Immobilized VEGF Fragment Promotes Microvasculature Formation in Poly(ethylene glycol) Diacrylate Hydrogels

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Statement of Purpose: Growth factors, often used to promote microvascularization, have inherent disadvantages such as immunogenicity and loss of bioactivity. Instead, a short bioactive peptide sequence derived from a growth factor protein could confer the same bioactivity and cellular response. A synthetic 15 amino acid peptide (KLTWQELYQLKYKGI, abbreviated QK) was designed by D'Andrea et al to mimic the region of vascular endothelial growth factor (VEGF) that binds VEGF receptor -1, domain 2 [1]. The QK peptide was immobilized via poly(ethylene glycol) (PEG) conjugation, and its bioactivity was shown to be similar to VEGF. Immobilized VEGF-mimicking peptide was then shown to promote endothelial cell tubule formation on 2D modified surfaces, tubule formation in 3D degradable hydrogels, and microvascular formation *in vivo* in a mouse cornea micropocket angiogenesis assay.

Methods: PEG-RGDS was made by reacting with PEG-SMC in dimethylformamide with diisopropylethylamine at a 1.1:1 PEG-SMC: RGDS molar ratio. MMP-degradable PEG polymers were synthesized in a similar manner by incorporating the synthesized peptide GGGPQGIWGQGK into the polymer backbone by reacting at a 2.1:1 molar ratio of PEG-SMC: peptide. PEGylated QK was synthesized similarly at a 10:1 PEG-SMC: QK molar ratio and precipitated with cold isopropanol. The levels of QK used corresponded to the bioactivity levels originally found in D'Andrea et al [1]. 2D studies were performed by seeding HUVECs (8.5 x 10^4 cells/cm^2) onto gels modified with (1) 30 µmol/ml PEG-RGDS, (2) PEG-RGDS with 173 nmol/ml PEG-QK, and (3) PEG-RGDS with 420 pmol/ml PEG-VEGF. Additionally, HUVECs (3 x 10^7 cells/ml) were encapsulated into degradable hydrogels containing (1) 3.5 µmol/ml PEG-RGDS, (2) PEG-RGDS with 152 nmol/ml PEG-QK, (3) PEG-RGDS with 760 nmol/ml PEG-QK, (4) PEG-RGDS with 400 pmol/ml PEG-VEGF. *In vivo* studies were performed by implanting a degradable hydrogel containing 3.5 µmol/ml PEG-RGDS and (1) 640 ng releasable VEGF per gel, (2) releasable VEGF with 6.4 ng PEG-VEGF per gel, (3) releasable VEGF with 128 ng PEG-QK into the cornea of *Flk1-myr::mCherry* transgenic mice as previously described [2].

Results: PEG-QK was shown to lead to HUVEC tubule formation 5 days after seeding onto the surface of modified hydrogels (Fig 1). HUVECs encapsulated into 3D protease-sensitive hydrogels were found to form tubule networks with cell-cell contacts between 22 and 32 hours after encapsulation (Fig 2).

In vivo vascular response to hydrogels incorporating both releasable VEGF, to initiate an angiogenic response from surrounding vessels, and covalently immobilized QK (Fig 3A), to prolong the response, showed an increase in vessel area and vessel percent coverage of tissue compared to hydrogels with releasable VEGF and immobilized VEGF.

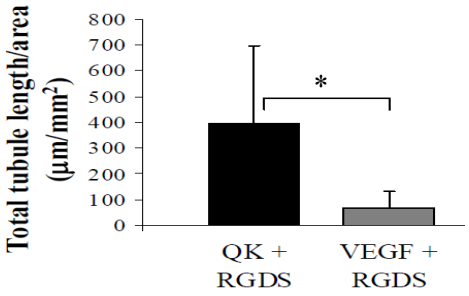

Figure 1: Total tubule formation at Day 5 is more robust on PEG-QK modified surfaces (* $p<0.05$).

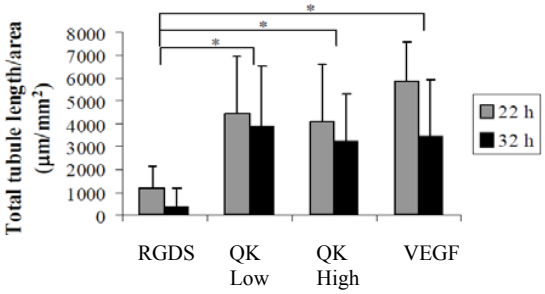

Figure 2: HUVEC tubule formation in 3D degradable hydrogels (*$p < 0.05$, comparison between levels at same timepoint) was enhanced with the addition of PEG-QK or PEG-VEGF.

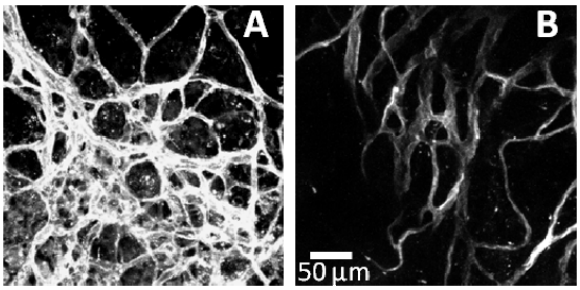

Figure 3: (A) Hydrogels with releasable VEGF and PEG-QK showed a more robust vascular response than hydrogels with releasable VEGF and PEG-VEGF (B).

Conclusions: An immobilized VEGF fragment induces endothelial cell tubule formation in 2D, 3D, and induces microvascular formation *in vivo*. Using PEG-QK, bioactive hydrogels can be designed to induce and support the formation of functional microvasculature for tissue engineering applications.

References:

1.D'Andrea LD et al. PNAS. 2005 Oct; 4;102(40):14215-14220.
2. Poche RA et al. Cold Spring Harb. Protoc. Apr; 2010(4):pdb prot5416.