Self-Assembled Synthetic ECMs Displaying Functional Protein Domains

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Statement of Purpose: Hydrogels created from self-assembling peptides provide synthetically defined, multi-component extracellular matrices for applications in 3D cell culture and regenerative medicine.[1,2] However, their ability to display functional ligands has been limited to short peptide sequences, whose specificity and affinity are significantly reduced compared to the well-folded proteins from which such peptides are derived. Here, we developed a means for producing self-assembled peptide matrices that display controlled quantities of well-folded protein domains. This work was based on a self-assembling peptide previously investigated within our laboratory, Q11, which forms fibrillar hydrogels in physiological conditions, and which has previously been functionalized with cell-binding peptides and chemical functionalities for cross-linking.[1,2] In the present work, we designed a self-assembling Q11 derivative displaying a phosphonate suicide ligand for the enzyme cutinase, thus enabling the chemically selective covalent immobilization of cutinase fusion proteins onto the self-assembled fibrillar network (Figure 1). Here, green-fluorescent protein (GFP)-cutinase fusion proteins were utilized to demonstrate this concept, which can be broadened to include previously designed cutinase fusion proteins displaying cell-binding domains from fibronectin.[3] This work is the first demonstration of cutinase-based protein immobilization in 3D systems.

Methods: *Peptide synthesis:* Cys-Q11 (CSGSGQQKF-QFQFEQQ) was synthesized using standard Fmoc-based solid phase protocols. *p*-nitrophenylphosphonate-(ethyleneglycol)$_5$-maleimide was synthesized and conjugated to Cys-Q11 to form phosphonate-Q11. *Protein expression*: A GFP-cutinase fusion protein was constructed with standard cloning techniques, containing an N-terminal cutinase domain, a $(GGGS)_3$ linker, a green fluorescent GFPuv domain, and a C-terminal His-tag. It was expressed in origami cells and purified on Ni-NTA columns. *Peptide characterization:* Phosphonate-Q11 identity was established with MALDI-TOF mass spectrometry, and fibrillization was observed with transmission electron microscopy (TEM). *Protein conjugation*: Q11 and phosphonate-Q11 were co-fibrillized into gels having 2mM total peptide. Gels were incubated with cutinase-GFP, and non-immobilized protein was removed by serial centrifugation and washing. Immobilized GFP was measured via fluorescence.

Results: Phosphonate-Q11 was successfully synthesized with excellent purity (Figure 2b), and it self-assembled in phosphate buffered saline (PBS) (Figure 2a). Individual fibrils were about 15nm wide, and they laterally entangled into tape-like parallel bundles with widths of 100-200nm, indicating that phosphonate decoration did not disrupt the peptide's capacity to self-assemble. After protein immobilization, gels incorporating 0.1mM phosphonate were strongly fluorescent, whereas unmodified 2mM Q11 gels were not (Figure 3). At the time of abstract submission, the phosphonate ligand is being dosed into Q11 backgrounds to achieve control over the quantity of protein immobilization, and the strategy is being applied to immobilize cutinase fusion proteins containing cell-adhesive fibronectin domains.[4]

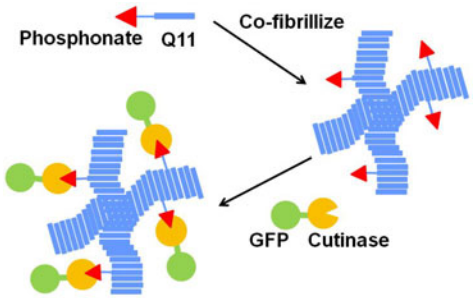

Figure 1. Schematic. A phosphonate-displaying self-assembling peptide (a) fibrillizes into hydrogels (b) able to covalently immobilize cutinase fusion proteins (c).

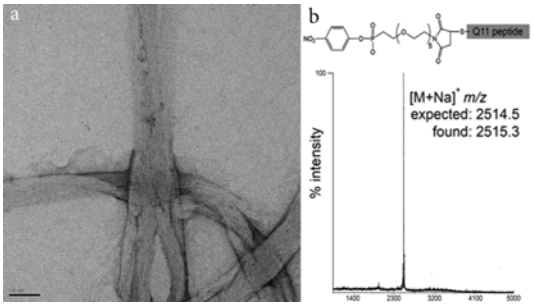

Figure 2. Phosphonate-Q11 formed self-assembled fibers in PBS (a, TEM; scale bar is 100 nm), and was chemically homogeneous (b, MALDI MS).

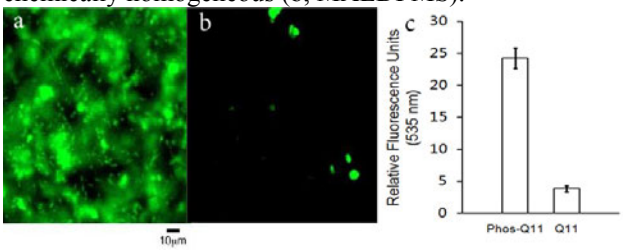

Figure 3. Fluorescence of hydrogels containing 5% phosphonate-Q11 (a) or 100% Q11 (b), both subjected to cutinase-GFP. Quantification in (c).

Conclusions: Cutinase fusion proteins were immobilized on self-assembled peptide matrices displaying phosphonate capture ligands. Cutinase-GFP was used as a model protein to demonstrate the strategy, which is currently being expanded to include ECM-specific protein domains as well.

References: **1.** Jung et al., *Biomaterials* 29, 2143-2151 (2008). **2.** Jung et al., *Biomaterials* 30, 2400–2410 (2009). **3.** Murphy et al., *Langmuir* 20, 1026-1030 (2004).