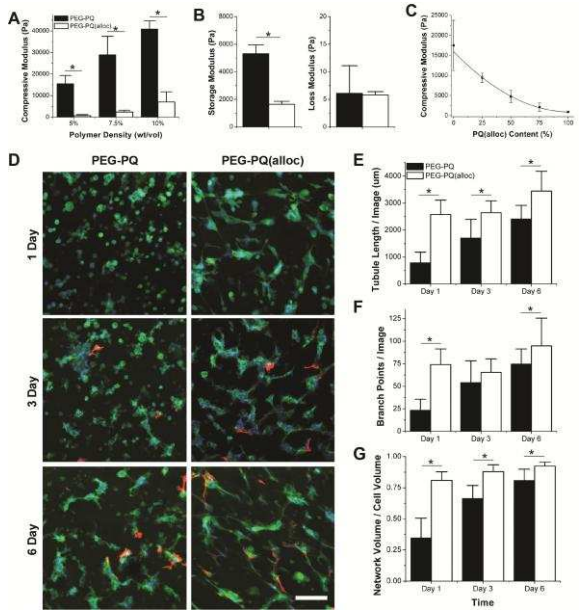

Figure 1. (A) Mechanical testing of PEG-PQ and PEG-PQ(alloc) hydrogels indicates significant changes in mechanical properties, supported by Rheology (B). (C) Ratiometric mixing of PEG-PQ and PEG-PQ(alloc) allows precise control of hydrogel mechanics. (D) Immunocytochemistry of EC:pericyte co-cultures after 1, 3, or 6 days (Red: αSMA, Green: CD31, Blue: DAPI; Scale bar = 100 μm). Enhanced network formation in PEG-PQ(alloc) hydrogel co-cultures as seen by tubule length (E), branch points (F), and network/cell vol. fractions (G), (*) indicates statistical significance.

Conclusions: This new method to precisely tune the mechanical properties of photopolymerized PEG hydrogels through peptide sequence decouples changes in mechanical properties from the degradative and diffusive properties. This independent control of mechanical properties was able to regulate the spreading and network formation kinetics of ECs, suggesting mechanical properties play equally important roles as biochemical cues in influencing many 3D cell fate decisions. The unique mechanical capabilities of PEG-PQ(alloc) hydrogels should lend their use to other applications where sufficiently compliant mechanical properties have been difficult to achieve in synthetic systems. Similarly, these hydrogels should enhance our ability to investigate the role of material compliance in vivo.