Design and Characterization of Porous MMP-sensitive Synthetic Hydrogels by Gelatin Leaching for Neovascularization Applications

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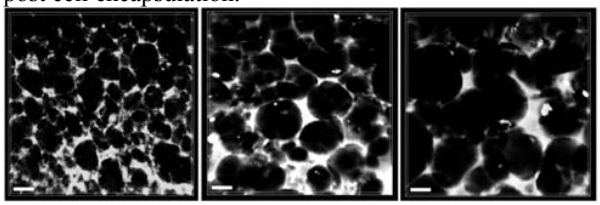
Statement of Purpose: The clinical success of hydrogel biomaterials is highly dependent on their ability to stimulate rapid and stable neovascularization. Poly (ethylene glycol) (PEG) hydrogels have been widely investigated for a number of tissue engineering applications because they can be tailored to match the mechanical properties of soft tissue and can be modified with peptides and proteins to mimic the natural extracellular matrix. The incorporation of matrix metalloproteinase (MMP) - sensitive peptide sequences render these hydrogels degradable and allow localized cell invasion by cell secreted proteases. While synthetic PEG hydrogels have been widely investigated on their ability to promote cell-mediated scaffold degradation and migration, the effect of pore size on vascularization has not been previously explored within these biomaterials. In this study, a gelatin particle leaching method was applied to generate porous MMP-sensitive PEG diacrylate (PEGDA) hydrogels. The resulting hydrogels were characterized for pore size, porosity, mechanical and degradative properties and neovascularization *in vitro*.

Methods: Gelatin beads were prepared using a gelatin-oil emulsion and mechanically sieved into templating sizes of 50-100 µm, 100-150 µm, and 150-200 µm. Gelatin bead diameters were characterized in the dry and swollen state. MMP-sensitive PEGDA hydrogels were generated by the covalent incorporation of an MMP-sensitive peptide GGVPMS↓MRGG (948.14 g/mol) (cleavage site in bold between the serine and methionine residues indicated by ↓) into the backbone of PEG using SVA chemistry. Hydrogels were prepared from precursor solutions of 3% (weight/vol (w/v)) MMP-sensitive PEGDA (MMP+) or MMP-insensitive PEGDA (MMP-) as a control, 225 mM triethanolamine, 37 mM N-vinyl pyrollidone, and 0.05 mM eosin Y as the photoinitiator and added to 15% (w/v) gelatin beads of pre-sieved templating sizes, photopolymerized using an Argon ion laser ($\lambda = 514$ nm) at a laser flux of 100 mW/cm^2 for 0.25 min. Gelatin was leached out of the hydrogels by incubating at 37 $^{\circ}$C in ddH_2O for 24 hrs. The resulting hydrogels were imaged with confocal microscopy, characterized for pore size and porosity, compressive modulus and degradation rate. Neovascularization within these hydrogels was evaluated using a co-culture model of human umbilical vein endothelial cells (HUVECs) and human umbilical artery smooth muscle cells (HUASMCs).

Results: MMP-sensitive porous PEGDA hydrogels were generated using templating bead sizes of 50-100 µm, 100-150 µm, and 150-200 µm, as described above. The inherent autofluorescence of residual eosin Y in the hydrogels allowed the visualization of the hydrogel pore structure (Fig. 1). The average hydrogel pore size and porosity was uniform between MMP-sensitive and MMP-insensitive hydrogels and was consistent with the swollen templating bead size. There was a statistically significant difference between the pore size groups and porosity increased with increasing pore size (Fig. 1). There were no statistical differences in hydrogel degradation times and compressive moduli among pore size groups (Fig. 2). *In vitro* data demonstrate that 24 hours post encapsulation, cells align along the pores of the hydrogel (Fig. 3).

Conclusions: A gelatin particle leaching method was applied to generate porous MMP-sensitive PEGDA hydrogels with controlled pore size, porosity, and good pore interconnectivity. The presented method allows for hydrogel photopolymerization in the presence of cells and shows preferential cell alignment along hydrogel pores post cell encapsulation.



Gelatin Bead Diameter (µm)			Avg. Hydrogel Pore Size (µm)		Avg. Hydrogel Porosity (%)	
Sieved	Dried	Swollen	MMP-insensitive	MMP-sensitive	MMP-insensitive	MMP-sensitive
50-100	86.39 ± 18.18	133.13 ± 23.28	134.49 ± 2.03	122.15 ± 2.34	54.38 ± 6.86	59.17 ± 6.73
100-150	115.01 ± 17.82	196.07 ± 26.46	187.06 ± 1.04	172.40 ± 19.05	65.97 ± 4.55	69.22 ± 3.50
150-200	135.40 ± 32.26	255.5 ± 33.09	247.98 ± 5.65	240.58 ± 4.61	69.50 ± 3.98	75.55 ± 4.60

Figure 1. Top) 3D Rendering of porous PEGDA hydrogels generated with templating beads of 50-100 µm, 100-150 µm, and 150-200 µm, respectively. Scale bar = 100 µm. Bottom) Characterization of sieved gelatin bead diameters and corresponding hydrogel pore size and porosity. Data are presented as mean ± standard deviation.

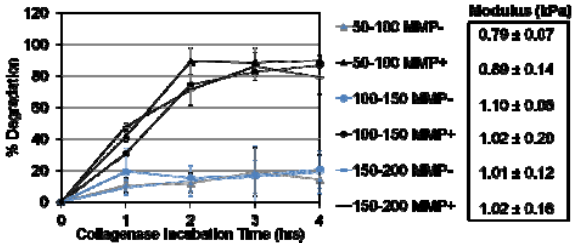


Figure 2. Degradation profiles (left) and compressive moduli (right) of porous PEGDA hydrogels with templating beads of 50-100 µm, 100-150 µm, and 150-200 µm. Compressive moduli are presented as mean ± standard deviation (n=4).

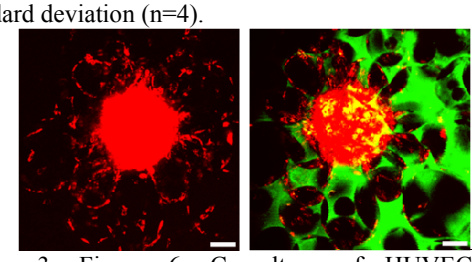


Figure 3. Figure 6. Co-culture of HUVECs and HUASMCs within porous MMP-sensitive PEGDA hydrogels. Scale bar = 100µm.