Enzyme-Mediated Chemical Modifications of Silk Fibroin Towards Advanced Biofunctional Silk Hydrogels

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Statement of Purpose: The use of horseradish peroxidase (HRP) enzyme to covalently crosslink silk fibroin (SF) via radical polymerization has given rise to a new class of silk biomaterials. Silk-based constructs are typically characterized by predominately beta-sheet conformations, yielding tough but opaque and brittle materials. In contrast, covalently crosslinked hydrogels exhibit minimal beta sheet content, optical clarity, and elastomeric properties. These SF hydrogels have exciting potential applications in drug delivery and regenerative medicine; however, the lack of biological epitopes limit advanced, functional biomaterial utility. The goal of this work is: 1) to enable the rational design of SF hydrogels with specific matrix properties by robust characterization of the hydrogel, and 2) to incorporate functional ligands into SF hydrogels by a facile and versatile method utilizing enzymes. First, fundamental characterization of the hydrogels was carried out to relate the crosslink density, diffusivity, and modulus to the reactant conditions. Next, ligands containing phenolic terminal groups were grafted onto tyrosine residues in the SF backbone via enzymatic addition, and the concentration, size, and charge of the ligands were varied to understand the design limitations.

Methods: Silk hydrogels were formed by radical polymerization of tyrosine residues in aqueous silk fibroin with HRP and hydrogen peroxide (H_20_2) (Figure 1B), as previously described[1]. The silk concentration and reactant concentration were varied to produce hydrogels with a range of matrix properties. Peptides with mono- or di-terminal tyrosine residues were incorporated into the hydrogels by enzymatic polymerization. A liquid chromatography tandem mass spectroscopy (LC-MS/MS) method was developed to quantify the crosslink density[2], fluorescent recovery after photobleaching (FRAP) was applied to assess the diffusivity of fluorescent particles through the hydrogel matrix, and rheology was used to measure the gelation kinetics and modulus of the hydrogels.

Results: In native SF hydrogels, LC-MS/MS analysis revealed that 28 to 56% of tyrosine residues participate in the crosslinking reaction, depending on the reaction conditions (Figure 1A), and rheological analysis showed that the modulus ranged from 100 to 150 Pa. FRAP results indicated that gels with higher crosslink density had lower diffusivity, and the mobile fraction for 40 kDa particles was between 70 and 80%. A range of peptides with terminal tyrosine groups modified with tyramine were successfully incorporated into silk hydrogels through enzymatic polymerization, both throughout the bulk and on the surface (depending on the synthesis procedure). The molecular weight, hydrophobicity, and charge of the ligands were varied, and the reaction efficiency and distribution were dependent on these variables. The addition of difunctional peptides to SF hydrogel resulted in increased crosslinking density and modulus (Figure 1C).

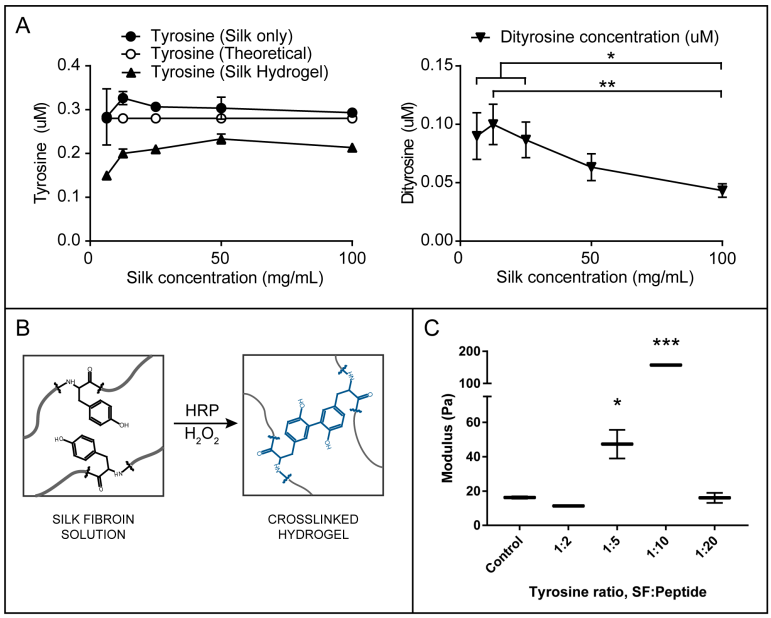

Figure 1: (A) Concentration of free tyrosine (left) and dityrosine crosslinks (right) by LC-MS/MS in hydrogels fabricated with different concentrations of silk. (B) Schematic of crosslinking reaction between tyrosine residues in silk via HRP/H_2O_2 radical polymerization. (C) Modulus of silk hydrogels with an increasing concentration of peptide, a tyrosine-terminal chain extender.

Conclusions: Characterization of silk hydrogels with rheology, LC-MS/MS, and FRAP resulted in a comprehensive understanding of the matrix properties, at both the molecular scale and macroscale, enabling gels to be designed with specific properties. Importantly, LC-MS/MS analysis revealed that a significant number of tyrosine residues remain unreacted after hydrogel synthesis, supporting the availability of these residues for further modification with functional ligands. Incorporation of difunctional peptides resulted in increased modulus, revealing that a higher crosslinking density can be achieved in modified gels than in native gels, with the peptides acting as chain extenders. This establishes the opportunity to design hydrogels with a greater range of mechanical properties, and to incorporate labile or cell-responsive conduits into the hydrogel matrix. Here we demonstrate that enzymatic polymerization can be used to incorporate a range of peptide ligands into SF hydrogels, ultimately expanding our potential to design advanced, silk-based biomaterials.

References:

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