Controlled Peptide Hydrogel Degradation via RAPID Sequence Design and Stereocomplexation

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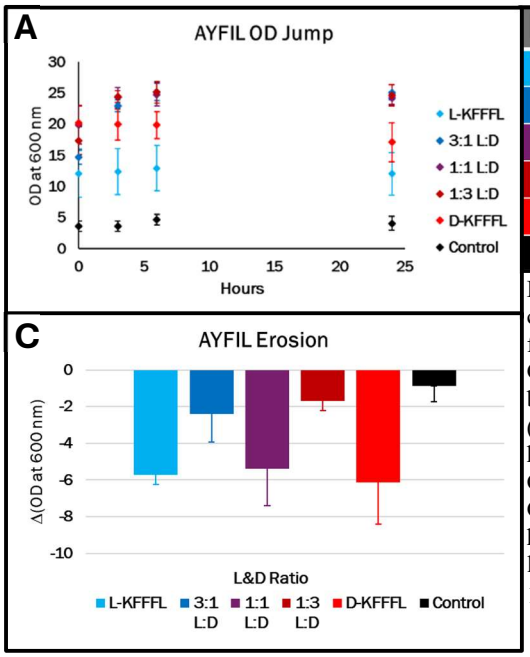
Statement of Purpose: Since the discovery of the first short peptide sequence that can gel on demand in 1990,[1] peptide-based biomaterials have been employed in a variety of regenerative medicine and tissue engineering applications. Unique to peptide-based biomaterials, incorporating $_D$- form peptide can be used as a tool to control degradation and to invoke cell immune responses, both pro-regenerative or cytotoxic.[2,3] Our group recently reported a gel-forming peptide sequence, KYFIL, where integration of $_D$- form peptide reduced proteolytic degradation in the presence of an enzyme, Proteinase K.[4] While those results assess proteolytic degradation, during cell culture physical degradation or erosion of hydrogels can occur from convective forces and simple dilution during media exchange steps. During 3D cell culture, Rapidly Assembling Pentapeptides for Injectable Delivery (RAPID) hydrogels sometimes degrade quickly. Here, we explore the erosion of three RAPID sequences, KYFIL, AYFIL, and KFFFL, in an acellular pseudo cell culture system with varying compositions of $_L$- form and $_D$- form peptide.



B L&D Ratio	% Change
L-AYFIL	7%
3:1 L:D	42%
1:1 L:D	20%
1:3 L:D	31%
D-AYFIL	-2%
Control	-2%

Fig 2. AYFIL hydrogel characterization (N=7-8 hydrogels) from two separate experiments. Control groups are 1X phosphate buffered saline. (A) Optical density (OD) change observed within 24 hours and (B) percent change of OD from 0 hours to 6 hours. (C) Change in OD at 600nm from 6 hours to 8 days indicating the largest amount of erosion occurs in 1:1 and pure $_L$- and $_D$- AYFIL.

Methods: 3 wt% (w/v) KYFIL, AYFIL, and KFFFL hydrogels were each prepared in five different formulations: pure $_L$- and $_D$- peptide, and intermediate blends of $_{L:D}$ at molar ratios of 3:1, 1:1, and 1:3. Hydrogels made in silicone molds with 1X phosphate buffered saline (PBS) were incubated under cell culture conditions for 8 days. Every two days, 1X PBS was replaced to simulate media exchange, brightfield microscopy images were taken to assess bulk hydrogel degradation, and optical density (OD) measurements at 600nm were taken to quantify hydrogel erosion (Figure 1). Four OD measurements were also taken within the first 24 hours.

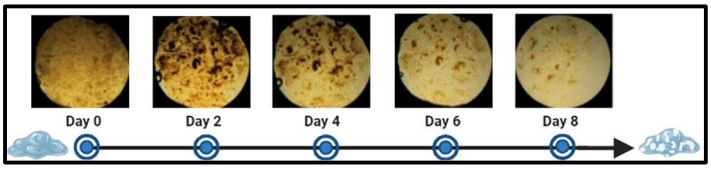


Fig 1. Brightfield microscopy images of a 1.5 wt% 1:1 $_{L:D}$- KFFFL hydrogel pseudo cell cultured over 8 days.

Results: OD measurements at timepoints <24 hours indicate an increase in OD for all $_{L:D}$ mixtures in AYFIL (Figure 2A), KYFIL(not shown), and KFFFL (not shown). For all three sequences, this difference in OD was supported via brightfield microscopy images and well plate images. Mixtures of $_{L:D}$ appear heterogeneous (Figure 1) compared to pure $_L$- and $_D$- hydrogels under the microscope, and well plate images show $_{L:D}$ mixtures appeared to be white and opaque compared to translucent pure $_L$- and $_D$- hydrogels (not shown). Quantification of the percent change of OD is highest for AYFIL $_{L:D}$ mixtures (Figure 2B) compared to the other two peptide hydrogel groups (not shown). Erosion for each peptide sequence was unique. For AYFIL, the 1:1 mixture, as well as pure $_L$- and $_D$- hydrogels eroded the most (Figure 2C). For KYFIL, pure $_L$- and $_D$- hydrogels eroded the most (not shown). For KFFFL, all experiment groups degraded similarly (not shown).

Conclusions: An increase in OD at early timepoints for $_{L:D}$ mixtures may suggest time-dependent morphology changes, or potentially a temperature induced change (e.g. annealing) in RAPID hydrogels. Differences in hydrogel erosion across $_{L:D}$ mixtures in peptide sequences are surprising but illustrate that only one or two different amino acids can impact bulk mechanical properties. However, regardless of sequence, pure $_L$- and $_D$- hydrogels eroded the quickest. Further work will examine if nanofiber morphology differences are driving differential degradation. Next steps also include cell encapsulation to understand the impact of the incorporation of $_D$-form peptide and peptide sequence on cell behavior.

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References: 1) Zhang, S. Interface Focus 2017, 7 (6) **2)** Griffin, DR. Nat. Mater. 2021, 20 (4), 560–569. **3)** Bomb, K. ACS Macro Lett. 2023, 12 (6), 725–732. **4)** Duti, IJ. J. Am. Chem. Soc. 2023, 145 (33), 18468–18476.