Spatial and Temporal Tuning of Synthetic Hydrogel Microenvironments for Promoting Neovascularization

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Statement of Purpose: The clinical success of hydrogel scaffolds is highly dependent on their ability to promote rapid, guided, and stable neovascularization within the biomaterial. While synthetic matrix metalloproteinase (MMP)-sensitive hydrogels of poly(ethylene glycol) (PEG) have been previously explored as matrices for the stimulation of neovascularization, the spatial and/or temporal presentation of immobilized biofunctional signals and biomaterial properties on neovascularization and depth of tissue invasion has not been previously explored. To this end we have developed novel biomaterial approaches for engineering (1) isotropic PEG scaffolds with enhanced and controlled protease-mediated scaffold degradation independent of alterations in mechanical and physical properties of the biomaterial leading to more rapid neovascularization and (2) PEG scaffolds with tunable gradients of immobilized cell adhesion ligands of YRGDS, MMP-sensitive domains, and elastic modulus that promote directed and guided 3D *in vitro* sprout invasion and neovascularization in the direction of the gradient. The ability of these scaffolds to promote neovascularization and tissue invasion *in vivo* was assessed using a subcutaneous rat implant model.

Methods: Scaffolds with isotropic and anisotropic properties were formed using free-radical photopolymerization in the presence of visible light (λ = 514 nm). The precursor consisted of 3-7.5 % wt/vol MMP-sensitive PEG diacrylate (PEGDA) (MW 5.2, 17, 18 kDa), 37mM NVP-vinyl pyrrolidone, 225mM triethanolamine, and 10-15 mg/mL Acryl-PEG-YRGDS (MW=3400 Da). Eosin Y (0.5-1 mM) was used as the photoinitiator. Isotropic scaffolds with tunable degradation rates were engineered using MMP-sensitive peptides that contained either one (SSite) or multiple (TriSite) protease-sensitive cleavage site repeats that were reacted with Acryl-PEG-SVA to form degradable SSite or TriSite PEGDA macromers that were subsequently crosslinked into scaffolds. Scaffolds containing gradients of immobilized ECM signals, mechanical properties, and MMP-sensitive degradation were formed by perfusion-based frontal photopolymerization (PBFP) through the perfusion and scheduled delivery of buoyant Eosin Y through the precursor solution using a glass frit filter disk resulting in the formation of a propagating polymer reaction front leading to gradients in crosslinking. Scaffolds formed by PBFP were sectioned in 2mm segments perpendicular to the gradient to quantify spatial variations in biomaterial properties and incorporated biofunctionality. Hydrogels were characterized in terms of elastic modulus, degradation rate by measuring the change in the wet weight of the hydrogels incubated with collagenase enzyme over time, and via radiolabeling to quantify gradients of immobilized YRGDS. *In vitro* neovascularization within hydrogels was quantified over three weeks using a co-culture model of human umbilical vein endothelial cells and human umbilical artery smooth muscle cells. Histological analysis and isolectin perfusion of subcutaneous implants harvested at 2-4 weeks were used to quantify tissue invasion and vascularization.

Results: Isotropic scaffolds formed with SSite or TriSite peptides resulted in independent tuning of hydrogel elastic modulus, but in statistically different hydrogel degradation times. TriSite gels supported vessel invasion over a wider range of compressive modulus (0.3-3.6 kPa) as compared to SSite gels with no invasion occurring above 0.4 kPa. *In vitro* data show that TriSite gels enhance vessel invasion as compared to SSite (Fig.1). Preliminary *in vivo* data indicate that TriSite hydrogels result in enhanced in vivo neovascularization as compared to SSite hydrogels of similar modulus. (Fig.1). Gradient hydrogels exhibited an 80.4% decrease in elastic modulus and a 56.2% decrease in immobilized YRGDS and MMP-mediated degradation. Degradation times ranged from 10-12 hours in more crosslinked regions to 4-6 hours in less crosslinked regions. *In vitro* sprout invasion within gradient hydrogels occurred bi-directionally with sprout alignment observed in the direction parallel to the gradient after three weeks in culture (Fig.2). Gradient hydrogels were sectioned in half in the direction perpendicular to the gradient and implants were oriented with the gradient either away or against the muscle wall. Preliminary *in vivo* data indicate that depth of tissue invasion is dependent on the properties presented at the tissue interface as well as the gradient orientation.

Conclusions: Spatial and temporal tuning of scaffold properties plays a critical role in controlling and directing neovascularization *in vitro* and *in vivo*. Future studies will investigate the incorporation of MMP-sensitive-peptide substrates with increased MMP specificity as well as isolating the effects of scaffold gradients and gradient combinations on neovascularization.

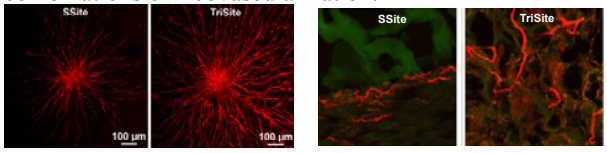

Figure 1. Neovascularization within SSite and TriSite PEG Hydrogels *in vitro* after 21 days in culture (left), and *in vivo* after 4 weeks (right).

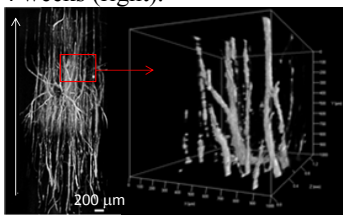

Figure 2. Flattened 3D mosaic renderings of *in vitro* vascular sprout formation in gradient gels. Right is a 3D image reconstruction of a region of the gel shown on the left (white arrow indicates the direction of the gradients).