Biogel Release to the Ocular Surface of Epithelial Growth Factors

John Byce, Anthony Sprangers, Sarah Reichert, Alex Johnson, Jeff Hlinka

Client: Dr. Neal Barney

Advisor: Professor Chris Brace

Abstract

Dry eye results from an imbalanced tear-flow system. Current treatment options function to reduce discomfort, but are incapable of repairing or replacing the epithelial cells that have been damaged. Epidermal growth factor has been shown to promote proliferation of epithelial cells on the ocular surface. Because of this, Dr. Neal Barney asked us to develop a biogel that is capable of releasing this growth factor to the eye while it degrades over a seven to ten day period.

We constructed poly(ethylene glycol) diacrylate (PEGDA) hydrogels to accomplish this task. First, we made gels of 10, 15, 20, and 25 percent weight PEGDA and allowed them to swell to equilibrium volumes. All swelling percentages were around 200% of the initial volumes, and no statistical difference was found. We chose to use 10 weight percent PEGDA gels for convenience.

Incorporating dithiothreitol (DTT) into the gels caused them to degrade in buffer solution at rates dependent on the amount of DTT. Gel ratios of 1:2, 1:3, 1:4, and 1:5 DTT:PEGDA were tested over a 13 day time period. It was determined that gels of a 1:2 ratio degrade the fastest, in 40 days.

A live/dead assay was performed to determine if the gels are toxic to the epithelial cells of the eye. After days one and two of testing there was no statistical difference in the amount of surviving cells between the control and gel. However, after this time period both conditions became infected with a fungus and further data could not be obtained.

Introduction

Significant dry eye is an affliction that affects over ten million Americans. Patients who suffer from this disease may experience symptoms of pain, light sensitivity, itchiness, redness and blurred vision[1]. Treatments currently available work to lessen these symptoms, but they do not repair the damage done.

Our client, Dr. Neal Barney, is an ophthalmologist at the UW Hospital. He prompted us to design and fabricate a biogel that is capable of dissolving on the ocular surface while delivering growth factors over a sustained period of time. This will serve to ease inflammatory symptoms while stimulating the regrowth of epithelial cells to replace those that have been damaged.

Figure 1: Eye anatomy and tear components.
Tears consist of moisturizing water, lubricating oils, antibodies, and mucus that evenly distributes these components. Dry eye can be caused by abnormal evaporation or deficient tear production. Symptoms stem from the destruction of epithelial cells due to the resulting inflammatory responses. From: http://www.vision-and-eyes.com/articles/eye-diseases/dry-eye-syndrome.php.

Design Criteria

- Gel completely degrades on the surface of the eye in 7-10 days
- Biocompatible with the ocular surface
- Complies with FDA regulations
- Facilitates a sustained release of a growth factor for 7-10 days
- Functions properly at 32-34 °C and a pH of 7-7.5
- Initial dimensions: 2x5x1 mm

Ergonomics

- Cannot cause physical discomfort while in use
- Cannot impede vision
- Easy and quick to apply with minimal training
- Minimal maintenance required after application
- Equally or more convenient than current treatment methods

Testing

Swelling

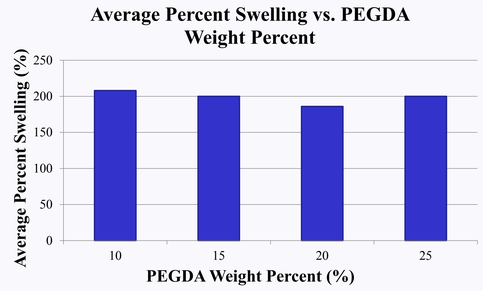

Chart 1: Swelling Results.
There is no statistical variance in swelling based on weight percent, so 10% PEGDA was chosen for future testing to minimize the amount of materials needed.

We made gels of 10, 15, 20, and 25 weight percent PEGDA. Three gels of each weight percent were submerged in buffer solutions for approximately 48 hours to allow for equilibration. Gel thickness, diameter, and wet mass were measured before and after equilibration. Change in volume for each hydrogel was calculated to determine the extent of swelling at each weight percent. An ANOVA analysis was conducted and returned a p-value of 0.73683, indicating that any variance present in the data was statistically irrelevant.

Degradation

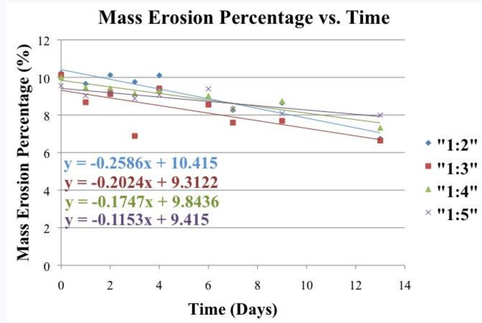

Chart 2: Degradation results.
The 1:2 hydrogel degrades the fastest. By extrapolation this gel is expected to fully degrade in 40 days. A gel with more DTT must be used to meet the design criteria.

The rate of degradation is dependent on the amount of dithiothreitol (DTT) incorporated into the gel. Including more DTT increases the the amount of bonds that are readily degradable in a physiologic environment. To determine the amount of DTT needed, we constructed gels with DTT:PEG ratios of 1:2, 1:3, 1:4, and 1:5, and observed their degradations over 13 days. Degradation, quantified as the percentage of dry mass to wet mass, was determined for each condition after 0, 1, 2, 3, 4, 6, 7, 9, and 13 days.

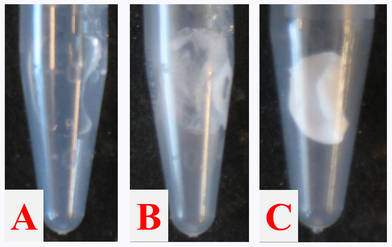

Figure 2: Freeze dry process.
A. Gel immediately after removal from buffer solution.
B. Gel after freezing for 10 minutes.
C. Gel after being in vacuum dryer for about 24 hours.

Cytotoxicity

To determine whether or not the gels will be toxic over the intended length of use, we performed tests on cell cultures at the UW Hospital. We grew cells in four wells with just the protein media necessary for them to survive, and in four other wells with the media and 1:2 DTT:PEG gels. After 1, 2, 5, and 7 days we removed the cells from one well of each condition, stained them, and counted 100 cells. Tallies were kept for the number of alive and dead out of the 100, and the results of the gel wells were compared to the controls to determine if the gels yielded cytotoxicity.

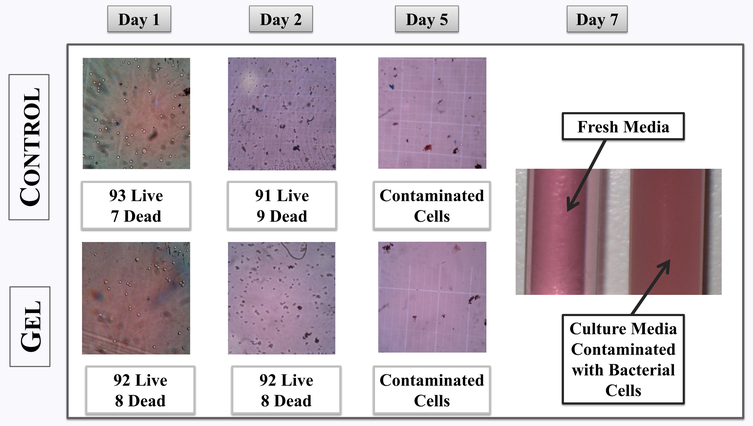

Figure 5: Live/Dead assay.
Toxicity testing was conducted over a 7 day period on cultured conjunctiva cells. No significant difference in the ratio of live to dead cells was observed between the conditions for the first two days. Further toxicity testing could not be performed due to a fungal and bacteria infection that killed all of the cells for both conditions.

Final Design

Figure 3: Fabricated PEG hydrogel.
Size of a 100 µL aliquot prior to swelling and degradation relative to the end of a pen.

Poly(ethylene glycol) (PEG) is a synthetic polyether. We chose to use it for our final design because it is amphiphilic, meaning it can be dissolved by both water and organic solvents[2]. The PEG was reacted with a derivative of acrylic acid to form acrylate-terminated chains. When these chains were exposed to ultraviolet radiation they created cross-linkages to form a gel that is stable in a physiologic environment. However, incorporation of the thiol dithiothreitol (DTT) prior to radiation forms biodegradable bonds. Therefore varying the ratio of PEG to DTT allows for a mechanism to control the rate of degradation[3].

Future Work

- Physiologic conditions
- Diffusion testing
  - Model protein
  - Radiolabeling
  - Fluorescence
- Drug delivery vehicle
- Clinical testing

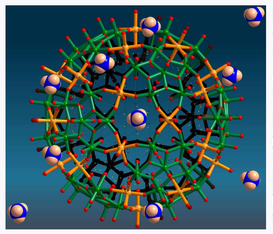

Figure 5: Drug delivery vehicle representation.
The growth factors will be encapsulated within microspheres that will be incorporated into the gel to promote a linear rate of release to the eye.
From: http://www.uni-bielefeld.de/chemie/ac1/AMU/magicspheres.htm

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2. Poly(ethylene glycol) and poly(ethylene oxide). (2010). Retrieved April 26, 2010, from http://www.sigmaaldrich.com/materials-science/material-science-products.html?TablePage=20204110
3. Hudalla, G. A., Eng, T. S., & Murphy, W. L. (2008). An approach to modulate degradation and mesenchymal stem cell behavior in poly(ethylene glycol) networks. *Biomacromolecules, 9*(3), 842-849.