Modulating Viscoelasticity and Ligand Presentation in Self-Assembled Matrices via Peptide Intermixing

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Statement of Purpose: Self-assembled peptide gels are promising materials for 3D cell culture and regenerative medicine, but the ECMs they are designed to mimic have complex structures and diverse properties. In particular, the spatial positioning of crosslinks and biochemical ligands make these natural ECMs difficult design targets for engineered peptide matrices. Well defined mixing and gelation procedures for creating modular, multi-component hydrogels from self-assembling peptides may provide new avenues to help recreate the diverse range of mechanical properties of native ECMs. These same strategies may also provide control over the spatial distribution of biochemical ligands[1]. Here we report techniques that significantly modulate stiffness and biochemical ligand positioning through straightforward mixing and gelation methods that do not require the development of new reagents or unique chemistries.

Methods: Q11 (Ac-QQKFQFQFEQQ-Am), myc-Q11 (EQKLISEEDLSGSG-Q11), RGDS-Q11 (GGRGDSGGG-(Q11)), biotin-RGD-Q11 (biotin-SGSG(RGD-Q11)), RDG-Q11 (GGRDGSGGG-(Q11)), Cys-Q11 (CSGSG-(Q11)), and Lys-Q11 (GGKSGSG-(Q11)) peptides were synthesized using Fmoc chemistry. For basic hydrogel formation, dry, lyophilized Q11-based peptides were first dissolved with water to initiate fibril formation. The soluble fibril solutions were then cast into molds by pipetting and carefully bathed in phosphate buffered saline (PBS), causing gelation. Oscillating rheometry was performed at 0.2% strain with logarithmically increasing frequencies (0.01-10 Hz). Dry Q11 peptides with different terminal ligands (e.g. RGD, myc) were individually dosed into backgrounds of base Q11 peptide to form fibrils with integrated functionalities. Identification of fibril species was observed by transmission electron microscopy (TEM) using biotinlyated and myc-tagged peptides labeled with avidin-gold (6 nm diameter) and anti-myc gold conjugated antibodies (15 nm). Human Umbilical Vein Endothelial Cells (HUVECs) and NIH 3T3 cells were seeded onto or within Q11 based gels and cellular growth levels were assessed by triplicate MTS assays after 3 days.

Results: Allowing Q11 peptides to fibrillize in water for different time points between 0.5-24 hr, followed by 2-24 hr durations of PBS exposure resulted in hydrogels with broad, controllable, 15-50 kPa ranges of storage moduli (not shown). We used TEM and dual labeling with two different sized gold particles to follow fibril formation. 10% (by molar concentration) biotin-RGD-Q11 and myc-Q11 were individually mixed dry and allowed to fibrillize with 90% non-functional Q11 in water, in separate containers for 24 hours. When those two fibril solutions were subsequently mixed and spotted on a TEM grid, we observed that they were maintained in two distinct fibril populations (Fig A, left bar). In contrast, if biotin-RGD-Q11, myc-Q11 and Q11 were all mixed as dry powders together, the majority population of the fibrils were "intermixed" with both functionalities (Fig A, 2nd bar, blue). As expected, control samples of either only biotin-RGD-Q11 or only myc-Q11 resulted in exclusive fibril species with 10-15% non-specific background labeling (Fig A). With these established mixing techniques, we seeded HUVECs on top of gels composed of fibrils with 10% integrated RGD-Q11. Gels composed of separately assembled 100% Q11 fibrils and 100% RGD-Q11 fibrils mixed in a 9:1 ratio were used as a control. Cell growth was significantly better (p=0.002) on the integrated RGD-Q11 based gels. Integrated RGD-Q11 also outperformed other controls: Q11 alone, integrated RDG and separately assembled RDG, but not the commercially available matrix, Geltrex(p=0.21) (Fig B). Similarly, NIH 3T3s grew significantly better when cultured within 3D gels comprised of integrated RGD fibrils compared to the same controls (not shown). Finally, these mixing techniques were used to generate hydrogels composed of separately assembled Cys-Q11 and Lys-Q11 fibrils. A maleimide-PEG-NHS crosslinker was then used to direct the formation of interfibril crosslinks (Fig C black squares), which improved gel storage moduli to over 120 kPa; 2-3 fold higher than controls, including crosslinked gels composed of Cys/Lys intermixed fibrils (Fig C).

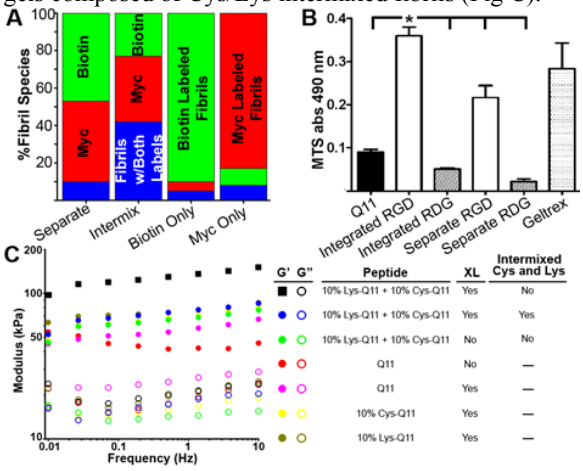

Figure A) Identification of fibril species types within different peptide samples (x-axis) using TEM and dual gold labeling. **B)** MTS data of HUVECs grown for 3 days on 2D hydrogel surfaces composed of intermixed and separately assembled RGD and scrambled RDG fibrils. **C)** Storage moduli of gels composed of different fibril species treated with and without a heterobifunctional crosslinker (Thermo Sci 22112, Rockford, IL).

Conclusions: Here we used straightforward strategies to demonstrate that the mechanical properties of peptide based hydrogels can be altered by controlling fibril assembly and subsequent exposure to PBS. Ligand functionality could be directed to different fibril species by controlling how self-assembling peptides are mixed, which affect chemical crosslinking and cell growth.

Reference: [1]Jung JP. Biomaterials. 2009. (12):240-10.