Exploring the Endothelial Glycocalyx

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Abstract

Atherosclerosis is a cardiovascular disease characterized by plaque build-ups in blood vessel walls. Interestingly, atherosclerosis develops at areas characterized by disturbed blood flow and is also associated with stiffening of the blood vessel wall. Therefore, mechanotransduction, conversion of mechanical forces into biochemical signals, is believed to be a key player in the development of the disease. Researchers are studying the role of the endothelial glycocalyx (GCX), a known mechanotransducer, on atherosclerosis development. The overall goal of the research is to effectively model both healthy and diseased blood vessels to accurately study the glycocalyx in atherosclerosis development. To do so, we will use polyethylene glycol (PEG) hydrogels polymers of different variables. By creating a representation of the GCX, scientists can better understand its function. The results obtained from experimentation will allow one to accurately model the endothelial GCX.

Background Info

The endothelial glycocalyx is an extracellular structure of endothelial cells composed of proteoglycans and glycoproteins. 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. Disturbed flow, which generates altered shear stresses, can lead to atherosclerosis, the buildup of fats and other substances in vessel walls. It is believed that these altered shear stresses lead to impaired glycocalyx-mediated mechanotransduction, affecting endothelial health and subsequently contributing to atherosclerosis development.

Hydrogels are soft gels made of polymers, such as polyethylene glycol, and water that can vary in stiffness and composition. They can be used to model various biological structures, such as the membranes of blood vessels, with varying stiffnesses. This allows for the synthesis of hydrogels that represent blood vessels at healthy or diseased states.

1)

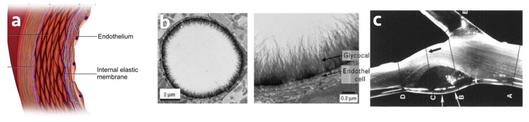


Figure 1: a. the wall of a blood vessel, made up of endothelial cells. **b.** the endothelial glycocalyx, the extracellular structure found on endothelial cells.**c.** flow patterns that can lead to the development atherosclerosis in the carotid artery.

Methods

2)

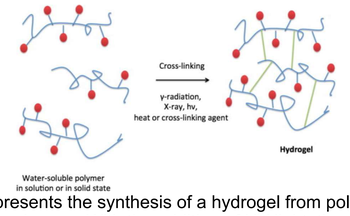
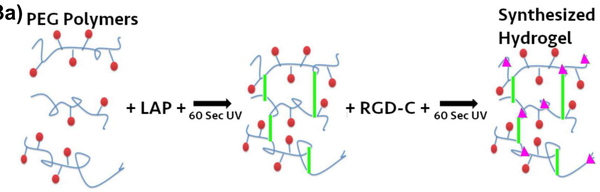


Figure 2. represents the synthesis of a hydrogel from polyethylene glycol (PEG) polymers. With the addition of UV light, the polymers combine with water to form a solid gel.

3a)



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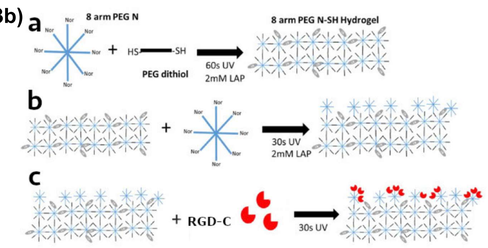
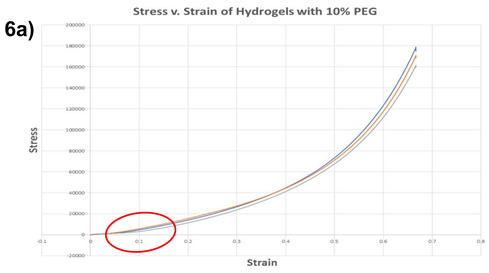


3c)

Figure 3: a. Synthesis of hydrogels using polyethylene glycol (PEG) polymers and LAP, a photoinitiator that causes cross-linking in the gels. The gels were then coated or mixed with RGD-C, a peptide that causes adhesion between the PEG polymers and cells. The result is a hydrogel that can be used to culture endothelial cells. **b.** Diagram of hydrogel synthesis using an 8-ARM PEG, rather than a regular PEG. The 8-ARM PEG has more polymers, which offers more binding sites for the RGD-C peptide. **c.** Electroforce Mechanical Tester, which can test the compressive moduli of hydrogels.

Results

6a)



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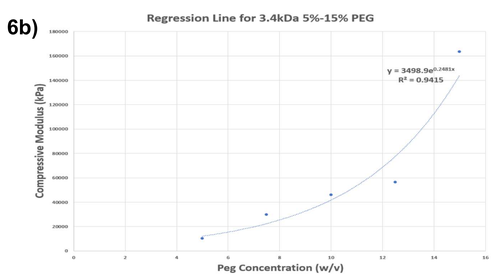

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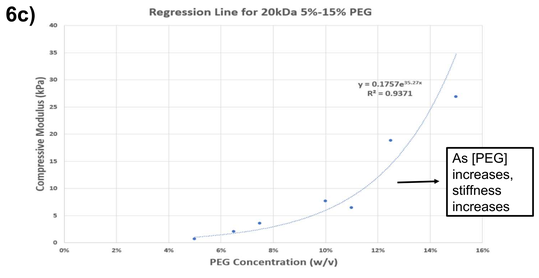

Figure 6: a. Example stress vs. strain curve of hydrogels with a 10%, 3.4 kDa PEG. The linear region of the curve can be used to calculate the compressive modulus for the specific percentage of PEG used. **b.** Regression line for the compressive moduli of 5%-15% PEG hydrogels with a molecular weight of 3.4 kDa. **c.** regression line for the compressive moduli of 5%-15% PEG hydrogels with a molecular weight of 20 kDa.

7)

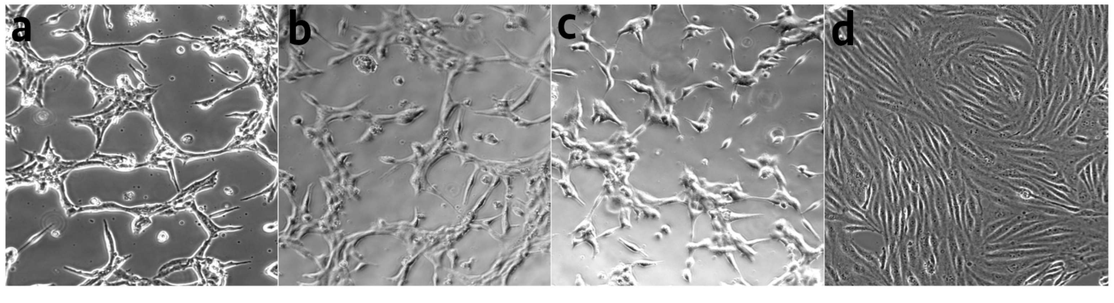

Figure 7 Human aortic endothelial cells cultured on hydrogels **a.** incubated in a collagen solution**, b.** synthesized with a Matrigel surface, **c.** synthesized with RGD-C proteins throughout the hydrogel, and **d.** synthesized with a RGD-C surface. Hydrogels in **a-c.** display good cell-cell adhesion but poor cell-substrate adhesion while hydrogel **d.** demonstrated strong cell-substrate adhesion.

8)

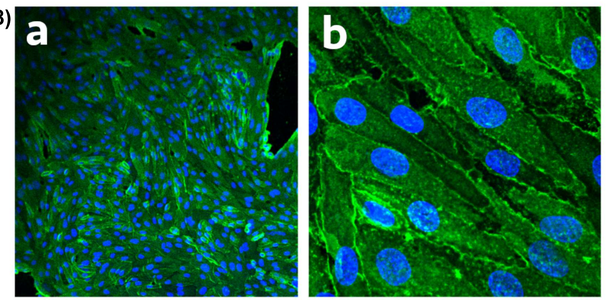

Figure 8. A **a.** 10x and **b.** 63x image taken with a Zeiss LSM 710 confocal microscope. The specimen is monolayer of human aortic endothelial cells (HAECs) cultured on a 10%, 3.4 kDa PEG hydrogel with an RGD-C surface coating. Cell **nuclei** are seen in blue (stained with DAPI), and the **glycocalyx** is seen in green (stained with wheat germ agglutinin [WGA]).

9)

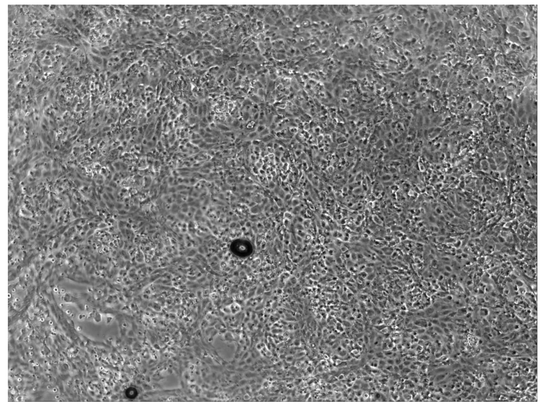

Figure 9. HAECs cultured on an 8-ARM PEG hydrogel. The cells were plated at a density of 250,000 cells/mL

Conclusions

- HAECs require sufficient binding sites to adhere to the gel surface, which promotes increased cell-substrate adhesion vs. cell-adhesion. This contributed to proper monolayer formation
- Lower MW PEG produces hydrogels with higher compressive moduli.
- Lower MW PEG gels offer more binding sites for RGD-C attachment
- Multi-arm PEG hydrogels can overcome issues related to limited binding sites

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