PEG Hydrogel Nanoparticles for Controlled Release of Angiogenic Peptides

Daniel A Young, Georgia Papavasiliou.
Biomedical Engineering Department, Illinois Institute of Technology, Chicago, IL, USA.

Statement of Purpose: The ability to promote rapid and stable neovascularization of implantable tissue engineered scaffolds remains a significant challenge. While previous studies have shown that the inclusion of growth factors in scaffolds is necessary for promoting scaffold neovascularization *in vivo*, their clinical use is limited by their susceptibility to enzymatic degradation and short *in vivo* half-life. A promising alternative is the utilization of peptide sequences designed to interact with the specific receptors that growth factors use to regulate neovascularization pathways. A short, alpha-helical peptide sequence, QK, has been developed which elicits the same pro-angiogenic cascades as vascular endothelial growth factor, VEGF, a key regulator of neovascularization (D'Andrea, LD. *PNAS*, *102*(40), 14215–20). QK has demonstrated increased stability in serum as compared to VEGF. Previous studies have demonstrated that sustained as well as soluble delivery of QK in scaffolds results in enhancements in endothelial cell invasion in scaffolds (Mulyasasmita, W. *TE:A*, *20*(15-16), 2102–14); however, the role of adjustments in sustained QK release profiles on scaffold neovascularization have not been explored. To address this issue we propose the inclusion of synthetic PEG hydrogel nanoparticle (NP) carriers within scaffolds that allow for tunable, sustained release of QK through mechanisms of diffusion and NP degradation.

Methods: Solid phase peptide synthesis with standard Fmoc chemistry was used to produce the QK peptide, followed by purification with reverse phase HPLC. QK-loaded PEGDA hydrogel NPs were created using inverse phase miniemulsion polymerization. The precursor solution was prepared with or without QK and included either a non-degradable poly(ethylene glycol) diacrylate (PEGDA) or a hydrolytically degradable poly(L-lactic acid) (PLLA) crosslinker (PEG-PLLA-DA) of similar molecular weight and N-vinyl pyrrolidone, potassium persulfate (KPS) as the thermal initiator, potassium phosphate salt, and Tween-20, a water-soluble surfactant. The organic phase consisted of cyclohexane and Span-80, an organic-soluble surfactant. The solutions were homogenized then ultrasonicated to produce a stable emulsion. The emulsion was transferred to a reaction vessel and bubbled with nitrogen gas for 1 hr at room temperature. To initiate polymerization, the reaction vessel was heated to 56 ºC and maintained for 4.5 hrs with constant stirring. NPs were washed 3 times in acetone and 3 times with water then stored lyophilized. Particle size distribution was quantified using nanoparticle tracking analysis on a Nanosight LM10 system. Zeta potential, an indicator of NP stability and surface charge, was measured in a Malvern Zetasizer Nano. Swelling ratio (Qw) of hydrogel NPs was quantified from the ratio of the equilibrium swollen and dry NP weights. Peptide release kinetics from NPs were quantified by placing QK-loaded NPs inside a dialysis cartridge (50 kDa MWCO), submerged in 15 mL of DIW in a sealed tube and gently shaken at 37 °C. At specified timepoints, the cartridge was removed and transferred to a new tube with 15 mL of fresh DIW. The dialysate was lyophilized and then resuspended at a higher concentration for QK analysis by HPLC.

Results: Two different precursor formulations were used to create NPs. These included hydrolytically degradable NPs synthesized using a PEG-PLLA-DA (MW = 3,400 Da) crosslinker as well as non-degradable NPs using PEGDA of molecular weight 3,400 Da. This allowed for comparable NP crosslink density, based on swelling ratio (Qw) measurements, and particle size, as indicated in Table 1. Zeta potential for the PEGDA NPs was found to be -29.6 ± 5.84 mV in DIW.

Macromer	Diameter	Qw
PEG-PLLA-DA (MW = 3,400 Da)	189.3 ± 111.6 nm	14.1 ± 1.4
PEGDA (MW = 3,400 Da)	169.4 ± 70.6 nm	17.2 ± 5.0

Table 1: *Effect of crosslinker on NP diameter and swelling ratio.*

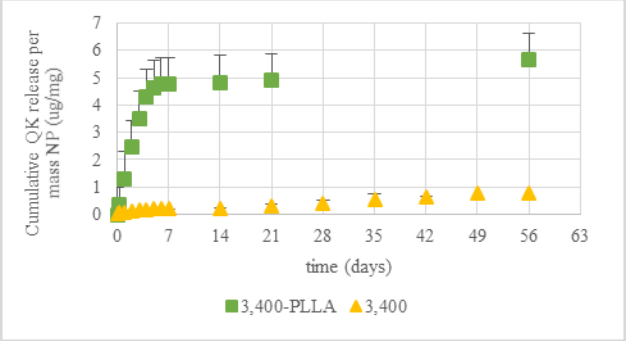

Figure 1. *Normalized QK release from NPs*

Measurements of QK cumulative release indicate that hydrolytically degradable NPs result in the fastest release, while the non-degradable PEGDA NPs formed with a 3,400 Da crosslinker allowed for sustained release up to 9 weeks (Figure 1).

Conclusions: Hydrogel NPs have been created allowing for sustained release of a pro-angiogenic peptide, QK. The release of the peptide can be controlled using a hydrolytically degradable crosslinker. The NPs possess a negative zeta potential, which is preferred as this will reduce cellular internalization and allow the released peptide to bind its extracellular receptors. Current work is investigating varied ratios of degradable to non-degradable crosslinker to further tune peptide release. Future work includes 3D *in vitro* studies to confirm the bioactivity of the released peptide.