Manipulation of Mechanical Properties in Protein-Polysaccharide Hydrogels

Marina Slawinski (PI: Ionel Popa)

Department of Physics, University of Wisconsin-Milwaukee, 3135 North Maryland Ave., Milwaukee, Wisconsin 53211, United States

Abstract

The ability to fine-tune the physical structure and mechanical properties of biomaterials is desirable for applications in tissue engineering as well as the design of cell scaffolds and smart materials. The porous and highly absorbent network of hydrogel materials offers an environment similar to the extracellular matrix (ECM), capable of supporting cell growth, while specifically tailored material properties can alter the physical form and mechanical behavior of the substrate. Here, we synthesize protein-based hydrogels using a photoactivated reaction to covalently cross-link bovine serum albumin (BSA). The addition of the polysaccharide, alginate during synthesis allows for the formation of a protein-polysaccharide hydrogel, with a primary network of covalent cross-links between BSA molecules and a secondary network obtained through the ionic interaction of alginate chains with calcium cations. In calcium chloride solution, alginate chains associate with calcium cations to form an insoluble gel within the covalent protein network, significantly increasing the stiffness of the material. When exposed to specific chelating agents, the calcium ions dissociate and the alginate chains revert to their water-soluble form, altering the physical structure of the protein-polysaccharide hydrogel. Characterization of mechanical response through force-clamp rheometry revealed a marked decrease in stiffness and strength as a result of the removal of the secondary network. This method of hydrogel synthesis demonstrates the ability to manipulate mechanical properties based on changes in aqueous environment and explores a controlled mechanism of biomaterial degradation.

Methods

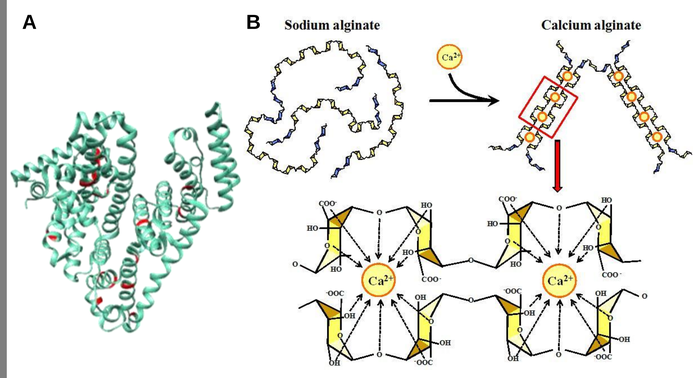

Figure 1: Protein-Polysaccharide Hydrogel Synthesis. Hydrogel samples are prepared from a solution containing bovine serum albumin (BSA) protein, the bovine analog of human serum albumin (HSA), and alginate – a polysaccharide derived from brown algae. **(A)** BSA proteins are covalently cross-linked via tyrosine residues (indicated in red) to form a primary network. The photoactivated reaction is catalyzed by the presence of ammonium persulfate (APS) and Tris(bypyridine) ruthenium(II) chloride $[Ru(bpy)3]^{2+}$. **(B)** Upon extrusion into calcium chloride solution, a secondary ionic network can form within the primary covalent network. Guluronate blocks of alginate (indicated in yellow) associate with divalent calcium cations to form an "egg-box" structure, converting alginate to its insoluble form. The time allowed for the initial formation of the primary network and for the networks to form simultaneously are carefully selected to yield hydrogels with desired mechanical properties.

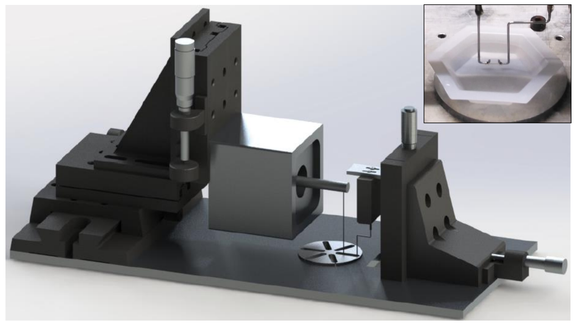

Figure 2: Instrument Setup. Rendering of the custom-made force-clamp hydrogel rheometer. Hydrogel samples are tethered to the hooks with sutures between a voice coil (left hook) and a force sensor (right hook). A PID feedback loop mediates the applied force to match parameters of the set force as hydrogel samples are subjected to tensile loading and relaxation. The applied force and percent elongation data are used to generate stress-strain curves. (Inset) A tethered 2 mM BSA hydrogel sample.

Confocal Laser Microscopy

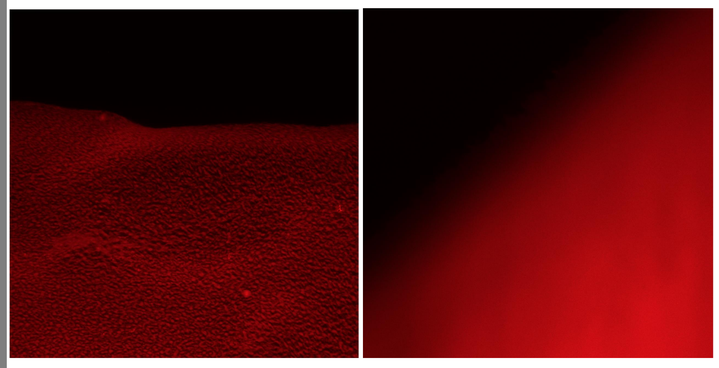

Figure 4: Confocal Laser Microscope Imaging. A 2mM BSA 2% w/v Alginate hydrogel sample was prepared as previously described (Figure 3a) and subsequently exposed to EDTA solution for 1 hour. A 2mM BSA hydrogel lacking alginate was prepared with 30 minutes of exposure to light. Both samples were then stained with 0.001 mg/mL Rhodamine B fluorescent dye overnight. Confocal laser microscope images reveal small, widely dispersed pores within the protein network of the 2mM BSA 2% w/v Alginate sample (left). These pores are not visible in images of 2mM BSA samples lacking alginate (right), indicating that the porous structure is likely left behind when the secondary calcium-alginate structure diffuses out. (Image credit: Jennifer Gutzman)

Mechanical Response Data

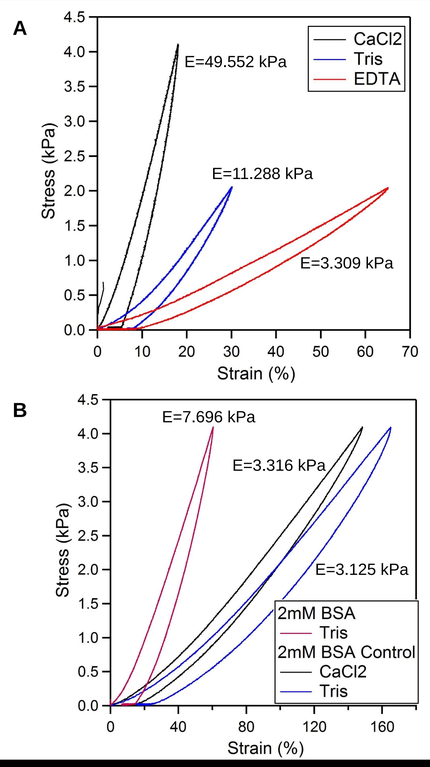

Figure 3: Stress-Strain Curves and Young's Modulus. Mechanical characterization of biomaterial samples is achieved through the analysis of stress-strain curves from force clamp rheometer measurements. Young's modulus, a measure of stiffness, is given by the slope of the linear part of the curve. **(A)** 2mM BSA 2% w/v Alginate hydrogel samples were prepared according to the following protocol: 1 minute of exposure to light followed by extrusion into CaCl2 solution and subsequent exposure to light for a total of 30 minutes to allow for simultaneous formation of both networks. The samples were subjected to force ramps in $CaCl_2$, Tris, and the chelating agent ethylenediaminetetraacetic acid (EDTA), and the respective Young's moduli were calculated. From $CaCl_2$ to Tris, there is a significant decrease in stiffness. This is likely attributed to the conversion of alginate back to its soluble form, causing the degradation of the secondary network. From Tris to EDTA, there is a smaller decrease in stiffness, likely due to the diffusion of alginate chains from the network as calcium cations are removed from the network. **(B)** 2mM BSA hydrogels lacking alginate were exposed to light for 30 minutes and measured in Tris in accordance with standard protocol. As a control, 2mM BSA hydrogels lacking alginate were also prepared in the same manner as 2mM BSA 2% w/v Alginate hydrogels previously described. The control samples were measured in $CaCl_2$ and Tris and the respective Young's Moduli were calculated, revealing no significant change between the solutions. This confirms that interactions between calcium cations and alginate chains, rather than residues of BSA, are responsible for altering the stiffness of the material. The 2mM BSA hydrogels prepared for the control have a lower Young's Modulus than those prepared without CaCl2 likely due to the early extrusion during preparation. Moreover, because the stiffness of the control samples resembles the stiffness of the protein-polysaccharide hydrogels in EDTA, it is likely that some residual alginate remains present within the network in Tris solution before exposure to EDTA, contributing to the greater mechanical properties.

Conclusions & Outlook

- Here, we synthesized dual-network protein-polysaccharide hydrogels with a primary covalent network of cross-links between BSA proteins and a secondary ionic network formed through the association of guluronate blocks of alginate with calcium cations. The secondary network can be removed in response to changes in aqueous environment, significantly altering the physical structure and mechanical properties of the material.
- Young's Modulus calculated from stress-strain curves indicate that the presence of the secondary calcium-alginate network greatly increases the stiffness and strength of the hydrogel material compared to pure BSA hydrogels of the same concentration (49.552 kPa vs. 7.696 kPa). Meanwhile, degradation of the secondary network leads to a significant decrease in mechanical properties.
- Confocal laser microscope images reveal changes in physical structure in accordance with the changes in mechanical behavior, demonstrating that degradation of the secondary network leaves behind a weakened porous structure.
- The protein-polysaccharide hydrogels are synthesized using biocompatible components, and the material properties can be manipulated in room temperature, aqueous solutions – resembling in vivo conditions. This is significant to the future of engineering biomaterials responsive to changes in their environment and to the development of smart materials for applications in soft-robotics.

Contact Information

Ionel Popa: popa@uwm.edu
Marina Slawinski: slawins6@uwm.edu