Single-step Chemistry to Independently Control Matrix Stiffness and Conjugation of Pro-angiogenic Peptides

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Statement of Purpose: Growth factor mimetic peptides are more stable and less expensive to synthesize than native growth factors, making them ideal bioactive components of engineered matrices. However, small peptides are highly diffusive, necessitating their tethering to the matrix. Previously described methods using heparin to tether native growth factors cannot be translated to small peptides that lack heparin-binding domains; therefore, novel cytocompatible conjugation chemistry must be developed. Furthermore, since cells respond to matrix stiffness, an ideal conjugation method would enable independent control of peptide concentration and material stiffness. We hypothesized that a tetrafunctional covalent crosslinker at sub-stoichiometric ratios would simultaneously encapsulate cells and immobilize bioactive peptides within an engineered protein hydrogel. We evaluated this conjugation strategy using a vascular endothelial growth factor (VEGF) mimetic peptide, QK [ref. 1], within a modularly designed elastin-like polypeptide (ELP) hydrogel, **Fig. 1**, to promote the proliferation and migration of human umbilicial vein endothelial cells (HUVECs). [ref. 2]

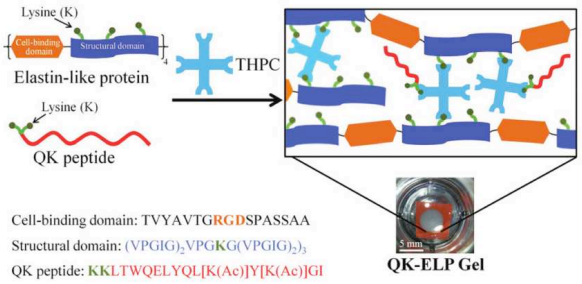

Figure 1. Schematic of QK-ELP biomaterial.

Methods: The ELP sequence was modularly designed with repeats of cell-adhesive and elastin-like domains with lysine residues positioned at regular intervals. The QK peptide included two N-terminal lysines to use as crosslinking sites, and internal lysines were acetylated for chemical protection. The ELP hydrogel (4% w/v) was formed upon the addition of an amine-reactive, tetrafunctional crosslinker, tetrakis(hydroxylmethyl) phosphonium chloride (THPC); QK-ELP gels were made in the presence of QK (10 nM, 1 µM, or 100 µM). Peptide conjugation efficiency and peptide diffusivity were determined using fluorophore-labeled QK. Shear moduli and swelling ratios were quantified for gels with varying QK concentration. HUVECs were seeded on QK-ELP gels as individual cells (15,000 cells/cm^2) or encapsulated within QK-ELP gels as spheroids (~500 cells/spheroid) in growth factor-depleted medium. Controls were pristine ELP gels without soluble QK (negative) and with soluble QK that was replenished (positive) or depleted at day 2 to mimic interstitial fluid transport. Immunostaining and confocal microscopy were used to quantify cell viability, proliferation, and spheroid outgrowth at day 4.

Results: Fluorescence recovery after photobleaching (FRAP) analysis of a soluble, FITC-labeled QK within a pristine ELP gel revealed a diffusivity of 61 ± 5 µm^2/s. This expected high diffusivity illustrates the speed at which these small peptides diffuse from gels *in vivo*. In contrast, conjugated QK showed no detectable diffusion, demonstrating effective immobilization. Rheological measurements of ELP gels with 0, 10 nM, 1 µM, and 100 µM QK were all found to have shear moduli of ~270 Pa and nearly identical swelling ratios of ~19, confirming that our conjugation strategy does not change gel mechanics. HUVECs seeded on 10 nM and 1 µM QK-ELP gels proliferated more quickly than on pristine ELP gels, decreasing the doubling time by 30%, **Fig. 2**. Thus, QK appears to maintain its bioactivity after conjugation. Surprisingly, 100 µM QK-ELP prevented mitotic behavior. The encapsulated HUVEC spheroids exhibited nearly 100% survival in all gels. While the 10 nM and 1 µM QK-ELP gels promoted three-dimensional outgrowth compared to pristine ELP gels, the 1 µM soluble QK had greater activity. This may be due to soluble QK penetration throughout the HUVEC spheroid, whereas conjugated QK activity is limited to the spheroid surface. However, when soluble QK was removed from the medium at day 2 to mimic *in vivo* diffusion and depletion, outgrowth was significantly less than that in the 10 nM and 1 µM QK-ELP gels. Analogous to the 2D results, 100 µM QK-ELP did not stimulate outgrowth.

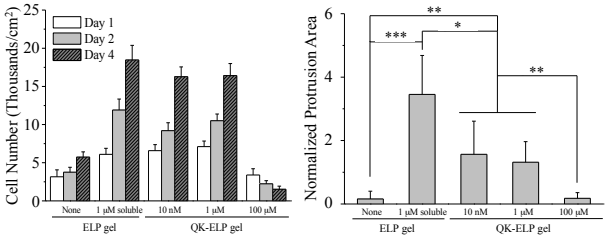

Figure 2. (Left) Proliferation of HUVEC on ELP gels. (Right) Outgrowth from 3D-encapsulated spheroids within ELP gels.

Conclusions: We report the use of a tetra-functional crosslinker to simultaneously encapsulate cells within an ELP hydrogel matrix and covalently tether QK peptides at tunable concentrations. This single-step procedure is simple and results in over 95% cell viability without impacting hydrogel stiffness or swelling. The tethered QK peptides retained their VEGF-mimetic function and stimulated HUVEC proliferation and outgrowth. As an increasingly large toolbox of biomimetic peptides become available, this simple chemistry can create tailored materials for regenerative medicine applications.

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References: 1) D'Andrea L *et al.* PNAS. 2005; 102(40): 14215-20. 2) Cai L, Dinh CB, Heilshorn SC. Biomater Sci. 2014; 2(5):757-65.