Hydrogels Designed to Provide Sustained, Stimuli-Responsive Release of Pro-Angiogenic Peptides

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Statement of Purpose: The inability to sufficiently encourage vascularization of tissue engineered constructs drastically limits their size, complexity, and ability to be scaled up to fully-realized clinical treatments. Tissue engineering, as well as a plethora of ischemic tissue disorders, may therefore benefit from controlled pro-angiogenic drug delivery. Thus, peptide sequences that mimic the bioactivity of growth factors such as vascular endothelial growth factor (VEGF) were synthesized and incorporated into poly(ethylene glycol) (PEG) hydrogels to provide sustainable peptide delivery in response to enzymatic activity (Fig. 1). These small peptides have greater stability over large proteins *in vivo*, and allow controlled release of higher doses due to their small size.

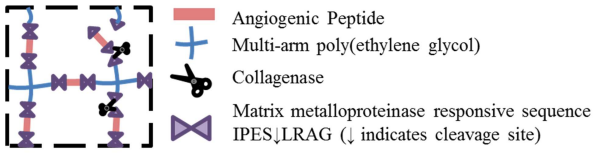

Fig. 1: Schematic depection of stimuli-responsive peptide delivery system. Peptide sequence IPESLRAG is susceptible to cleavage by Matrix metalloproteinases (MMPs) 1, 2, 3, 7, 9 & 14 [1].

Methods: Peptides were synthesized via solid phase techniques (Liberty1, CEM, Matthews NC). After cleavage in trifluoroacetic acid-based cocktails, correct synthesis was confirmed using matrix-assisted laser desorption ionization time of flight mass spectrometry. Pro-angiogenic efficacy was assessed using the human umbilical vein endothelial cell (HUVEC) proliferation and tube formation assays. HUVEC proliferation was tracked after 3 days of treatment in basal media with or without peptide or control agent (positive control VEGF, negative controls scrambled peptide and the angiogenic inhibitor sulphoraphane). To assess tube formation, HUVECs were seeded on reduced growth-factor matrigel and treated for 8 hours before imaging. Norbornene-functionalized PEG was synthesized as previously described [2], and crosslinked into hydrogel networks via the enzymatically-degradable linker IPESLRAG via thiol-ene reactions between terminal cysteine amino acids and norbornene functionalized PEG (Fig. 1). Proof-of-principle enzymatically responsive hydrogel degradation upon incubation with collagenase was then demonstrated.

Results: All peptides investigated except for T7 (angiopoietin mimic) resulted in significant increases in HUVEC proliferation (Fig. 2A). This increase in proliferation was similar to that resulting from treatment with VEGF, and is sequence-specific as the scrambled peptide did not affect proliferation. Treatment with VEGF encouraged development of smooth, fully connected tube networks while control media results in minimal, disconnected tubes. All peptides investigated except for Ten2 (tenascin X mimic) and the scrambled peptide significantly increased HUVEC tube length over basal media (Figure 2B, quantification not shown), with T7, Qk (VEGF mimic), and $SPARC_{118}$ (Secreted Protein Acidic and Rich in Cysteine mimic) resulting in the largest increases to 2.1, 2.1 and 2.0 fold of control media, respectively.

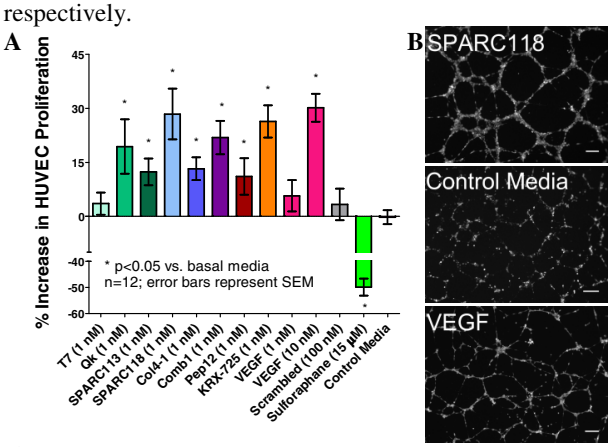

Fig. 2: Angiogenic peptide screening. A) Increase in proliferation over basal media upon treatment with select peptides. B) Representative fluorescent images (calcein AM) of HUVEC tube formation. Scale bar = 200 μm.

Collagenase-responsive hydrogel degradation was then achieved through use of the enzymatically-responsive peptide sequence IPESLRAG (Fig. 3).

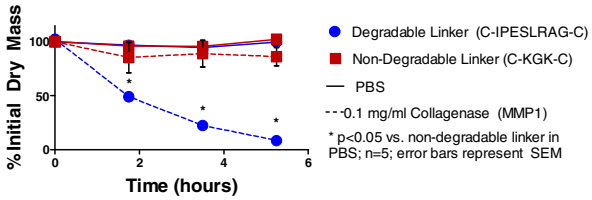

Fig. 3: Enzymatically-responsive hydrogel degradation. Super-physiological concentrations were used in this proof-of-principle study.

Conclusions and Future Directions: Ten peptide sequences identified from literature which mimic the function of pro-angiogenic proteins were identified, synthesized using solid phase chemical techniques, and assessed for pro-angiogenic potential. Qk and $SPARC_{118}$ resulted in the greatest increases in both proliferation and tube formation, but all peptides except T7 and Ten2 significantly increased both measures. Enzymatically-responsive hydrogel degradation was achieved through inclusion of cleavable peptide substrates, demonstrating release mechanism feasibility. In addition, the promising angiogenic peptide $SPARC_{118}$ has been incorporated into and released from PEG hydrogel networks via the degradable linker IPESLRAG. Our current research is focusing on quantification of temporal peptide release and demonstration of the released peptides' biological efficacy *in vitro*. Additional future work will study the pro-angiogenic capabilities of these hydrogels *in vivo* in subcutaneous implants and hindlimb ischemia.

References:

1. Hubbell, J.A. *Biomaterials*. **31**(30) 2010.
2. Fairbanks, B.D. *Adv Mater*. **21**(48) 2009.