Fibrillizing Peptide Matrices with Modular Construction Enabling Multifactorial Optimization

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Statement of Purpose: Self-assembling biomaterials are increasingly investigated as synthetic matrices for 3D cell culture and as scaffolds for Tissue Engineering. However, in many self-assembling systems, biological properties such as ligand presentation cannot be adjusted without also influencing other physicochemical properties such as viscoelasticity.[1] Owing to this, we have sought to develop modular, co-assembling scaffolds with independent control over such factors.[2,3] This modular strategy provides a route for systematically adjusting and optimizing materials whose interactions with biological systems are driven by multiple factors. The objective of the present work was to investigate co-assembling peptide scaffolds displaying precise combinations of multiple different fibronectin- or laminin-derived cell-binding sequences and a nitric-oxide (NO) releasing peptide to systematically identify combinations of these factors that most effectively promote the rapid endothelialization of synthetic surfaces. These materials were explored with Full Factorial Experiment (FFE) designs, enabling their high-throughput statistical optimization. This strategy is useful because it enables the systematic tuning of multiple scaffold properties for controlling biological processes.

Methods: *Peptides:* Fusion peptides containing N-terminal cell-binding GGRGDSGGG, GGYIGSRGGG, and GGIKVAVGGG sequences and C-terminal self-assembling Q11 sequences were prepared as previously described.[3] *Factorial Experiments:* Multi-component gels were optimized with 2-level/4-factor (2^4) FFEs, in which 16 formulations of various levels of each peptide were generated in culture inserts. The response measure was proliferation of endothelial cells (HUVEC) by MTS assay, and FFEs were analyzed using JMP statistical software (SAS Institute Inc.) to identify significant factors, interactions between factors, and to create contour plots. *NO Release:* Cys-Q11 was synthesized and reacted with $NaNO_2$ at pH 3. Nitric oxide release from the resultant nitrosothiol peptide gels was measured by Griess assay.

Results: The proliferation of HUVECs on Q11 matrices was complexly modulated by the three N-terminally functionalized cell-binding peptides. Contour plots were created for all interactions; two examples are provided in Figures 1a-b. The highest HUVEC proliferation, marked **X** in Figure 1a, corresponded to a formulation combining 6 mM RGD-Q11/1.5 mM IKVAV-Q11/2 mM Q11 (phase image of HUVECs at 3d shown in Figure 1c). All other formulations showed lower HUVEC proliferation (the **X** in Figure 1b corresponds to Figure 1d). Analysis of t-ratios (Fig. 1e) showed that each peptide significantly contributed to HUVEC proliferation, with the exception of Q11 itself. Interestingly, the interactions of YIGSR-Q11 with all other factors were antagonistic, with those with RGDS-Q11 and IKVAV-Q11 being significant. This indicated a deleterious effect of YIGSR, but only in the context of RGD and IKVAV, a result that would be more difficult to clearly observe in materials that are not as easily adjusted within factorial experiments as the modular materials reported. NO release from Cys-Q11 gels was maintained for at least up to 9 days in HUVEC medium (Figure 1f), and at the time of abstract submission, this peptide is being combined with the ligand-bearing peptides to determine the effect of NO release on HUVEC responses to the ligand combinations.

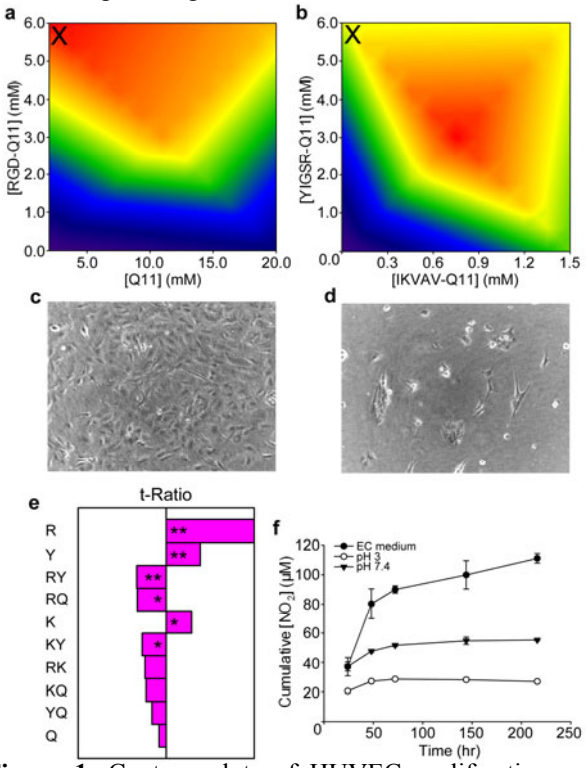

Figure 1. Contour plots of HUVEC proliferation as a function of Q11 and RGD-Q11 concentrations (a) or IKVAV-Q11 and YIGSR-Q11 (b). Red: high proliferation; blue: low. Corresponding phase contrast images of HUVECs at the formulation **X** in (a) and (b) are shown in (c) and (d), respectively. (e) t-ratios estimated up to 2nd order interactions, where R= RGD-Q11; K=IKVAV-Q11; Y=YIGSR-Q11; Q=Q11. ** $p<0.01$ and *$p<0.05$, n=3. (f) Cumulative nitrite (NO_2) release from NO-Cys-Q11 gels by Griess assay (n=2, means±SD).

Conclusions: The groundwork for systematically tuning the responses of endothelial cells to multi-component biomaterials was established. In combination with previous work,[3,4] this modular system enables us to explore a multitude of formulations via Design of Experiment approaches for tuning hydrogel properties with multiple biological (cell-binding ligands), mechanical (stiffness), and therapeutic (NO) factors.

References: 1. Collier JH. Soft Matter 2008;4:2310-2315. **2.** Lee JS. et al. Acta Biomater. 2009; in press. **3.** Jung JP. et al. Biomaterials 2009;30:2400-2410. **4.** Jung JP. et al. Biomaterials 2008;29:2143-2151.