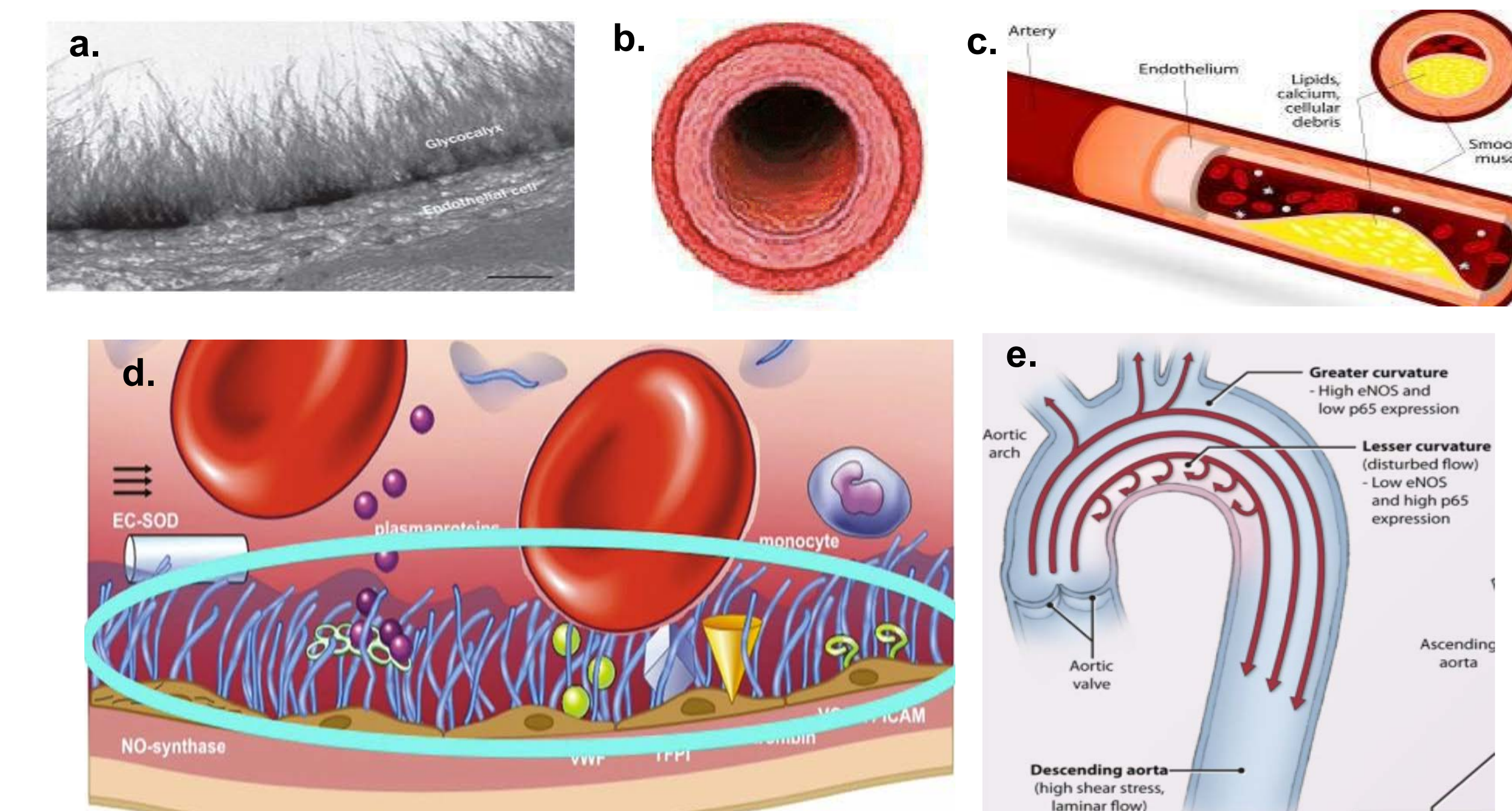


## Abstract

The planned experiments were intended to contribute to the development of synthetic surfaces (hydrogels) that mimic the real life mechanical properties of healthy or diseased blood vessel walls, particularly in hypertension. Hydrogel synthesis, mechanical testing, cell culturing, immunocytochemistry and biological microscopy were all techniques utilized to study how endothelial cells, which line blood vessel walls, adhere, proliferate, survive, and express important features on the hydrogels. We used advanced biological microscopy techniques to image the hydrogel cell units. The image processing software ImageJ or Fiji was used for data collection and analysis. We are striving to not only create hydrogels that effectively imitate healthy and diseased human blood vessels, but to also analyze and understand the role that the endothelial glycocalyx (GCX) plays in preventing cardiovascular disease, like atherosclerosis. We hope these hydrogels are used in uncovering potential treatments in heart disease.

## Background

The endothelial glycocalyx is an extracellular structure of endothelial cells composed of proteoglycans, glycoproteins, and glycosaminoglycans (GAGs). Altered stiffnesses lead to impaired glycocalyx-mediated mechanotransduction, affect endothelial health and contribute to the development of heart disease, such as atherosclerosis and hypertension [1]. Hydrogels are soft gels made of monomers, such as polyethylene glycol (PEG), and water that can vary in stiffness and composition, allowing for the representation of blood vessels at healthy or diseased states. Our goal is to fabricate gels at 2.5 kPa and 10 kPa to represent healthy and diseased conditions respectively.



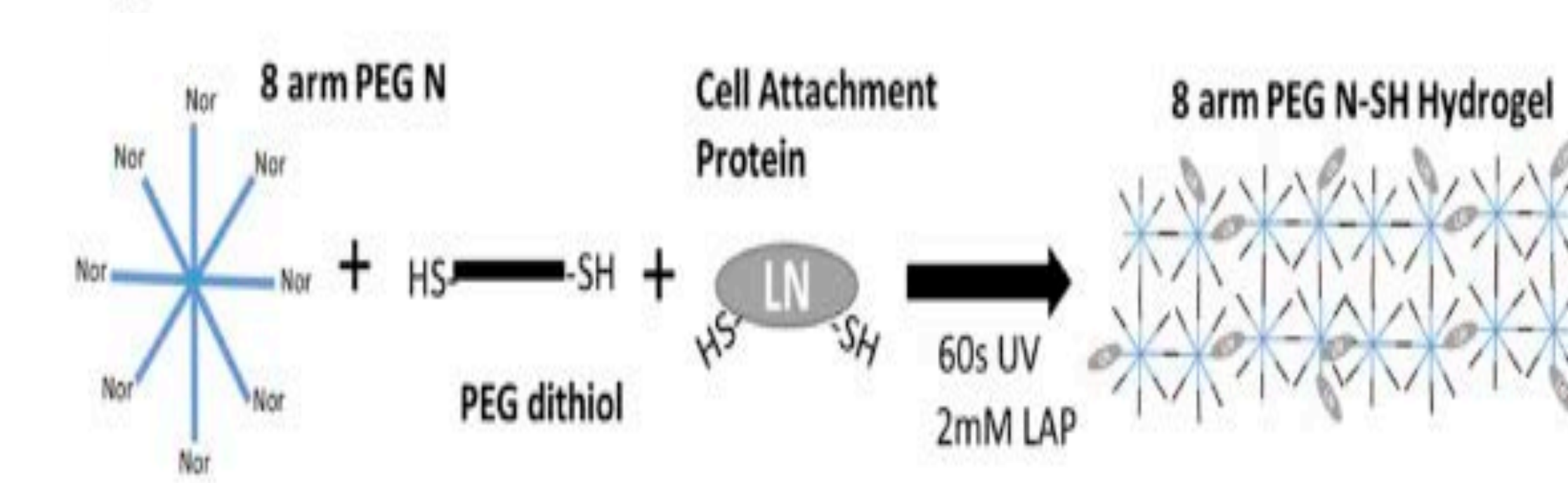
**Fig. 1:** a. The endothelial glycocalyx, the extracellular, hair-like structure found on endothelial cells from www.truemd.com. b. Cross-section of a normal, unclogged human artery from www.researchgate.net. c. Build up of plaque in atherosclerosis. Adapted from www.medicalnewstoday.com. d. The glycocalyx functions as a barrier, preventing fats and proteins from depositing in vessel walls [2]. e. When blood flows through vessels, shear stress is applied to the surface of endothelial cells, including their glycocalyx, which can affect the expression of the glycocalyx. For example, in the aortic arch, disturbed flow patterns and low shear stress can lead to atherogenesis [3].

## Objective

- 1) Create hydrogels that effectively imitate healthy and diseased human blood vessels.
- 2) Determine how the physiological relevance of hydrogel stiffness facilitates the proliferation of endothelial cells.
- 3) Have a thorough understanding of endothelial glycocalyx expression on different hydrogel surfaces.

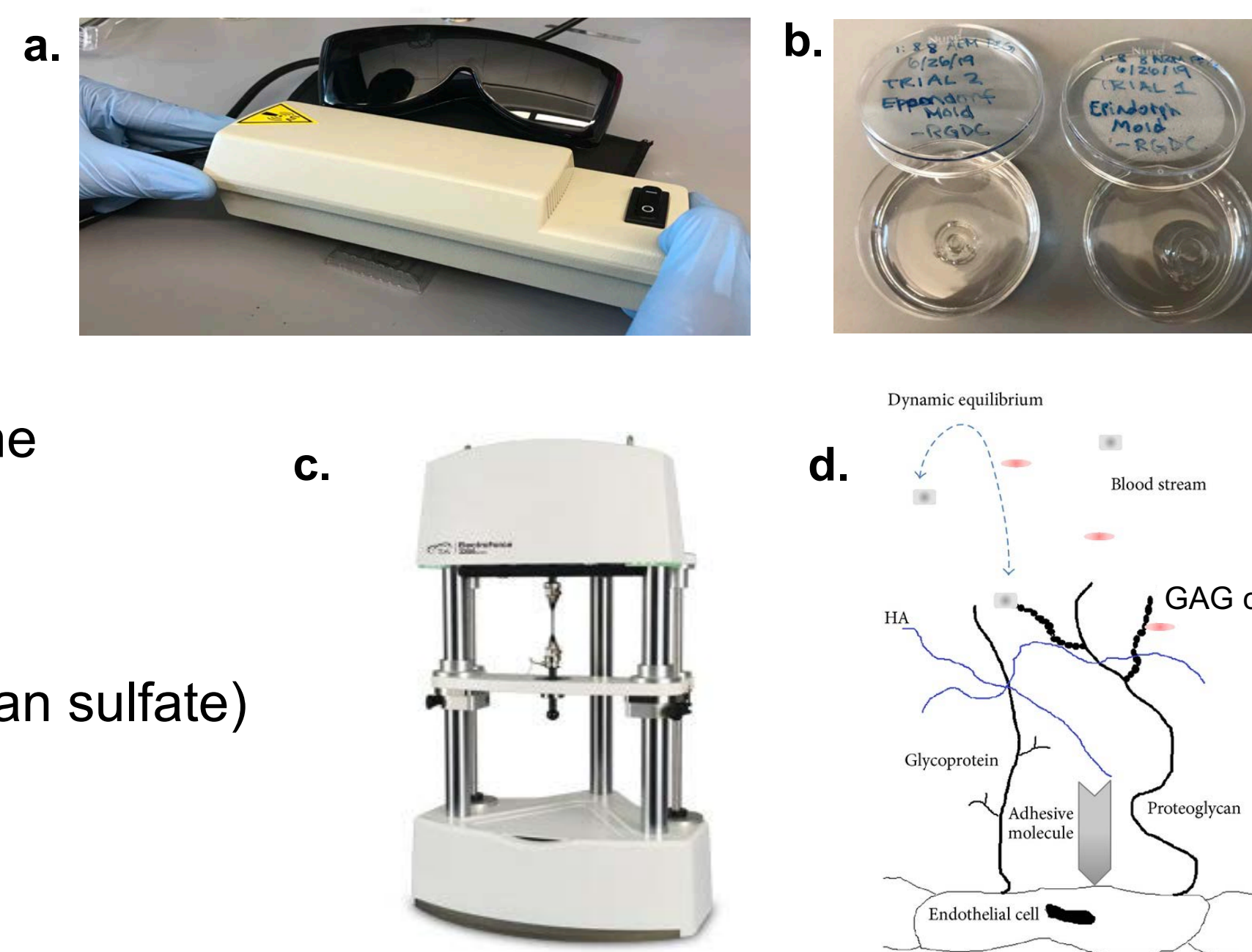
## Experimental Methods/Skills Set

### Protocol for synthesizing 8-arm PEG hydrogels



**Fig. 2:** Steps to synthesizing 8-arm PEG hydrogels. 8-arm PEG is a monomer that combines with another monomer, PEG dithiol, a crosslinker, to form a hydrogel after 60 seconds of UV and LAP photo light initiator.

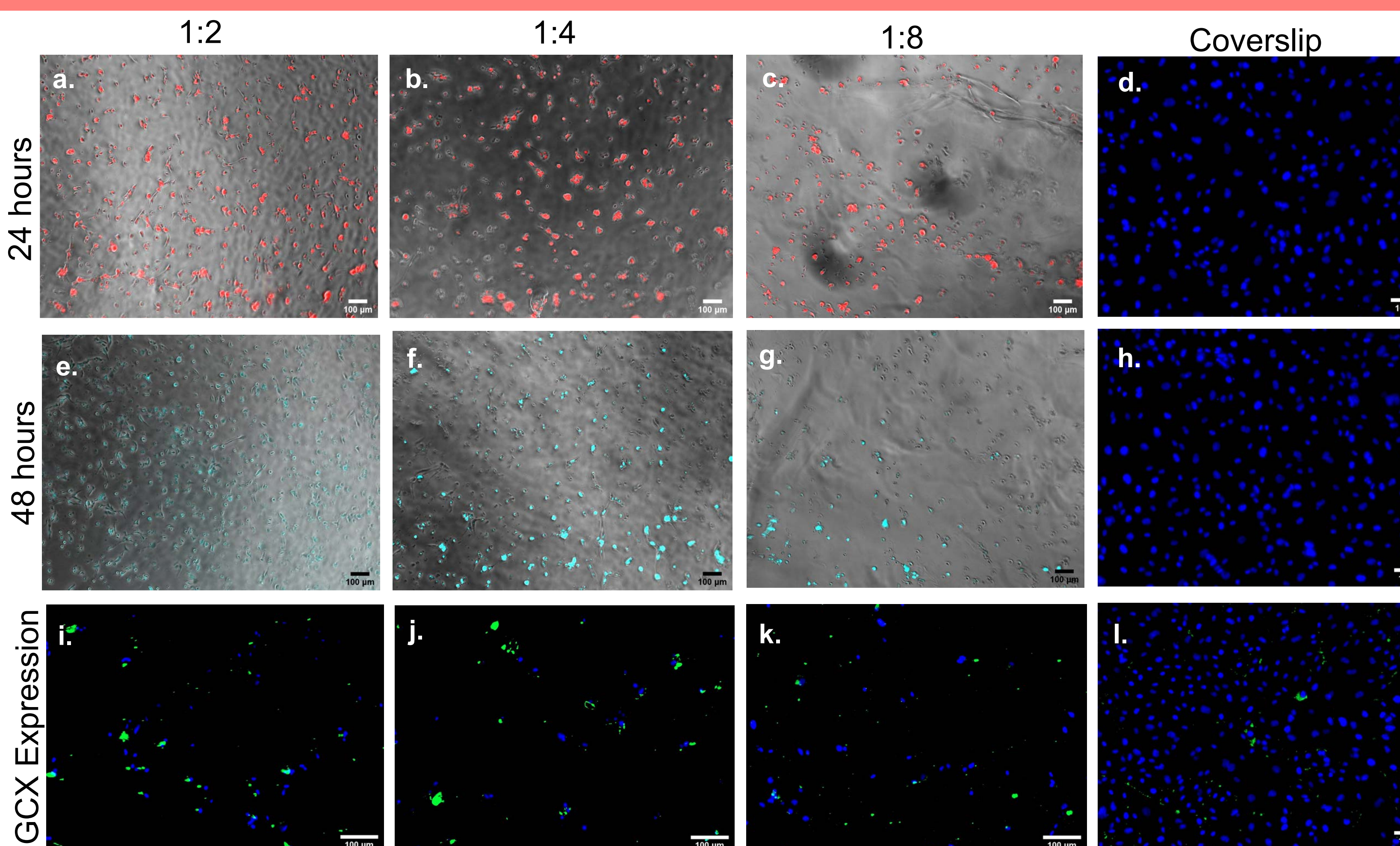
- Concentrations of 1:2, 1:4, 1:8  
 - 8-arm PEG : PEG-Dithiol ratios
- **Swelling Rates**
- **Cell culturing**  
 - Add RGDC solution for cell attachment
- **Compressive Strength Testing**  
 - Electroforce Mechanical Tester machine  
 - Compressive Moduli in kPa
- **Immunocytochemistry**  
 - HCAEC fixing  
 - GAG component staining steps (heparan sulfate)  
 - Fluorescent microscopy to image gels  
 - Cell nuclei (DAPI) labeling/staining



**Fig. 3:** a. UV light source used to create hydrogels. b. Hydrogels that exhibit swelling. c. Electroforce mechanical testing machine from www.tainstruments.com. d. Location of the GAG chain on the endothelial cell and what we were staining for from www.researchgate.net. e. Fluorescent microscope used to image cells and endothelial glycocalyx.

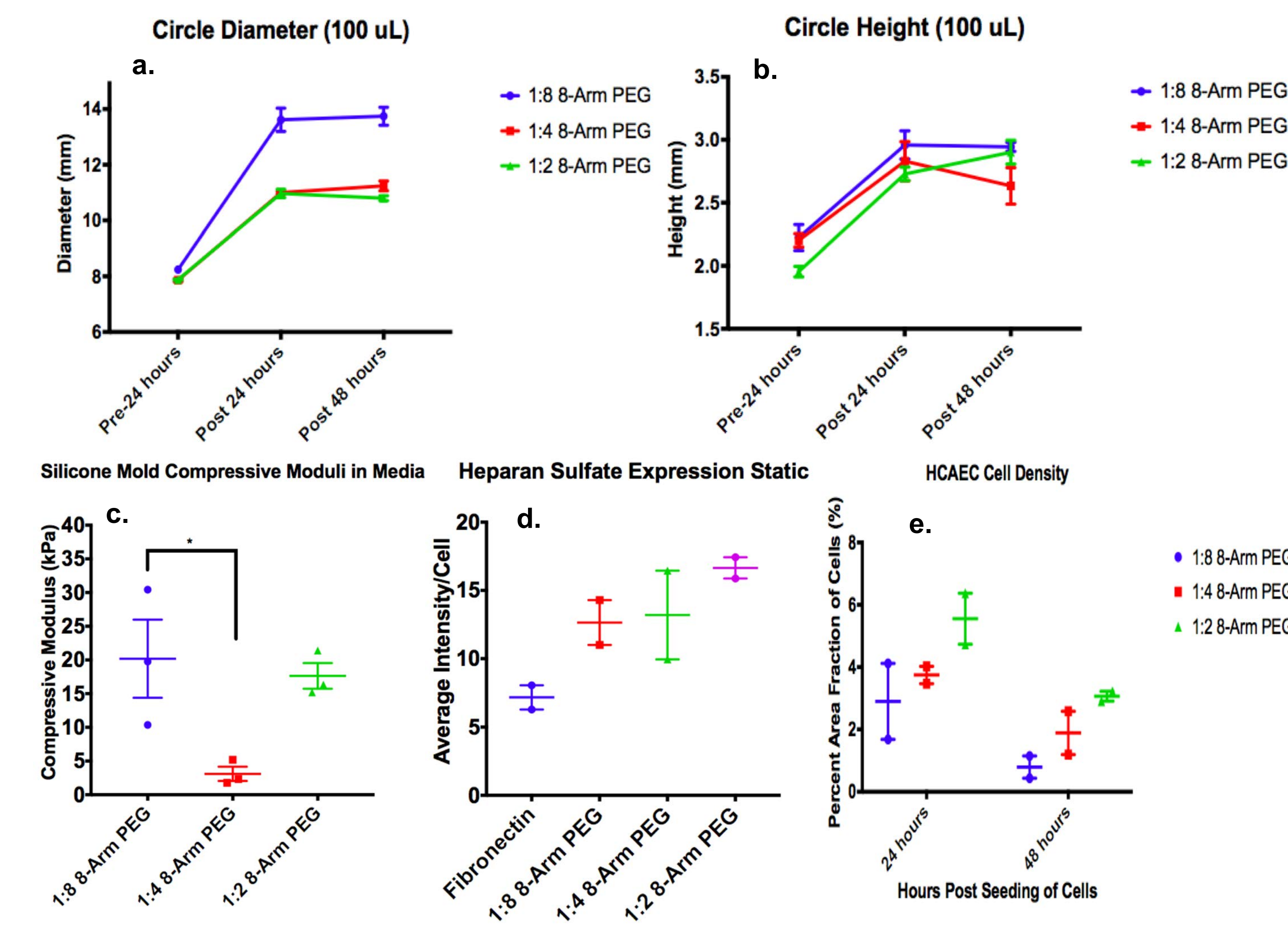
- **Data Analysis**  
 - ImageJ/Fiji  
 - Excel Spreadsheets

## Results



**Fig. 4:** Cell densities. a. Percent area fraction of cells (red) on a 1:2 8-Arm gel post 24 hours. b. 1:4 8-Arm gel post 24 hours. c. 1:8-Arm post 24 hours. d. Cells present on the glass coverslip post 24 hours. e. Percent area fraction of cells (teal) on a 1:2 8-Arm gel post 48 hours. f. 1:4 8-Arm post 48 hrs. g. 1:8 8-Arm post 48 hours. h. Cells on the coverslip post 48 hours. i. GCX expression on 1:2 8-Arm gel. j. GCX expression on 1:4 8-Arm gel. k. GCX expression on 1:8 8-Arm gel. l. GCX expression on the control cover slip with fibronectin.

## Results Continued



**Fig. 5:** a. Swelling rate of Eppendorf mold gels in terms of circle diameter. b. Swelling rate of Eppendorf mold gels in terms of circle height. c. Compressive moduli data from the mechanical testing on the silicone mold gels in cell media. d. Expression of heparan sulfate stain in each concentration of gels vs. the control of fibronectin on a cover-slip. e. Cell density expression of human coronary arterial endothelial cells on hydrogels post 24 and 48 hours.

## Conclusion and Future Steps

In conclusion, our fabricated hydrogels did not precisely reach the stiffness requirements needed to appropriately mimic a blood vessel, and the corresponding data did not corroborate our hypothesis that stiffness will decrease with decreasing PEG concentration. In addition, the mechanical testing results showed no particular stiffness pattern.

Some **future steps** that can be considered are:

- What mold to use
- Using a 4-arm PEG instead of the 8-arm to adjust the kPa stiffness
- Instead of using PEG, make Gelatin-Methacrylate (GELMA) based hydrogels which won't swell, and can help with cell density.
- Inserting these hydrogels with cell monolayer in customized parallel flow chamber
- Increase cell concentration on hydrogels

## Acknowledgments

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## References

- [1] Mitra, Ronodeep et al. "Glycocalyx in Atherosclerosis-Relevant Endothelium Function and as a Therapeutic Target." *Current atherosclerosis reports* vol. 19,12 63. 10 Nov. 2017. doi:10.1007/s11883-017-0691-9 [2] "Background." *GlycoCalyx*, www.glycocalyx.nl/background.php. [3] Cybulsky, Myron I, and Philip A Marsden. "Effect of Disturbed Blood Flow on Endothelial Cell Gene Expression." *Arteriosclerosis, Thrombosis, and Vascular Biology*, 1 Sept. 2014. www.ahajournals.org/doi/full/10.1161/atvbaha.114.304099.