Enzymatically-Responsive Delivery of Pro-Angiogenic Peptides for Therapeutic Vascularization

Amy H. Van Hove[1-2] and Danielle S.W. Benoit, PhD[1-4]

[1]Department of Biomedical Engineering; [2]Center for Musculoskeletal Research; [3]Department of Chemical Engineering; [4]Department of Biomedical Genetics; University of Rochester, Rochester, NY USA

Statement of Purpose: The inability to sufficiently vascularize tissue engineered constructs drastically limits their size, complexity, and potential for clinical translation. Therefore, tissue engineering approaches could benefit from pro-angiogenic approaches. Peptides that mimic the bioactivity of large proteins allow for delivery of higher concentrations of angiogenic factors and overcome growth factor stability issues. However, tight temporal control over growth factor availability is critical for the formation of mature, stable vessels [1]. Thus, poly(ethylene glycol) (PEG) hydrogels were designed to provide sustained, stimuli-responsive release of pro-angiogenic peptides in response to matrix metalloproteinases (MMPs) that are expressed at increased levels by cells commonly used in tissue engineering and ischemic tissues (Fig. 1A) [2]. Exploiting this delivery system, we have designed gels that deliver the pro-angiogenic peptides $SPARC_{113}$ and $SPARC_{118}$ (mimics of SPARC, the secreted protein acidic and rich in cysteine [3]), and translated this technology *in vivo*, characterizing hydrogel degradation and efficacy upon subcutaneous implantation, as detailed herein.

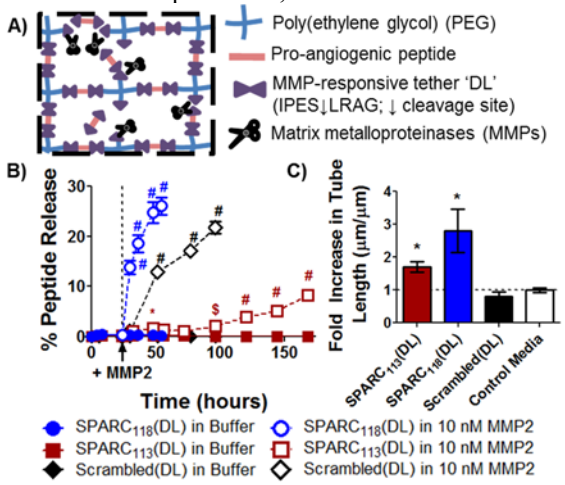

Fig. 1: A) Schematic of stimuli-responsive peptide delivery system. B) Enzymatically-responsive peptide release (n=6). C) Degraded hydrogels induce HUVEC tube formation *in vitro* (1/7,000th gel/well, 100-240 nM free peptide drug; n=9). * $p<0.05$, \$ $p<0.001$, and # $p<0.0001$ (B - vs. buffer at same time point, C- vs. control media), error bars = SEM.

Methods: Norbornene-functionalized PEG and MMP-releasable pro-angiogenic peptides flanked by thiol-containing cysteine amino acids (degradable linkages, 'DL') were synthesized and used to form hydrogels as previously described [2]. Enzymatically-responsive peptide release (high performance liquid chromatography; HPLC) upon incubation with MMP-2 was tracked until complete gel degradation. *In vitro* efficacy of degraded hydrogels was assessed using the human umbilical vein endothelial cell (HUVEC) tube formation assay. To facilitate *in vivo* tracking, hydrogels were labeled using a cysteine-functionalized fluorophore (Texas Red; $\lambda_{580/620}$ nm), implanted subcutaneously in mice, and degradation monitored using a Xenogen live animal imaging system (IVIS). One week after implantation, hydrogels were collected and vascular ingrowth assessed via hemoglobin (Hb) quantification [4]. All animal procedures were approved by the University of Rochester's University Committee of Animal Resources.

Results: Enzymatically-responsive release of the pro-angiogenic peptides $SPARC_{113}$ and $SPARC_{118}$, and a scrambled control peptide was achieved by flanking peptides with 'DL' substrates. Hydrogels were stable in buffer, and degradation and peptide release only occurred upon treatment with MMP-2 (Fig 1B). Interestingly, peptide drug affected the time to hydrogel degradation and amount of drug fully released from the network (144 hr and 8.3% for $SPARC_{113}$(DL), 30 hr and 26% for $SPARC_{118}$(DL)). Both $SPARC_{113}$(DL) and $SPARC_{118}$(DL) gels released bioactive peptide at levels sufficient to induced HUVEC tube formation *in vitro* (Fig. 1C).

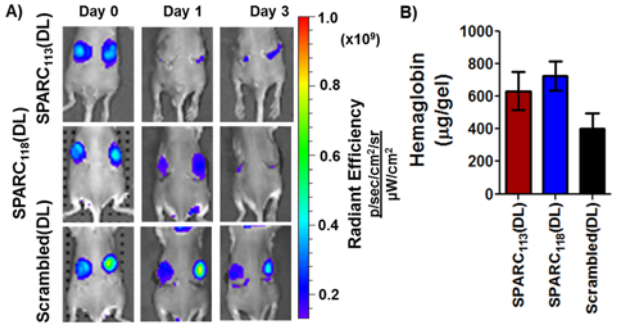

Fig. 2: Enzymatically-responsive hydrogels A) degrade and B) induce vascularization *in vivo* (n= 4 mice, 8 gels). \$ $p<0.001$ vs. Scrambled(DL), error bars = SEM.

Hydrogels formed with $SPARC_{113}$(DL) and $SPARC_{118}$(DL) degraded significantly faster than Scrambled(DL) gels (Fig. 2A; quantification not shown), and caused trending increases in vascularization (Fig. 2B; 630, 720, and 400 μg Hg/gel, respectively). Additional replicates and control groups (PBS, soluble VEGF) are underway to detect relevant differences given the assay variability and subsequent power calculations.

Conclusions and Future Directions: Pro-angiogenic peptides $SPARC_{113}$ and $SPARC_{118}$ were incorporated into PEG hydrogels via the degradable linker IPES↓LRAG, and enzymatically-responsive hydrogel degradation, peptide release, and therapeutic potential of the hydrogel system demonstrated *in vitro*. Preliminary *in vivo* studies demonstrate promising increases in vascular ingrowth into pro-angiogenic peptide releasing hydrogels. Our current research is focused on confirming our *in vivo* results, and increasing the efficacy of the hydrogels by developing methods to increase both drug concentration and delivery duration by increasing hydrogel crosslinking density.

References: **[1]** Silva, E.A. *et al. Biomaterials*. 31(6) 2010. **[2]** Van Hove, A.H. *et al. Biomaterials*. 35(36) 2014. **[3]** Sage, E.H. *et al. J of Bio Chem*. 278(39) 2003. **[4]** Mendes, J.B. *et al. J Biomed Mater Res B*. 83B(2) 2007.