Enzyme-Mediated Peptide Decoration of Silk Fibroin-based Hydrogels

Meghan McGill[a], James M. Grant[a], David L. Kaplan[a]
[a]Department of Biomedical Engineering, Tufts University

Statement of Purpose: Covalently crosslinked silk fibroin (SF)-based hydrogels exhibit a tunable modulus and mesh size, optical clarity, and elastomeric properties[1,2], making them versatile scaffolds for tissue engineering and drug delivery applications. However, native SF proteins lack biologically active domains, which limits their ability to interact with the environment. Here we utilize enzymatic crosslinking reactions as a simple and versatile method of conjugating peptides with terminal tyrosine groups to the tyrosine residues in SF **(Figure 1A)**. The purpose of this work is to (1) understand the design space of the conjugated peptides, assessing how reaction efficiency, spatial distribution, and protein secondary structure change with peptide properties (length, hydrophilicity, and number of tyrosines), and (2) demonstrate the utility of this method *in vitro*.

Methods: Aqueous silk fibroin solution and solutions of peptides with terminal tyrosine groups **(Figure 1B)** were crosslinked with horseradish peroxidase (HRP) and hydrogen peroxide (H_2O_2). Fluorescein isothiocyanate (FITC)-labelled peptides were incorporated into the hydrogels and leaching was studied over a five day period using a fluorescent plate reader. Additionally, the spatial distribution and leaching of FITC-peptides from peptide SF hydrogels were studied under fluorescent microscopy. Peptides with isotopically labelled tyrosine groups (^{15}N) were crosslinked into silk hydrogels, hydrolyzed, and studied under liquid chromatography tandem mass spectroscopy (LC-MS/MS) to quantify dityrosine crosslinks by a method previously developed[2]. Fourier-transform infrared spectroscopy (FTIR) was used to study the changes induced in the secondary structure of SF by different peptides at varying molar ratios. Finally, the utility of this method was explored *in vitro* with SF hydrogels conjugated to matrix metalloprotease (MMP)-cleavable peptides.

Results: Experiments using FITC-peptides conjugated to SF hydrogels revealed that leaching of the peptides from the hydrogels increased as the molar ratio of peptide to silk was increased, and that leaching was higher in shorter peptides than longer peptides. These properties can be exploited to create gradient-patterned gels, as seen in **Figure 1C**. LC-MS/MS analysis of peptides with isotopically-labelled tyrosine groups revealed the presence of both SF-SF dityrosine bonds as well as SF-peptide dityrosine bonds, confirming that the peptides were bound into the hydrogel and not only entrapped (**Figure 1D**). Preliminary FTIR results (not shown) revealed that peptides with two terminal tyrosine groups (effectively short crosslinkers) induced the formation of β-sheet structures in the SF hydrogels, whereas hydrogels formulated without peptides had minimal β-sheet content.

Conclusions: We demonstrated that enzymatic polymerization can be used to incorporate a range of peptides into SF hydrogels. The first part of this work focused on the use of model peptides to study the reaction efficiency, spatial distribution, and effect on protein structure. Ongoing work focuses on an MMP-cleavable peptide hydrogel studied *in vitro* to demonstrate a specific application of the proposed method. Ultimately, this work expands our ability to design advanced, silk-based biomaterials that can interact with the local environment.

References:

1. Partlow, BP et al. *Adv Funct Mater*. 2014; 24(29): 4615-4624.
2. McGill, M et al. *Acta Biomater*. 2017; 63:76-84.

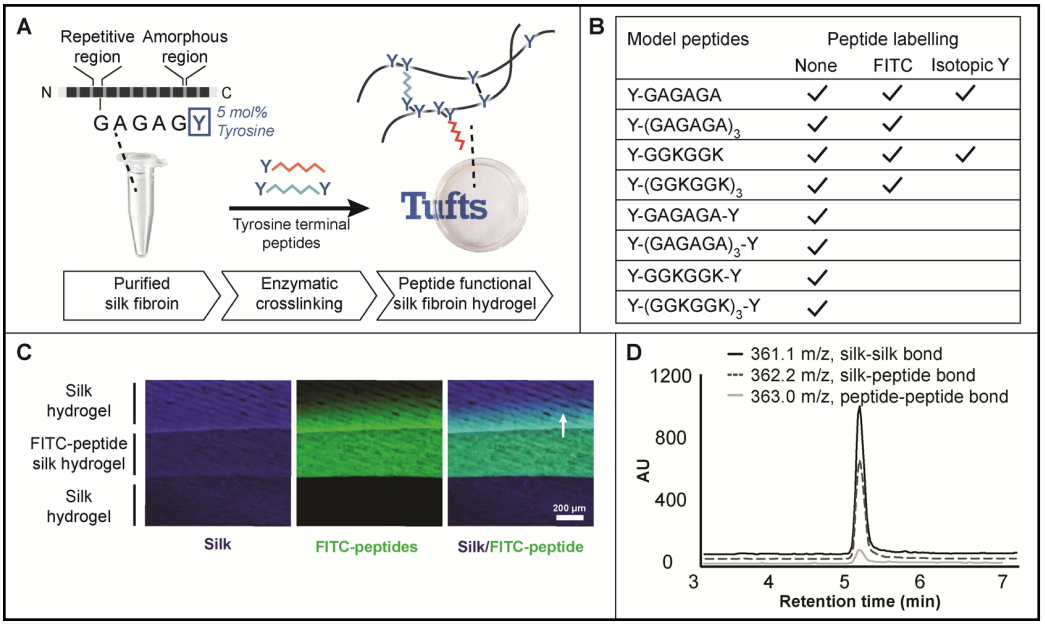

Figure 1 (A) Schematic of the enzymatic functionalization of silk fibroin (SF) via tyrosine crosslinking. **(B)** Summary of model peptides with varying hydrophilicity, length, and tyrosines assessed in this work. **(C)** Fluorescent imaging of layered hydrogels functionalized with FITC-peptides, demonstrating controlled leaching. **(D)** LC-MS/MS analysis of isotopically-labelled peptides, showing covalent crosslinking between peptide and SF.