Developing a 3D Retinal Tissue Scaffold Using PEG-Based Hydrogels

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Background and Introduction

Growing interface between cell biology and bioengineering

There is a need for *an ex vivo 3D* model of the retina in order to better understand retinal disease. Standard *in vitro* cell culture methods are 2D; however, the cells in our body interact with each other in a 3D environment. Therefore, 2D cell culture methods do not provide an accurate representation of how cells interact *in vivo*. To better understand the dynamic and complex 3D extracellular environment in which our cells interact *in vivo*, biologists have been collaborating with material scientists and tissue engineers to design 3D tissue models using different types of natural and synthetic biomaterials. Our project aims to develop a method to create a 3D retinal tissue model using poly(ethylene) (PEG) based hydrogels, which are a synthetic biomaterial that can be fine-tuned to create a biomimetic scaffold of the target tissue.

Requirements for creating a tissue scaffold

- Biodegradable
- Bioactive/cell-adhesive
- Mimics mechanical properties of extracellular matrix (ECM) of target tissue
- Incorporation of signaling components that can promote a healing environment, such as cell-adhesion ligands, protease-sensitive domains, growth factors, and cytokines

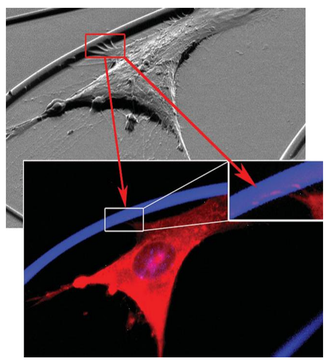

Figure 1: Image of biomimetic scaffold which favors cell attachment and growth.

Poly(ethylene glycol) (PEG) is a useful polymer to create hydrogels

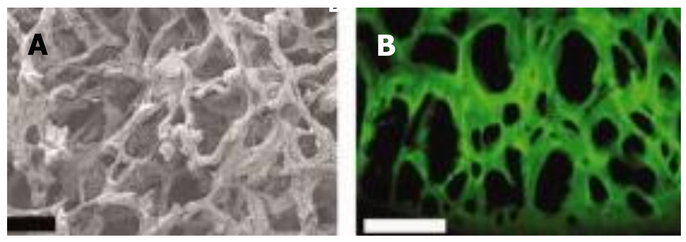

Figure 2: (a) SEM micrograph of PEG-PLL hydrogel. **(b)** 20-µm cross section of PEG-PLL hydrogel reacted with FITC to demonstrate pore structure. The scale bar is equivalent for both **(a)** and **(b)** .

Hydrogels make excellent candidates for creating tissue scaffolds because they are biodegradable, can be fine-tuned to mimic the extracellular matrix (ECM) of the target tissue, have a highly porous architecture, and can be rendered bioactive.

Research Objectives

- Develop a method to synthesize a poly (ethylene) (PEG)-based hydrogel that is fast and does not require UV light
- Incorporate poly (L-lysine) into the hydrogel to make it cell-adhesive
- Develop a technique to produce the hydrogel into the 3D, multi-layered structure of the retina

Methods

A schematic of the hydrogel formation is presented in Figures 3 and 4. PEG is an inert substance and was made cell-adhesive by incorporating poly (L-lysine) (PLL) into the hydrogel. In order to achieve this, we mixed the PLL with VS-PEG-NHS, a polymer precursor for forming the PEG hydrogel crosslinking reaction (Figure 3). The VS-PEG-NHS and the PLL were each dissolved in phosphate buffer (PBS) at 10% w/v. The two polymers were mixed at a VS/PLL 1:1 ratio. 3M NaOH was added to obtain a final pH of 10.5, the pKa of the lysine side chain. The solutions were vortexed, and then left for 2 hours to react, and subsequently lyophilized. Then the hydrogel was formed via a Michael-type addition of PEG-diester-dithiol onto the 1-arm VS-PEG-PLL polymer (Figure 4). Each polymer precursor was dissolved in a 0.3 M triethanolamine (TEA) solution of pH 8 and 20% w/v. The ratio of VS/SH was 1:1. Immediately after mixing, the solution was vortexed for 15 secs and quickly transferred to the center of a glass slide, and casted into a specific shape (Figure 5). Gelation occurred in under 30 secs at room temperature.

Preparation of Poly(Ethylene Glycol) (PEG)-Based Hydrogels

Incorporation of poly (L-lysine) (PLL)

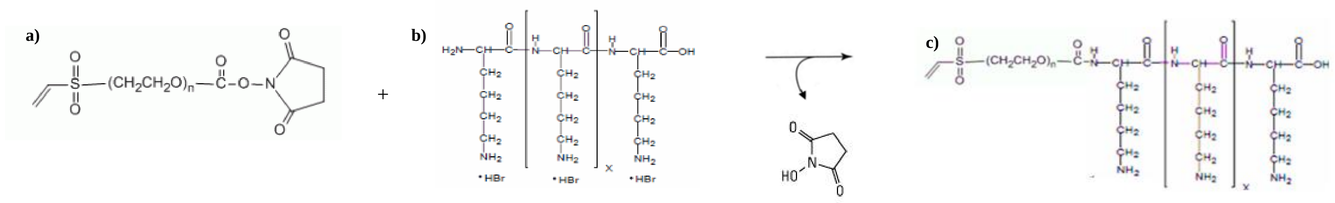

Figure 3: Reaction scheme for incorporation of PLL. **(a)** VS-PEG-NHS polymer crosslinker mixed with **(b)** PLL in pH 10.5 buffer solution, and allowed to react for 2 hours to form **(c)** VS-PEG-PLL, a precursor for the hydrogel formation.

PEG hydrogel polymerization

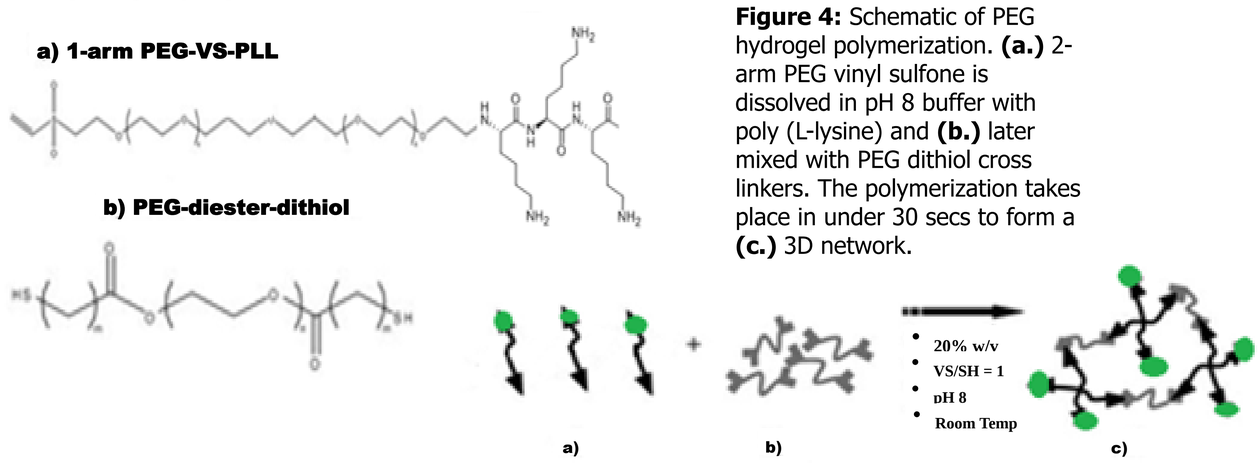

Figure 4: Schematic of PEG hydrogel polymerization. **(a.)** 2-arm PEG vinyl sulfone is dissolved in pH 8 buffer with poly (L-lysine) and **(b.)** later mixed with PEG dithiol cross linkers. The polymerization takes place in under 30 secs to form a **(c.)** 3D network.

Hydrogel Casting Technique

Figure 5: Images of PEG hydrogel casted into different shapes to demonstrate versatility of casting technique

- Casting technique allows hydrogel to be produced into virtually any 3D shape
- Provides the potential to produce the hydrogels into the multi-layered structure of the retina in future works

Future Work

- Characterize the mechanical properties of the PEG-PLL gel
- Culture retinal progenitor cells (RPCs) on the hydrogel
- Utilize the casting technique to produce the hydrogels into the multilayered structure of the retina

Conclusion

- Synthesized VS-PEG-SH hydrogel via Michael-type addition reaction in under 30 seconds without exposure to UV light
- Rendered PEG hydrogel bioactive through incorporation of PLL into the PEG-VS hydrogel precursor
- Developed casting technique to produce hydrogel into virtually any desired shape

References

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Acknowledgements

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