Physical Network Crosslinking of Collagen-based Bio-inks for Cartilage Bioprinting

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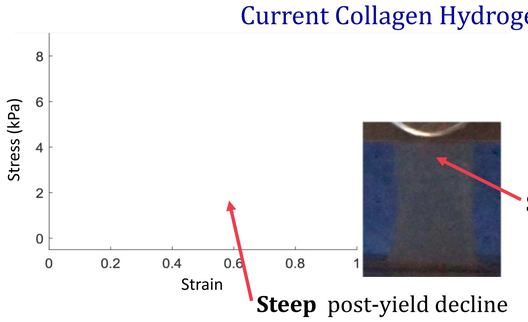
Introduction

- The application of collagen-based bioinks to 3D bioprinting is limited by their poor mechanical properties and slow gelation.
- Collagen has been shown to significantly improve both the mechanical performance, and cytocompatibility of zwitterionic polymer hydrogels.
- Non-mammalian collagens, such as those found in mussel byssal threads, achieve exceptions toughness and extensibility through metal-ion complexation[1].
- This work builds on the potential for ionic crosslinking in hydrogel systems through two distinct strategies: zwitterionic microgel-collagen composites[2], and collagen-alginate conjugates.

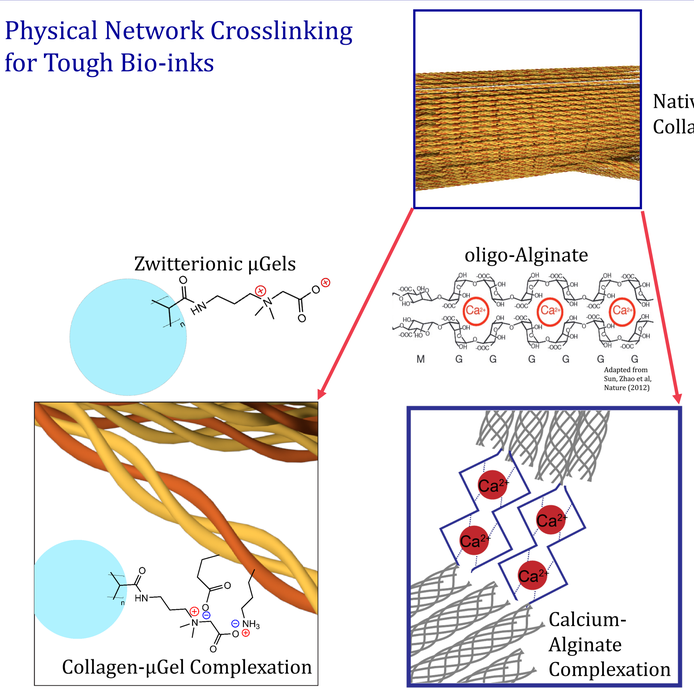
Current Collagen Hydrogels



Objective

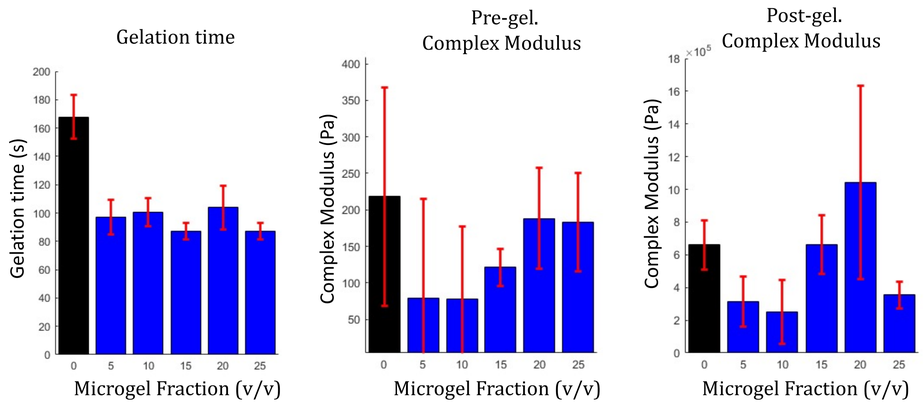
Improve the toughness of collagen hydrogels for cartilage bioprinting through physical network crosslinking

Physical Network Crosslinking for Tough Bio-inks

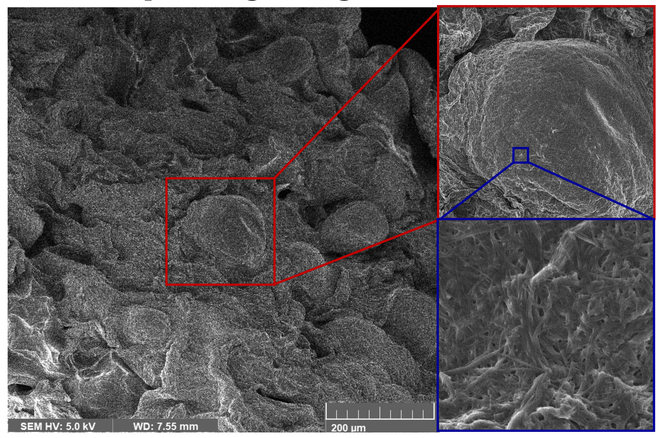


Results

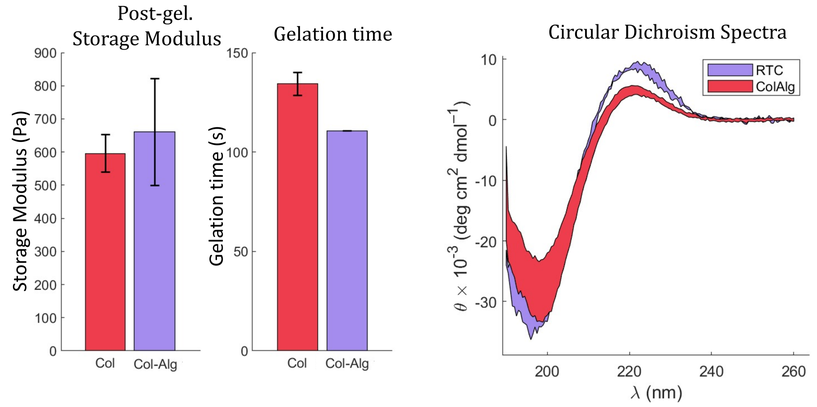
µGel-Collagen composites have **decreased gelation time** and **increased modulus**



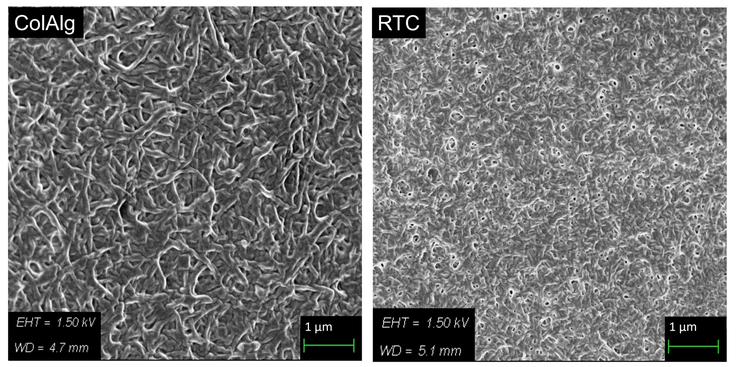
Robust collagen **fibrillar architecture**, with collagen fibrils **encapsulating microgels**.



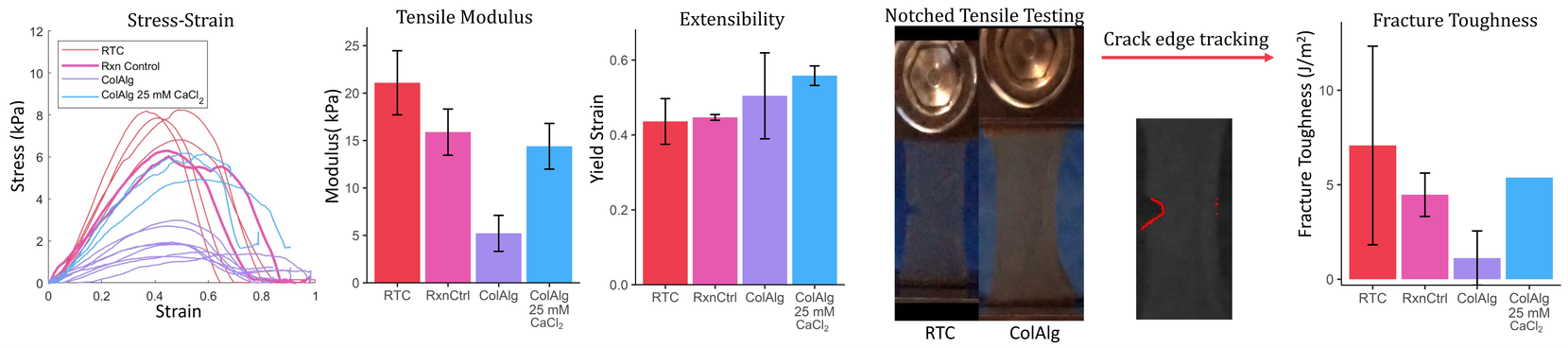
Collagen retains **fibrillar and helical structure** and **thermal gelation** following alginate conjugation



Scanning electron microscopy confirms preserved fibrillogenesis



Collagen-alginate gels exhibit **calcium dependent** increases in **modulus and toughness**



Discussion & Conclusions

- Collagen-µGel complexation led to decreased gelation time, while increasing post-gelation mechanics compared to collagen gels.
- Collagen fibrillar architecture was preserved in µGel composites, with close collagen-µGel interaction likely contributing to higher post-gelation complex modulus.
- Alginate conjugation resulted in decreased gelation time, with no change in post-gelation storage modulus. Likewise, circular dichroism showed preservation of collagen helical structure.
- SEM of conjugate gels showed preservation of fibrillogenesis. This, along with rheological performance, suggests collagen helical architecture is not adversely affected by alginate conjugation.
- Alginate conjugation resulted in calcium dependent increases in tensile modulus and toughness, though not recovering fully to the level of native collagen gels. Calcium crosslinked conjugate gels also exhibited increased extensibility compared to native collagen gels.
- These findings show the potential for ionic crosslinking strategies to improve printability and mechanics of collagen hydrogels for 3D bioprinting applications. Additionally, these bio-orthogonal crosslinking strategies are not likely to mitigate the cytocompatibility of collagen bioinks.

Methods

µGel-Collagen Composites

- Zwitterion µGels were synthesized by passing poly(carboxybetaine) hydrogels through 50 um micronic steel mesh filters.[2]
- Dialyzed, lyophilized, and reconstituted µGels were mixed with rat tail tendon collagen from 5-25% (v/v)

Collagen-Alginate Conjugates

- Oligomeric alginate was conjugated to collagen through reductive alkylation n-terminus of collagen chains.
- Tensile mechanics and fracture sensitivity was determined through notched tensile testing.
- Crack edges were tracked with MATLAB.

- Pre- and post-gelation shear mechanics were analyzed via torsional shear rheology.[3]
- Nanostructure was determined by scanning electron microscopy (SEM).[3]

Significance

Physical crosslinking improves gelation kinetics and post-gelation mechanics while preserving robust fibrillar architecture in collagen hydrogels. Thus, these strategies have the potential to modulate toughness while maintaining cytocompatibility of collagen bio-inks.

References

1. Holten-Andersen et. al, *Langmuir* (2009) 2. Sinclair et. al, *Adv Mater* (2018) 3. Slyker et. al, J Biomed Mater Res (2022)

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Acknowledgments

Cornell Center for Materials Research CCMR (DMR1719875) Ruth L. Kirschstein Predoctoral Individual Fellowship (F31) NIH National Institute of Dental and Craniofacial Research