Using Peptide Stereochemistry to Control Physical Features of Self-assembling Hydrogels

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Statement of Purpose: Tunable hydrogels create tissue-appropriate microenvironments for cell delivery, growth, and differentiation. Rapidly Assembling Pentapeptides for Injectable Delivery (RAPID) hydrogels[1] consist of five amino acid long peptide sequences that assemble into β-sheet fibers, forming physically crosslinked hydrogels under physiological conditions. RAPID gels flow when subjected to shear and quickly recover[1], making them excellent candidates for injectable cell delivery. In addition, RAPID gel mechanical properties are highly tunable by pH, amino acid sequence, and concentration, suggesting potential uses in diverse biological contexts. Previous literature of the $(FKFE)_2$[2] and MAX1[3] sequences, which also form β-sheet fibers, demonstrated that racemic mixtures of peptides create unique assembly mechanisms and morphology by forming non-twisted ribbon fibers useful for cellular microenvironments. This suggests the variable of stereochemistry influences physical characteristics of hydrogels. Here, we determined the rheological and morphological properties of RAPID hydrogels made from pure L- and D- KYFIL enantiomers, as well as various ratios of the enantiomeric mixtures.

Methods: Peptides examined via rheology were synthesized and purified (>98% purity) by GenScript Biotech. Pure L- and D- hydrogels were prepared by mixing peptide with 1X PBS and adjusting pH with sodium hydroxide until a 3 % (w/v) gel with pH 7.4 was obtained. Enantiomeric mixtures were prepared similarly, except dry peptides were first dissolved together in ultrapure water (90%) before adding 10X PBS (10%). Thirty minutes following sample preparation, rheological properties of these hydrogels were measured at room temperature by frequency sweeps (0.1 – 100 rad/s) at a constant strain of 1% and strain sweeps (0.01 – 100 %) at a constant angular frequency of 10 rad/s. Peptides used for transmission electron microscopy (TEM) were synthesized on a Liberty Blue peptide synthesizer using Rink Amide Resin, treated with trifluoroacetic acid, washed with diethyl ether, dried under vacuum, and purified via high performance liquid chromatography (HPLC). Lyophilized peptides were combined with 1X PBS and pH adjusted to 7.4 to form 1.5 % (w/v) gels. The hydrogels were negatively stained and imaged via TEM to determine average fiber width and pitch.

Results: Frequency sweeps showed that 3 % (w/v) L-KYFIL and D-KYFIL gels possessed similar G' (36.7 +/- 5.8 kPa, 27.6 +/- 3.8 kPa) and G'' (4.9 +/- 0.53 kPa, 3.8 +/- 0.73 kPa) values (Figure 1). The 3:1 and 1:3 enantiomeric mixtures declined in stiffness to G' of 15.8 +/- 8.8 kPa and 17.2 +/- 6.8 kPa, respectively. The 1:1 racemic mixture exhibited the lowest storage (3.6 +/- 1.1 kPa) and loss moduli (0.29 +/- 0.08 kPa) of all samples. These results contrast MAX1 and (FKFE)2 hydrogels, in which stereocomplexed gels exhibit higher moduli than the hydrogels from the constituent peptides. TEM images revealed D- and L-KYFIL, assembled into twisted ribbons but the racemic mixtures formed plate-like structures. Blends of D-and L-MAX1 and $(FKFE)_2$ peptides retained the fibrillar morphology of the constituent peptides, and accordingly, the stereocomplexed hydrogels exhibited greater moduli due to the increased stiffness of individual fibers. Here, loss of the fibrous morphology upon stereocomplexation of KYFIL translated to a reduction in moduli of KYFIL stereocomplexed hydrogels.

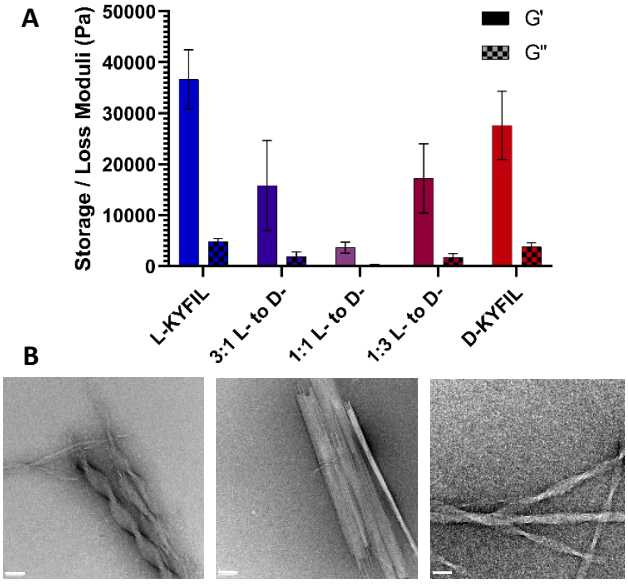

Figure 1. (A) Average storage and loss moduli of each KYFIL enantiomeric ratio from 10 – 100 rad/s (n=3). (B) Representative TEM images of 1.5 wt% L-KYFIL (left), 1:1-KYFIL (middle), and D-KYFIL (right) hydrogels. L- and D-KYFIL gels assemble into twisted ribbon structures, while racemically mixed RAPID hydrogels assemble into plate-like structures. Scale bars = 50 nm for all panels.

Conclusions: L-and D-KYFIL gels have nearly identical stiffnesses, the 1:3 and 3:1 KYFIL mixtures showed intermediate stiffnesses, and the 1:1 racemic mixture possessed the lowest stiffness. The plate-like morphology of KYFIL explains the lower stiffness of 1:1 L/D-KYFIL gels. Because KYFIL, MAX1, and $(FKFE)_2$ form similar enantiomeric structures but assemble differently in 1:1 mixtures, the effect of racemization is likely sequence dependent.

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References: 1) Tang JD. JACS. 2019;141:4886-4899. **2)** Swanekamp R. Chem. Commun. 2014;50:10133-10136. **3)** Nagy KJ. JACS. 2011;133:14975-14977.