# Rheology of Hydrogels Made with Healthy and *COL3A1* Mutant Cell-Derived Matrix for vascular Ehlers-Danlos Syndrome

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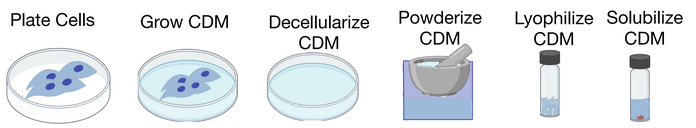
## Introduction

- Hydrogels made with animal decellularized extracellular matrix (dECM) proteins have been used in microfluidic devices to investigate and model vasculature.
- Use of human dECM is limited by the availability of human tissue for research.[1]
- Our lab has been making hydrogels using cell derived matrix (CDM) as an *in vitro* model for human ECM.[2]
- Rheology informs mechanical stability during gelation by monitoring the storage and loss moduli.[3,4]
- Rheological properties like stiffness and yield strain inform the utility of hydrogels for use within microfluidic vascular disease models for Vascular Ehlers-Danlos syndrome (vEDS), a rare disease caused by mutations in the COL3A1 gene.[3]
- Native ECM exhibits stress relaxation properties that can potentially be seen in our CDM hydrogels.

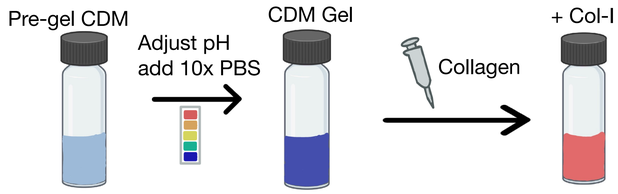
**Design and implement procedures to rheologically characterize healthy and COL3A1 mutant CDM to compare gelation & mechanical characteristics.**

## Cell Derived Matrix Hydrogels

Human dermal fibroblasts were plated and cultured to generate CDM. After a week, confluent cell cultures were decellularized to harvest CDM.[4] The CDM was then powdered, lyophilized, and solubilized for 12 hours to be used as a CDM pre-gel solution to make hydrogels.




Previous studies suggest that hydrogels from CDM without the additions are too compliant for use in microfluidic devices, and collagen can be added to stabilize gels.[2]

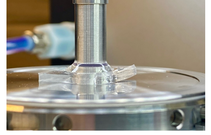


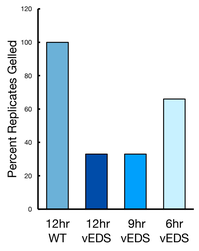

Four gel formulations were tested in our studies:

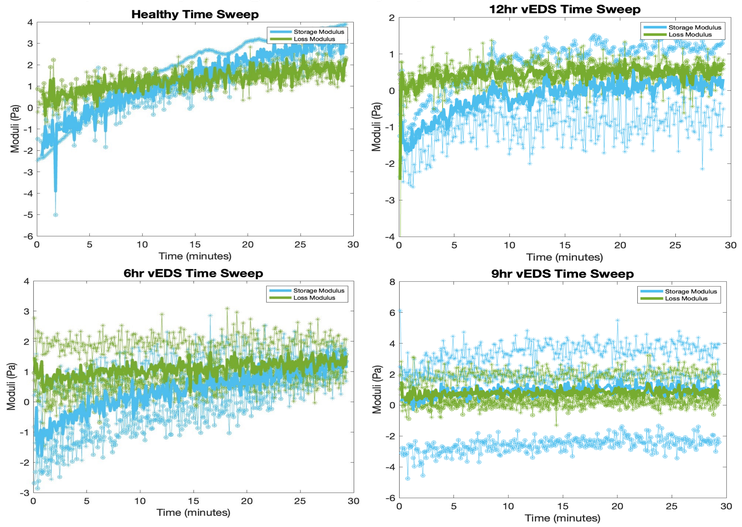
1) Healthy CDM only
2) vEDS CDM only
   - 6-, 9-, and 12-hour solubilization time
3) Healthy CDM + Collagen I (4:1 Ratio)
4) vEDS CDM + Collagen I (4:1 Ratio)

## Gelation of CDM hydrogels

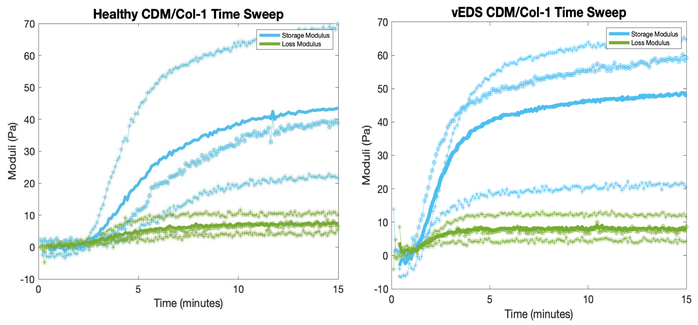
Method: Time sweep on CDM only and CDM+Col gels at 37°C, 1% strain, and 0.7HZ and 1Hz, respectively using Discovery Hybrid Rheometer-20 (DHR20).





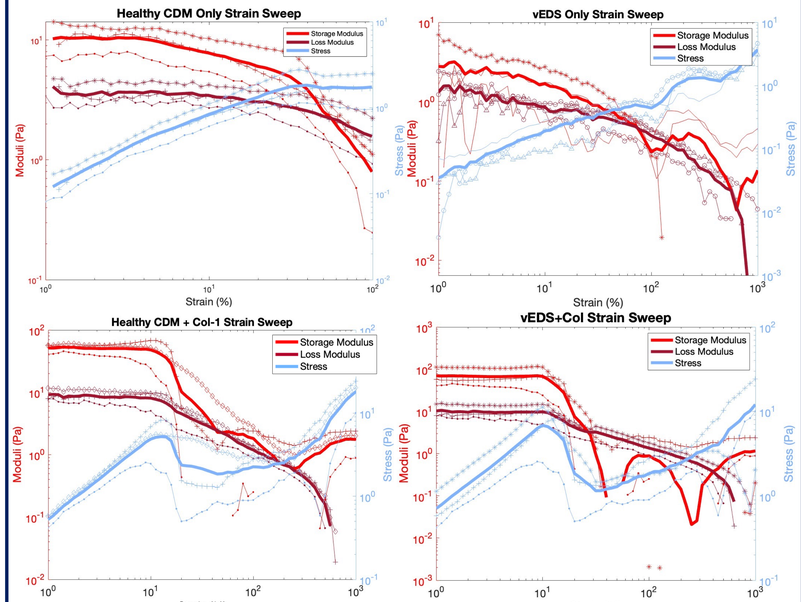

Healthy CDM only hydrogels successfully stabilized into gels. Different solubilization times were tested for vEDS CDM hydrogels, we observed a 6-hour solubilization time the most likely to gel.



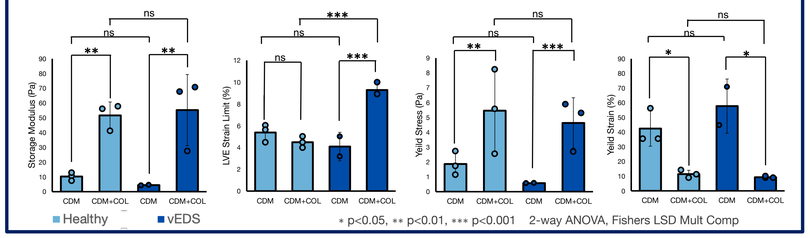

The addition of collagen to the CDM showed consistent gelation. The gels stabilized quickly and with a higher storage modulus than the gels without collagen.

## Rheological Mechanical Characterization

Method: Strain sweeps from 1%-1000% were performed on fully formed gels at 1Hz and 37°C



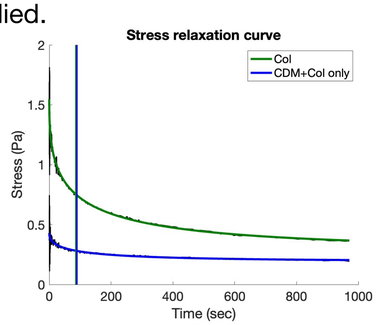

The yield strain of the CDM only gels was higher than the gels with collagen, while the yield stress was much lower. There was not a significant difference between the healthy CDM and vEDS CDM hydrogels with and without collagen.




## Stress Relaxation

Evaluating viscoelastic properties of CDM hydrogels can inform the biomimetic properties of gels and evaluate properties of diseased CDM. Stress relaxation evaluates viscoelasticity by measuring the stress response when a constant strain is applied.

Method: A 3% strain was applied for 1000s, and the data was fitted using a standard linear solid model derived with a finite loading rate to find the stress relaxation constant for samples.[5]




## Conclusion

- Healthy CDM only hydrogels gelled more consistently than vEDS CDM. A lower solubilization time (6-hr) increased the gelation percentage of vEDS hydrogels.
- There was not a significant difference in mechanical properties between healthy and vEDS CDM gels. The inconsistent gelation in vEDS hydrogels made mechanical properties harder to compare.
- Further tests will be completed to evaluate the stress relaxation properties of vEDS and healthy CDM hydrogels.

## References & Acknowledgements

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