Co-assembling Peptides as Defined Hydrogel Microenvironments for Endothelial Cells

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Statement of Purpose: Self-assembling biomaterials have been widely investigated as defined matrices for use in wound healing and tissue regeneration applications. However, many current self-assembling biomaterials cannot independently control properties such as ligand presentation without simultaneously affecting other physicochemical and biological properties such as viscoelasticity.[1,2] The objective of this work is to develop a strategy for using a modular peptide-based biomaterial incorporating fibronectin- and laminin-derived cell-interactive sequences to enhance endothelial cell attachment, spreading, and growth without significantly affecting mechanical properties of the hydrogels. This strategy is important because it allows the systematic tuning of multi-component hydrogels, where materials' physicochemical (e.g. viscoelasticity) and biological (e.g. ligand presentation) properties can be precisely controlled for the systematic investigation of cell-matrix interactions.

Methods: *Peptide Synthesis:* The peptides Q11 (QQKFQFQFEQQ) and Q11 peptides bearing RGDS or IKVAV ligands on their N-termini were synthesized on a CS Bio 136 synthesizer using standard Fmoc-based solid-phase peptide synthesis. Peptide *m/z* values were verified with ESI or MALDI-TOF mass spectrometry. *Quantitative Measurement of Peptide Entrapment:* Precursor peptide solutions and hydrogels were analyzed using HPLC, where relative peptide concentrations were determined by peak areas at 215nm. *TEM and Circular Dichroism (CD):* The fibrillization and secondary structures of the 10% mixtures of Q11 and the N-terminally functionalized peptides were identified using TEM and CD. *Oscillating Rheometry:* Cylindrical hydrogels were formed on the lower plate of a rheometer using a custom made template, and their moduli were measured at 0.1% strain with oscillating frequencies from 0.01 to 10 Hz. *Colloidal Gold Staining:* The N-terminally functionalized peptides were biotinylated and labeled with streptavidin-conjugated colloidal gold, then visualized by TEM. *Endothelial Cell Cultures and Characterization:* The attachment, spreading, and growth of endothelial cells were investigated in cultures of primary human umbilical vein endothelial cells (HUVECs). The attachment and spreading of HUVECs were measured after 1 h of seeding and the growth of HUVECs was analyzed using MTS-based proliferation assay at 64 h of seeding.

Results: Incorporating 10-50% of N-terminally functionalized peptides within Q11 matrices did not affect gel formation. Mixing 10% of the ligand-bearing peptides, i.e. RGD-Q11 and IKVAV-Q11, with 90% Q11 changed neither fibillization nor folding in comparison to 100% Q11. The 10% mixture of ligand-bearing peptides did not alter the mechanical properties of gels (Figure 1).

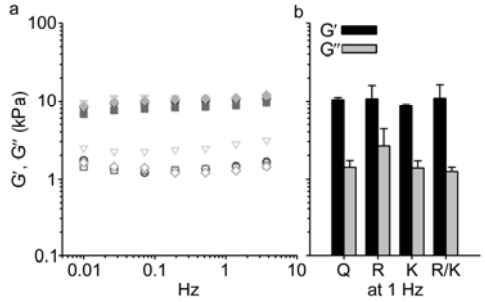

Figure 1. Oscillating rheometry of Q11 gels. Measurement of G′ (filled symbols) and G″ (open symbols) of peptide gels by frequency sweep (a). In (b), statistical analysis of multiple gels at 1 Hz, 30 mM total peptide, Q11 (●, ○, "Q"), 10% RGD-Q11/90% Q11 (▼, ▽, "R"), 10% IKVAV-Q11/90% Q11 (■, □, "K"), and 5% RGD-Q11/5% IKVAV-Q11/90% Q11 (◆,◇,"R/K"). (n=3 independent gels per group, means±SD)

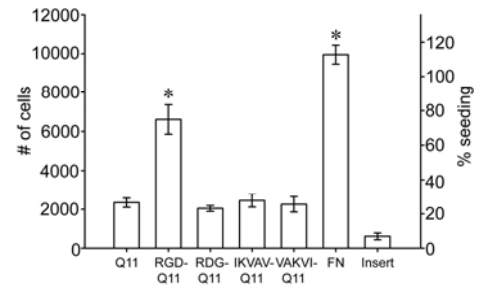

Figure 2. MTS-based proliferation assay at 64 h on 10% functionalized peptides in the context of 90% Q11, fibronectin adsorbed on Q11 (FN), and culture insert (Insert) (n=5, means±SD). ∗p<0.01, ANOVA with Tukey HSD post-hoc test.

By labeling biotinylated peptides (e.g. biotin-RGD-Q11) with streptavidin-conjugated colloidal gold, we observed that the ligands were displayed on fibrils and their presentation was quantitatively controlled by mixing at different ratios. The attachment of HUVECs was significantly increased on 10% RGD-Q11/ 90% Q11 gels and moderately increased on 10% IKVAV-Q11/ 90% Q11 gels. We observed increased spreading of HUVEC for 1 h on 10% RGD-Q11/ 90% Q11 gels. The proliferation of HUVECs was also significantly increased for 64 h after seeding on 10% RGD-Q11/ 90% Q11 gels over 100% Q11 and scrambled-sequence peptide gels (Figure 2).

Conclusions: This modular system enables us to self-assemble different ligand-bearing peptides into a defined matrix without altering the mechanical properties of the hydrogels. Within gels of consistent storage moduli, the functionalized ligands displayed on modular Q11 fibrils significantly modulated HUVEC attachment and proliferation. In combination with previous work in our laboratory aimed at specifically controlling matrix mechanics through chemoselective cross-linking,[3] this enables independent control over ligand presentation and mechanics, allowing for systematic tuning of multi-component hydrogels.

References: 1. Collier JH. *Soft Matt.* 2008. 2. Straley KS. et al. *Soft Matt.* 2008. 3. Jung JP. et al. *Biomaterials* 2008;29:2143-2151.