Biogel Release to the Ocular Surface of Epithelial Growth Factors

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Abstract

Dry eye is an affliction that results from an imbalance in the tear-flow system of the eye. Although there are treatment options currently available, they mainly function to reduce discomfort and are incapable of repairing epithelial cell damage. Therefore our client, Dr. Neal Barney, proposed that we design and fabricate a new treatment option that involves the extended release of growth factors from a biogel. Through research, we discovered three gels that could potentially be used for this application; poly(ethylene glycol) hydrogels, collagen shields, and poloxamer hydrogels. Based on the results of our design matrix, we chose to pursue the poly(ethylene glycol) hydrogel for our final design. After creating this gel, we performed various tests to determine the amount of swelling, the rate of degradation, and cytotoxicty to a cell culture. The results were that it swells to 200% its initial volume, degrades in 40 days at 20 °C, and does not cause cell death over two days of use (further testing of toxicity was unable to be obtained due to an infection of the cells).

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Background

Keratoconjunctivitis, commonly known as dry eye, is a disorder of the tear film caused by abnormal evaporation or deficient production of tears on the ocular surface. This condition results in the damaging of the corneal epithelium as well as symptoms of discomfort[1]. Dry eye disease can be divided into two major categories, Aqueous Deficient Dry Eye and Evaporative Dry Eye.

Aqueous Deficient Dry Eye (ADDE) refers to symptoms onset by insufficient production of lacrimal tears. ADDE can also result from a lack of water secretion by the conjunctiva, the membrane that lines the eyelids. Two subclasses of ADDE, Sjögren Syndrome and Non-Sjögren Syndrome Dry Eye, underline the causative mechanisms responsible for the insufficient tear production[2]. Sjögren Syndrome is defined as an autoimmune disease causing white blood cells to attack vital moisture-producing glands, such as the lacrimal and salivary glands. This autoimmune response is further classified into primary and secondary Sjögren Syndrome. Primary refers to the presence of a sole autoimmune disease affecting the tear film production, whereas secondary refers to the coupling of primary Sjögren Syndrome with another autoimmune disease[3]. Non-Sjögren Syndrome Dry Eye is insufficient tear production in the absence of an autoimmune disease. This classification of dry eye is predominantly a result of age-related dry eye, in which the function of the lacrimal gland is dissipated by old age. Less common forms of Non-Sjögren Syndrome Dry Eye are a result of systemic drug use, reflex hyposecretion resulting from sensory or motor block, and scarring over wounds on the conjunctiva[2]. All of the former conditions result in disruption of lacrimal gland production, and produce symptoms of ADDE.

The second major classification of dry eye, Evaporative Dry Eye (EDE), is the onset of dry eye symptoms due to abnormal evaporation of water from the exposed ocular surface[2]. EDE can be further divided into two subclasses. The first, intrinsic EDE, is the obstruction of the regulatory system of evaporation. This includes Meibomian Gland Disease, meager lid congruity and dynamics, low blink rate, and the use of systematic retinoids[2]. Extrinsic EDE, the second subclass, is a result of pathological effects on the ocular surface that increase evaporation. Included in this subclass are contact lens wear, ocular surface diseases, and vitamin A deficiency[2].

Both EDE and ADDE result in the disruption of proper function in the lacrimal functional unit. Disruption of the lacrimal functional unit induces tear hyperosmolarity and tear film instability on the ocular surface. Tear hyperosmolarity is an abnormal concentration of electrolytes and proteins in the ocular tear film, resulting from excessive evaporation. Hyperosmolarity promotes inflammatory events and the release of inflammatory mediators, which can be damaging to epithelial cells on the ocular surface. Damaged and destroyed epithelial cells, in turn, cause tear film instability. Tear film instability then induces greater tear hyperosmolarity[2]. This heightened tear film hyperosmolarity will propagate to even greater hyperosmolarity through the previously described repetitive cycle. Dry eye symptoms of irritation and discomfort result from epithelial cell injury that stimulates nerve endings in the cornea[2]. Symptoms heightened by this cyclic nature cause dry eye disease to result in extreme irritation of the ocular surface. As the initial causative mechanism of tear film instability and tear hyperosmolarity can range from autoimmune deficiencies to contact lens wear, dry eye symptoms can vary greatly in severity.

Current Treatments

Treatments available today for dry eye disease range from simple eye drops to surgical procedures. They can be divided into categories based on method of application. These include tear supplementation, tear retention, tear stimulation, biological tear substitutes, and anti-inflammatory therapy. Many of these treatments are used in synergy because none of them provide relief for all the symptoms associated with chronic dry eye syndrome.

Tear Supplementation

This type of treatment is known as artificial tears, which do not have exactly the same composition as natural tears. Drops, such as TheraTears®, are also known as lubricants and can be bought over-the-counter[4]. Several factors go into the production of lubricants such as electrolyte composition, osmolarity, viscosity agents, and the presence or absence of preservatives. The most common electrolytes, potassium and bicarbonate, help maintain corneal thickness and normal epithelial ultrastructure. People with dry eye have a higher tear film osmolarity, so it is important that this is regulated in the drops. The purpose of viscosity agents in artificial tears is to lengthen the time of application. Preservatives increase the shelf life of artificial tears by preventing the development of infectious materials. Lubricants that have preservatives, such as benzalkonium chloride (BAK), should be avoided as they damage the corneal and conjunctival epithelium[5].

Tear Retention

The two most common treatments within the tear retention category are punctal occlusion and the use of contact lenses. Punctal occlusion involves inserting a dumbbell-shaped silicone plug into the opening of the lacrimal punctum, the entrance to the drainage channel that empties

into the nose. This method aids in retarding tear clearance, improving corneal staining, and decreasing tear osmolarity. Punctal plugs, however, may cause adverse effects by being installed improperly or coming out of place. Both of these factors can lead to infection within the eye. Specialized contact lenses help protect and hydrate the corneal surface in patients with severe dry eye. These lenses are made of silicone and rubber, or are gas-permeable sclera-bearing hard contact lenses. Healing of corneal epithelial abnormalities and improved vision and comfort has been reported in patients who suffer from dry eye and use these specialized contact lenses. Nevertheless, as with regular contact lenses, there is a possibility for infection if they are not properly sterilized before use[5].

Tear Stimulation

Dry eye can also be treated through the use of secretagogues, which are chemicals that promote secretion of mucus, an important component of natural tears. Diquafosol is the most researched topical pharmacological agent and has been shown to be capable of stimulating aqueous and mucous secretions in animals and humans. Specifically, diquafosol has been proven to stimulate mucus release from goblet cells, which are glandular epithelial cells that primarily function to secrete mucus[5].

Biological Tear Substitutes

The two biological tear substitutes used in the treatment of dry eye are serum and salivary gland autotransplantations. Serum is the component of blood that remains after clotting and contains growth factors. While the specific growth factor may not target dry eye, concentrations of 20 to 100% of serum have been used as biological tear substitutes. Salivary gland autotransplantation is a surgical procedure that involves a graft of the salivary gland to replace a

deficiency in mucus. This procedure is only used as a last resort in patients with severe dry eye because it can potentially lead to epithelial defects due to the hypoosmolarity of saliva[5].

Anti-Inflammatory Therapy

Cyclosporine and corticosteroids are used in anti-inflammatory therapy. Cyclosporine was initially recognized in dogs that developed spontaneous Keratoconjunctivitis; a clinical trial showed a 200% increase in conjunctival goblet cell density in treated eyes. Cyclosporine is a component of Restasis®, the only FDA approved medication used for the treatment of chronic dry eye. Restasis® has been reported to help patients produce more natural tears that lubricate the ocular surface longer than regular tears[4]. Corticosteroids also have been demonstrated to improve symptoms associated with dry eye. Prescription drugs containing corticosteroids, such as Lotemax®, are available but long-term use is discouraged because it can lead to in an increase in eye pressure and the development of a cataract[4].

Problem Statement and Motivation

Tears are a necessary component of the ocular surface. They consist of water for moisture, oils for lubrication, mucus to ensure an even spreading of these components, and antibodies that help resist infection along with specialized proteins. If an adequate supply of tears is not present, dry eye may result. Significant dry eye is an affliction that affects over ten million people in the United States alone. Patients who suffer from this medical condition often experience symptoms such as pain, light sensitivity, a gritty sensation, a feeling of a foreign body or sand in the eye, itching, redness, and blurring of vision[6]. Although there are treatment options currently available, they mainly work to lessen the symptoms and prevent further damage from happening. They do not help treat the causes of dry eye or repair the damage that has already

been incurred. Our client, Dr. Neal Barney, has therefore proposed that we design and fabricate a dissolving biogel that is capable of sustained release of epidermal growth factor. This growth factor will work to maintain healthy epithelium and restore the ocular surface tissue that has been previously damaged.

Design Requirements

The design requirements governing this project are outlined in the Project Design Specifications in the appendix, and explained in detail here. As with any device intended for medical use on human subjects, the design requirements are strict and precise. Deviation from the design requirements may result in harm to the patient. Therefore, the first requirement for our design is safety. This device must not be harmful to the ocular surface of its users. All materials must be biocompatible, with no disruption to the physiological and biological function of the eye, and must comply with the standards of the Food and Drug Administration.

Along with essential safety requirements, our design must meet a set of performance requirements. The device must be biodegradable on the ocular surface of the human eye, as it is intended for one time use. This device must also be capable of a sustained release of growth factor for nearly its entire degradation range of one to two weeks. The necessary release rate of growth factor is currently unknown, and will require clinical trials to determine what dosage is the most effective for epithelial cell renewal on the cornea.

Accuracy and reliability are also of great importance in governing our final product. Our hydrogel must be exact and consistent in both its degradation and growth factor release rate. This means it must completely degrade within one day of the specified degradation period. As growth factors stimulate cell proliferation, failure to precisely control release rate could be extremely harmful to the patient as it could result in cancer. The controlled release and degradation must

function with consistency from patient to patient. Failure to do so may result in a buildup of hydrogel on the ocular surface or premature degradation of the hydrogel.

The final design of our product is expected to have a similar shelf life to comparable products. This includes proper function of the gel while being stored in a room temperature environment for up to 24 months. Once applied to the ocular surface, the device should have an optimal life in service of seven to fourteen days. This guideline is more for ease of use than function, and alterations from it are acceptable but not ideal.

Ergonomics is a major factor regulating the ability for our design to compete in the current market. As a large percentage of users may be elderly, the device must be easy to administer and require minimal to no maintenance. This includes removal from packaging, insertion between the lower eyelid and conjunctiva, and the lack of having to adjust or reinsert the gel after initial application. Our design must also be comfortable while in use and not impede any aspect of the patient's vision. Failure to be ergonomically sound will result in a treatment method that cannot compete with over-the-counter eye drops, and other current dry eye treatments.

The product design must be made to function on the ocular surface of a human patient. A typical ocular surface contains lacrimal fluid with a pH range from 7 to 7.5. The normal temperature range of the eye is 32 to 34 °C.

The final category in guiding our design construction is physical dimensions and appearance. The hydrogel is expected to cover a 2x5 mm surface area, with a density and thickness that may vary as needed. The weight of the hydrogel must be as small as functionally possible in order to be comfortable and not physically straining on the patient. The device must not have a distracting appearance, and should not be noticeable when placed on the eye.

Design Alternatives

Hydrogels are gels that have water as their liquid component, and as such are common in biomedical applications. Incorporating various entities into the hydrogel can influence the behavior of cells within the body. More specifically, factors such as cell adhesion ligands and soluble growth factors can be diffused into the gel network and released upon the degradation of the hydrogel in a physiological environment[7].

Poly(ethylene glycol) Hydrogel

Poly(ethylene glycol) (PEG) hydrogels have the potential to be highly effective in three dimensional cell culture applications because they are easy to control their degradation rates and they are relatively simplistic. In order to form these hydrogels, excess amounts of PEG-diacrylate chains are reacted with the thiol dithiothreitol (DTT) in a phosphate-buffered saline (PBS) solution. This reaction yields PEG polymer chains that terminate with acrylate molecules. The terminating acrylates are capable of being

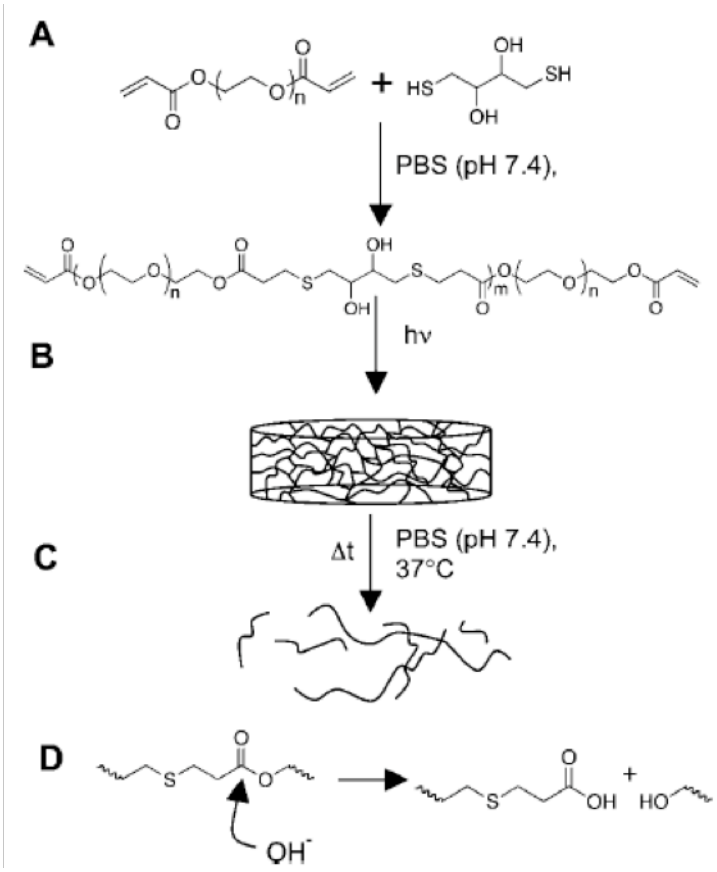

Figure 1: PEG Formation and Degradation[7]
(A) Addition reaction between poly(ethylene glycol) diacrylate and dithiothreitol.
(B) Photocross-linking of acrylate-terminated polymers.
(C) Degradation of hydrogel over time.
(D) Degradation products.

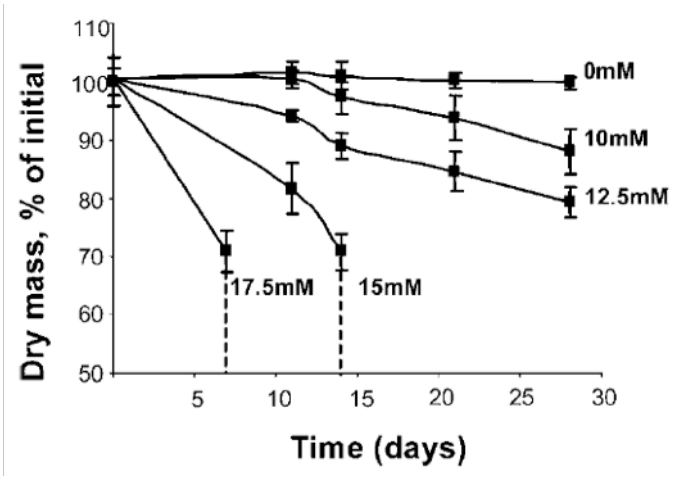

Figure 2: Hydrogel Degradation Over Time[7]
Hydrogels were prepared with 8 kiloDaltons PEG-diacrylate chains and the indicated concentrations of DTT. Vertical dashed lines indicate complete degradation of the hydrogel.

photocross-linked into a hydrogel network via exposure to ultraviolet radiation. These photoinduced cross-links can withstand months of exposure to aqueous solutions at physiologic pH and temperature without significant degradation. However, the dithiol bridges that are formed via the reaction between PEG-diacrylate and DTT are hydrolytically labile[7]. Therefore, the rate of degradation can be easily manipulated to be faster by increasing the quantity of DTT reacted with excess PEG-diacrylate and vice versa. The stepwise formation and degradation of PEG hydrogels is demonstrated in figure 1. The effect of varying the amount of DTT reacted is shown in figure 2.

Once the hydrogel network is established, the treatment that is to be administered to the patient can be incorporated into the hydrogel by soaking the gel in a solution containing the substance and allowing for natural diffusion to occur. Introducing the medicated hydrogel into a biological environment will enable the gel to swell as it degrades, and in doing so the integrated treatment factors will be able to diffuse out[7].

Collagen Shields

Collagen was first investigated as a way of protecting the eye and administering medications to the eye in the 1980's because of its abundance and biocompatibility. In the human body collagen plays an important role in the formation of tissues, organs and other support

systems[8]. Since this protein is present naturally, it is non-toxic and easily recognized by the body's immune system. It can then be effortlessly degraded or reabsorbed. Some benefits of collagen include the fact that it is non-antigenic, haemostatic and can be easily incorporated with synthetic polymer systems. Collagen is referred to as a biological 'plastic' because of its high tensile strength, ease of modifiability and minimal expressibility[9]. An example of a collagen shield can be seen in figure 3.

Figure 3: Oasis Collagen Shield[10]
The collagen shield fits onto the corneal surface of the eye in the same was as a contact lens.

Originally, collagen shields were developed as a bandage for corneal wounds to protect the healing ocular surface from damage caused by blinking. They began being used as drug delivery systems when it was discovered that medications could be entrapped, via diffusion, within the collagen matrix. Collagen shields successfully encase these medications through a natural process called cross-linking[9]. When collagen is subjected to glutaraldehyde or chromium tanning, cross-linking occurs to create interstices that allow for the entrapment of drugs, medications and other proteins[10]. Varying the amount of exposure time to the cross-linking method can easily regulate this property. The cross-linking effect of collagen increases the durability of the shield and makes it more resistant to degradation. This results in an increase in dissolving rate, drug release control and the duration of drug contact time with the biological environment. For this reason, collagen shields have been successfully used to deliver a steady supply of antibiotics and steroids to the ocular surface. Collagen shields have also served as a mild treatment for chronic dry eye solely by means of lubrication as the shield dissolves slowly over time[9].

Degradation rates for current collagen shields exceed three days and have been developed to dissolve over a period of one week. The process of degradation is relatively simple. Tears flush through the collagen shield and break down the cross-linked proteins, resulting in the dissolution of the outer layers. A thin film made of a collagen and tear solution then forms on the ocular surface and acts as a lubricant to minimize the rubbing done during blinking. This layer is slowly absorbed and degraded by the body but is replenished by further degradation of the shield. This slow cycle of breaking down and absorbing the collagen allows for optimal contact time between the drug and epithelial cells. In clinical trials, this process has shown great success and resulted in faster speed of epithelial healing than other conventional methods such as eye drops[9].

Although collagen has many benefits, there are some discrepancies as well. The natural hydrophilicity of collagen leads to excessive swelling, which results in unintended rapid release of small molecules[9]. Potentially, the shield may take a week to dissolve but release all of its encapsulated medication in the first three days. Along with this, there is variability in enzymatic breakdown of collagen from person to person. Due to the cross-linkage and entrapment properties, the patient has reduced visual activity that only dissipates as the shield dissolves. Also, since this product is similar to a contact lens, it may become blurry over time due to protein adsorption to the collagen shield surface. Another problem is the high cost of pure one type collagen[9].

Poloxamer Hydrogel

A poloxamer is a triblock copolymer consisting of a single polyoxypropylene (PO) and two polyoxyethylene (EO) blocks as shown in figure 4[11]. The number associated with the

poloxamer corresponds to the number of monomer units, each with varying physical and chemical properties[12].

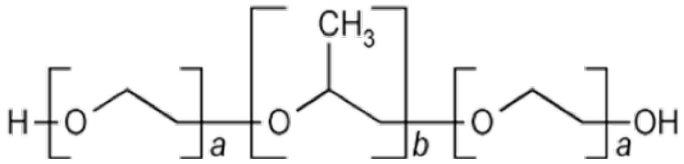

Figure 4: Poloxamer[12]
The monomer sequence $[-H(C_2H_4O)(C_3H_6O)(C_2H_4O)_OH-]$of all poloxamers, the number of units gives the poloxamer varying properties.

The designed poloxamer hydrogel is composed of Poloxamer 407. A Poloxamer 407 hydrogel can be easily formulated by adding the required amount, depending on desired weight percent, of Poloxamer 407 with deionized water at 5 ^oC[11,13]. Poloxamer 407 was chosen because of its limitless thermo-reversibility characteristics[11]. As the temperature increases, micelles form within the gel and become arranged in different manners depending on Poloxamer 407 concentration. This is shown is figure 5. Changing the percent composition of Poloxamer 407 in solution can alter the solution-to-gel transition temperature[13]. The designed hydrogel would be altered to have a transition temperature of approximately 32 ^oC, which corresponds to roughly 15 to 17 wt%[13]. This would allow the gel to be packaged and administered as an eye drop, which would then form a gel when in contact with the ocular surface.

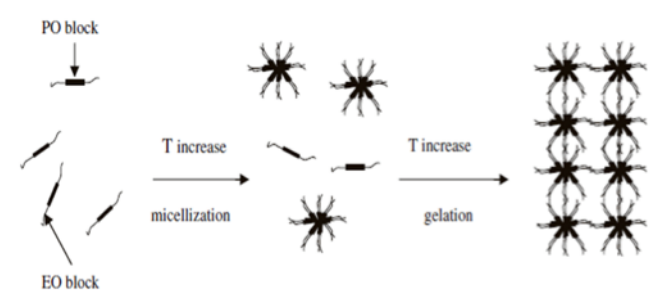

Figure 5: Schematic of Poloxamer 407 Gelation as Temperature Increases[13]
The micelles contain a hydrophobic, PO block core with a hydrophilic, EO block exterior.

Along with thermo-reversibility, the Poloxamer 407 gel is non-toxic and allows for incorporation of both hydrophobic and hydrophilic drugs[13,14]. Soaking the gel in solutions enables the incorporation of growth factors by allowing for drug uptake by simple diffusion. The

United States Food and Drug Administration considers Poloxamer 407 to be an inert ingredient when used for ocular drug delivery[13].

The degradation of the Poloxamer 407 hydrogel is relatively fast compared to the poly(ethylene glycol) gel. Medication diffusion rate of the Poloxamer 407 gel depends solely on gel degradation, which can range from two to six hours depending on the concentration of Poloxamer 407. If drug delivery vehicles such as liposomes or microspheres are incorporated into the hydrogel, this rate is no longer dependant on gel degradation, but rather on drug diffusion[13]. This allows for longer sustained release of medication with ocular residency time up to 24 hours[15,16]. Degradation of the Poloxamer 407 hydrogel can be altered to increase the degradation period to about seven days with the addition of cross-links amongst poloxamers[17].

Design Matrix

A design matrix was used to properly assess which design alternative would be the best choice to pursue for the final design. This allowed for a quantitative evaluation of how well each option satisfied the design criteria specified by our client. The five categories considered for the design matrix were biocompatibility, degradation control, drug release control, cost of materials, and patient ergonomics. However, it is important to note that a key criterion has not been included in the design matrix. Although it is necessary for the chosen alternative to be capable of initially incorporating the growth factors, all of the options accomplish this via simple diffusion. As this property is the same for all three alternatives, they would have received the same point value and therefore it would not have helped differentiate between them. Based on the results obtained, as tabulated in table 1, we have chose to pursue the PEG hydrogel for our final design.

Table 1: Design Matrix

The maximum point values are indicated in parentheses in the row headings. As the PEG hydrogel received the maximum overall point value, it is the alternative that will be pursued for the final design.

Design	PEG Hydrogel	Collagen Shield	Poloxamer
Biocompatibility (25)	20	25	20
Degradation Control (25)	25	20	15
Drug Release Control (30)	25	20	15
Cost of Materials (5)	5	2	5
Patient Ergonomics (15)	10	10	15
Total (100)	**85**	**77**	**70**

Biocompatibility

Biocompatibility is the ability for a material that is introduced into a biological environment to perform its intended function without eliciting any undesirable effects. This category was allocated one fourth of the total points in the design matrix because having a product that works effectively but causes harm to the user would be futile. Collagen shields are composed of collagen, the most naturally abundant protein in the body, and were therefore assigned the maximum point value. The poly(ethylene glycol) and Poloxamer 407 hydrogels are biologically inert and allow for the molecules encapsulated in their networks to be introduced to the biological environment without nonspecifically interacting with other molecules. For this reason, they received 20 of the 25 possible points.

Degradation Control

The ease at which the rate of degradation of the biogel can be altered is important for conforming to the client's specified treatment period of seven to fourteen days. If testing is done and it is determined that the rate at which the growth factors dissolve from the gel is greater or less than this time interval, it will be necessary to adjust the degradation rate accordingly. For this reason, degradation control was assigned one fourth of the total points. PEG hydrogels received all of these points, as the rate of degradation can be manipulated by simply varying the

amount of dithiothreitol that reacts with PEG-diacrylate in order to form PEG polymer chains. Collagen shields were given a score of twenty out of twenty-five because changing the cross-linkages within the shield can easily alter the degradation rate, however the process of changing cross-linkages is slightly more complicated and time-consuming than PEG hydrogel. The Poloxamer 407 hydrogel was given a fifteen out of twenty-five due to its rapid degradation without cross-linking amongst poloxamers.

Drug Release Control

When biogels come into contact with a physiologic environment, the medications incorporated into their networks are released via diffusion. This is related to the degradation of the gel, however, smaller molecules could completely diffuse out of the gel before total disintegration occurs. As the main goal of the project is to medicate the corneal surface with an adequate amount of growth factors, this category was allotted thirty percent of the possible points in the design matrix. PEG hydrogels were given the highest amount of these points because the gel pores swell as a result of the polymer chains breaking, which then enables the medication to diffuse out more rapidly. If a slower diffusion is desired, more DTT can be incorporated into the chains so that the rate of swelling is decreased. Collagen shields were given a value of twenty because they can easily release a drug over a set period of time. However, the natural hydrophilic nature of collagen leads to excessive swelling, which results in undesired rapid release of medication. Also, the release rate of collagen shields varies between individuals as a consequence of enzymatic degradation. The Poloxamer 407 hydrogel was given the lowest value, because in order to meet design specifications for drug release a drug delivery vehicle must be incorporated into the gel.

Cost of Materials

In comparison to the other categories considered, the cost of materials had a relatively minimal impact on choosing which alternative to pursue. Accordingly, in the design matrix it was only allocated five percent of the total points. This is because many of the treatment options that are currently available for dry eye are somewhat expensive and the project budget was stated to be upwards of $400 to $500. The PEG and Poloxamer 407 hydrogels could feasibly meet these requirements in mass production, as the materials needed to make them are fairly common and therefore relatively inexpensive. They both were given the maximum possible point values for this reason. Collagen shields were only assigned two of the five points because even though collagen is abundantly available, the high cost of pure type one collagen is substantially high in comparison with the other two designs.

Patient Ergonomics

The two main elements of patient ergonomics that needed to be considered for this project were ease of application and patient comfort once applied. This category was given fifteen percent of the points in the design matrix. The Poloxamer 407 hydrogel received the highest point value for patient ergonomics because the gel is in a liquid state at room temperature and can be applied to the ocular surface as a drop. The PEG hydrogel was assigned ten points because its application would likely be via insertion between the lower lid and corneal surface or by swiping the gel on the inside of the lower eyelid. This could potentially be a challenge to elderly and pediatric patients. Also, if the swelling is immense, the bulging gel could cause patient discomfort. Collagen shields were given a ten out of fifteen because due to their similar nature to contact lenses, some of the patients may have difficulties applying them to the ocular surface. In addition, they cover the pupil and can therefore result in blurry vision if there is a

high cross-linking density within the shield or an extensive amount of protein adsorption to the shield surface.

Final Design

Poly(ethylene glycol) (PEG) is a synthetic polyether. We chose to use it for our final design because it is amphiphilic, meaning it contains both components that readily interact with water and those that do not. Therefore, it can be dissolved by both water and organic solvents[18]. The PEG was reacted with a derivative of acrylic acid to form acrylate-terminated chains known as poly(ethylene glycol)-diacrylate (PEGDA). For our gels we used 8 kDa molecular weight PEGDA. Depending on the weight percent desired for the gel, we measured out a specific mass of the PEGDA, and made it into a solution with photo initiator IC59C2959 and a buffer solution that maintained a pH of 7.4. This solution alone could be aliquoted onto a slide with a one-millimeter spacer and exposed to ultraviolet radiation for three minutes to create cross-linkages and form a gel that can swell but is stable in a physiologic environment[7].

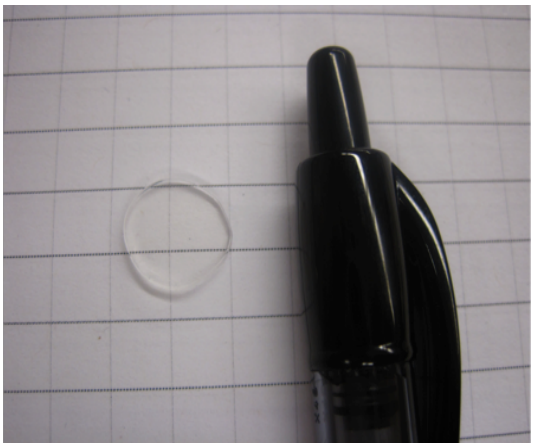

Figure 6: Fabricated PEGDA Hydrogel
Size of a 100 µL aliquot prior to swelling and degradation relative to the end of a pen.

However, incorporation of the thiol dithiothreitol (DTT) prior to radiation results in the formation biodegradable bonds. In order to make gels of this type, we made solutions with desired amounts of PEGDA, DTT, and buffer, tested them to ensure the pH was above 7, and put them in an incubator at 37 °C for one hour. Having the correct pH allows for the addition reaction to occur between PEGDA and DTT, and the elevated temperature increases the rate of the

reaction. After incubation, photo initiator IC59C2959 was added and ultraviolet radiation was used again to create gels[7]. A fully constructed PEGDA hydrogel can be seen in figure 6.

Growth Factors

The introduction of growth factors to the ocular surface is a promising method of treatment, as it reduces irritation through the restoration of cells. Experimental treatments using growth factors to restore corneal epithelial cells have proved effective, but are limited by the fact that they are applied as drops. Growth factor eye drops have minimal contact with the epithelial cells, and are flushed away promptly by renewing tear film. As a result, high concentrations must saturate the tear film in order to stimulate the desired effect. These highly concentrated drops may induce negative side effects such as uncontrollable proliferation. A sustained release of growth factors alleviates this limitation, as it enables the use of smaller concentrations and maximal contact time with the corneal epithelial cells. The lower concentration greatly reduces the possibility for side effects, and allows for uninterrupted treatment. Epidermal growth factor (EGF) is the most promising growth factor for this application, as it mainly functions to differentiate and proliferate epithelial cells[19].

As previously described in the description of the design alternatives, growth factors diffuse out of hydrogels as they swell and degrade. Therefore, the rate of diffusion is a property of both the rate of swelling and the molecular size of the growth factor. EGF has a relatively low molecular weight of 6000 Daltons[20]. This factor may cause an undesirable rapid diffusion, which would result in the gel releasing all of the growth factors encapsulated within its network prior to complete gel degradation. To decrease the rate of diffusion of EGF, the composition of the gel must be altered to decrease swelling. However, changing the structure of the gel in order to accomplish this will also increase the overall degradation time. An alternative would be to

incorporate a drug delivery vehicle, which would act as a secondary release mechanism and slow down the rate of diffusion. This concept is described in more detail in the future works section.

Testing

Swelling

The weight percent of PEGDA in the total solution was chosen through the execution of a swelling test. This experiment was conducted by calculating the volume of 10, 15, 20, and 25% PEGDA hydrogels before and after equilibration.

Prior to gel construction, calculations were made to determine the actual amount of PEGDA (mg), buffer solution (µL), and photoinitiator (µL) needed to create gels of the necessary weight percentages. 500 µL of total solution was prepared for each weight percent to ensure enough gel samples of 100 µL aliquots would be available to detect statistical variance in our data. As 500 µL of total solution was used, the 10% PEGDA solution required 50 mg of powdered 8 kDa PEGDA. The 15, 20, and 25% solutions, therefore, needed 75 mg, 100 mg, and 125 mg PEGDA, respectively. For proper gelation, each solution also contained 10% photo initiator IC59C2959, resulting in 50 µL for our 500 µL solutions. The final ingredient, buffer solution consisting of 20 mM Hepes, 150 mM NaCl, and 10 mM $CaCl_2$, was calculated as the total amount of solution subtracted by the amount of photo initiator and PEGDA. Actual values used in gel construction are displayed in table 2 below.

Table 2: Amounts of Materials Used for Swelling Testing
Values were previously calculated then adjusted to these values to yield more accurate weight percent solutions based on the actual mass of PEGDA used.

Weight Percent PEGDA (%)	Mass of PEGDA (mg)	Volume of Photo Initiator (µL)	Volume of Buffer Solution (µL)
10	49.65	49.7	397
15	75.60	50.4	378
20	100.23	50.1	351
25	125.56	50.2	326

The desired amounts of powdered 8 kDa PEGDA were weighed using an analytical balance and placed into a 1.5 mL capped plastic vial. Additions of the indicated quantities of buffer solution and photo initiator were then added via a micropipette. This was followed by agitation with a vortex machine to ensure even mixing of the solutions. Samples were agitated until there was no visible powder remaining in solution. After complete agitation, a 100 µL aliquot of solution was placed in the center of a glass slide with a 1.06 mm spacer, previously cleaned with absolute ethanol. A second microscope slide was then placed on top to compress the solution to a uniform thickness. Figure 7 shows a visual depiction of the slides used in gel construction. The final step in gel synthesis was ultraviolet (UV) light curing. To ensure full cross-linking occurred, all gels were placed under an UV lamp for three minutes.

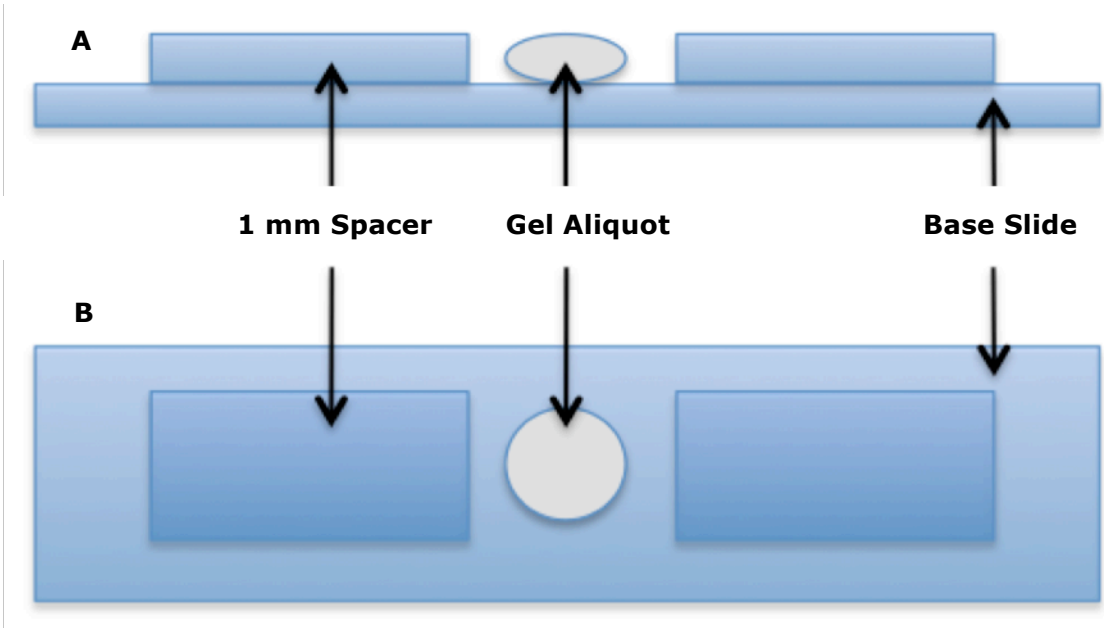

Figure 7: Representation of Spacer Slides
Slides were used to contain solutions and create a uniform gel thickness when exposed to ultraviolet radiation. (A) Side view. (B) Top view.

Three gels of each weight percent were fabricated using the above methods. Initial mass, diameter, and thickness of each were recorded immediately after UV exposure. Mass measurements were obtained using an analytical balance, while diameter and thickness were

acquired using calipers. The gels were submerged in labeled wells containing 2 mL of buffer solution and were left to swell to equilibrium. Equilibrium was obtained after approximately 24 to 36 hours, so the gels were left for 48 hours to ensure full equilibration. They were then removed from the buffer solution and the measurements were repeated. Both initial and final measurements are shown in table 3.

Table 3: Measurements for Swelling Testing

Mass measurements were made by wiping gels with weighing paper to remove solution from the surface, and then using an analytical balance. Dimensional measurements were made by using a calipers to measure in inches and then converting to millimeters.

Well	Initial Mass (mg)	Initial Diameter (mm)	Initial Thickness (mm)	Equilibrium Mass (mg)	Equilibrium Diameter (mm)	Equilibrium Thickness (mm)
10 A	96.10	11.11	1.06	168.40	14.29	1.35
10 B	86.15	9.53	1.06	161.30	11.91	1.59
10 C	87.00	10.72	1.06	150.34	12.70	1.35
15 A	93.12	9.53	1.06	175.26	12.30	1.51
15 B	92.34	11.11	1.06	181.66	13.34	1.35
15 C	89.24	11.91	1.06	178.58	14.29	1.43
20 A	79.88	11.11	1.06	189.29	13.65	1.43
20 B	80.09	10.72	1.06	175.02	13.65	1.27
20 C	90.93	11.91	1.06	199.17	14.13	1.19
25 A	93.93	11.11	1.06	199.83	14.61	1.27
25 B	90.97	10.32	1.06	202.78	12.46	1.43
25 C	96.04	11.51	1.06	221.52	14.29	1.35

The percent changes in volume of the three gel samples at each weight percent were averaged to achieve an average percentage of swelling. The 10, 15, 20, and 25% PEGDA solutions yielded averages of 208.04, 200.24, 185.61, and 200.24% swelling, respectively. These averages can be seen in chart 1. As the average swelling percentages were close in magnitude, an ANOVA analysis was conducted to determine the statistical significance of the variance. Execution of the ANOVA produced a p-value of 0.737, which clearly indicates that any variance observed in the data is statistically irrelevant. This means that all of the tested weight percent PEGDA gels had statistically equal swelling of approximately 200%. It can therefore be

concluded that our initial gel must be constructed with dimensions no greater than one half of the maximum dimensions depicted in the design specifications to account for a swelling factor. This result also leads us to the freedom to choose any desired weight percent of PEGDA for further production of our gels. We chose to fabricate all further gels with 10% PEGDA to limit excess material consumption.

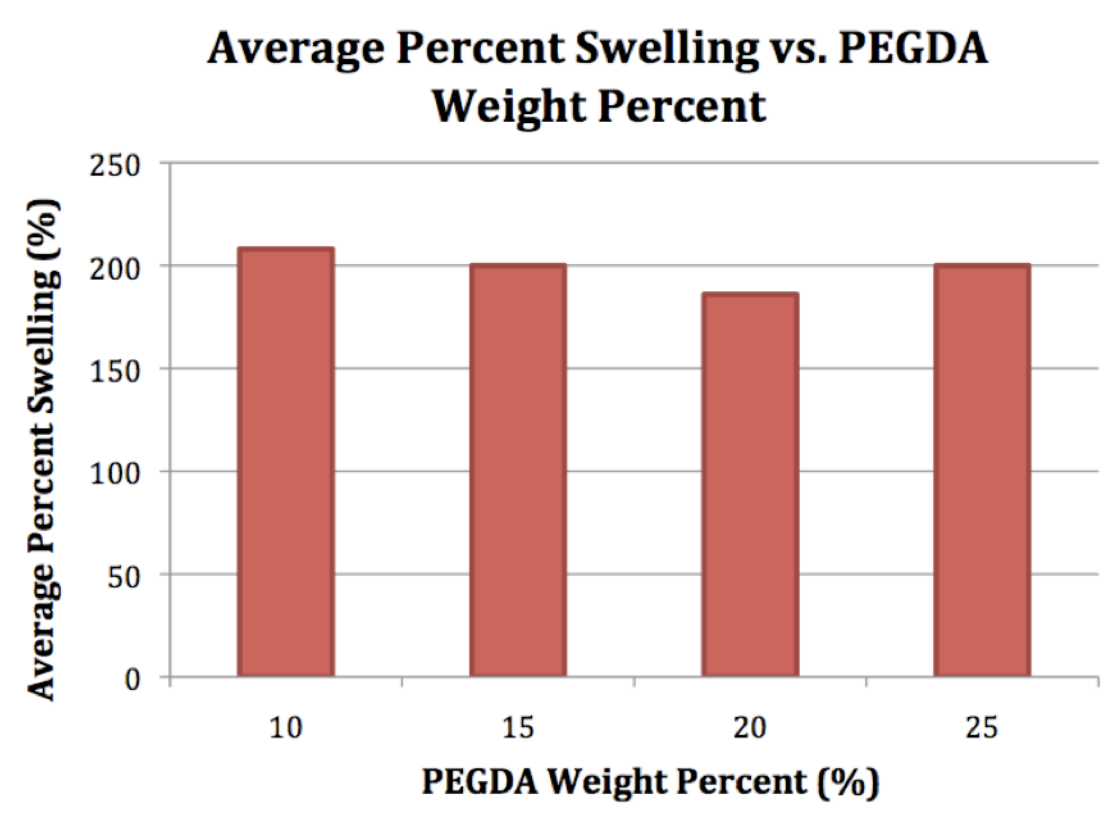

Chart 1: Average Swelling Percentages
Based on the ANOVA analysis there is no statistical difference in swelling between the various weight percentages.

Degradation

Varying the ratio of DTT to PEGDA within a hydrogel results in a change in the degradation rate. When reacted with PEGDA, DTT binds to the available acrylate groups of PEGDA chains. A large amount of DTT results in a high percentage of occupied acrylate groups. When a PEGDA solution is exposed to UV light, cross-linking occurs between unoccupied

acrylate groups. A solution that contains a high ratio of DTT:PEGDA has a large number of acrylate groups that are already occupied, resulting in fewer groups available for cross-linking during UV exposure. The degree of polymerization within hydrogels containing both PEG and DTT is governed by the equation:

$$X = \frac{(1+r)}{1+r-2\cdot r\cdot p}$$

The degree of polymerization (X), is both a factor of the ratio of DTT:PEGDA (r) and the extent of reaction (p). Experimentation conducted by Matt Parlato at the University of Wisconsin-Madison's Department of Biomedical Engineering has estimated the value of p for this reaction to be approximately 0.9 or 90%[21]. The plot of this equation is shown in chart 2, and indicates that

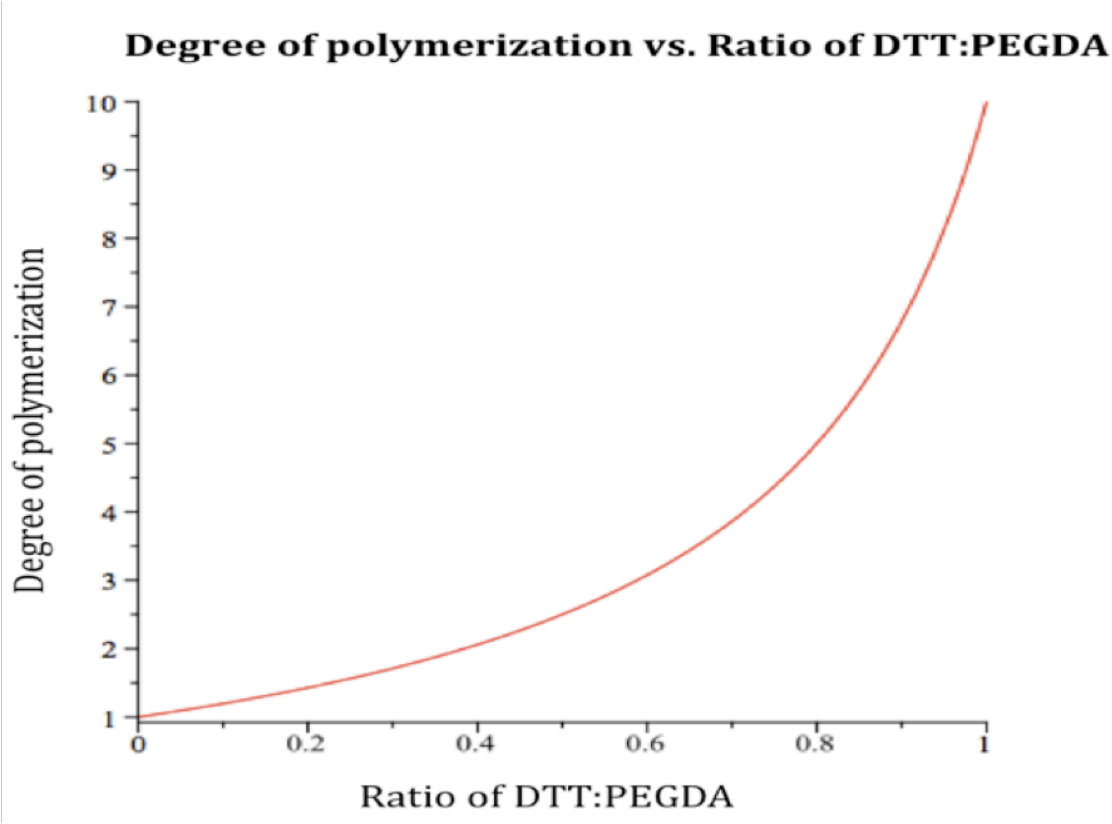

Chart 2: Plot of Polymerization Equation
As demonstrated, the relationship between the ratio of DTT:PEGDA and the degree of polymerization is exponential

an increase in the ratio of DTT:PEGDA causes an increase in the degree in polymerization. As an increase in the degree of polymerization corresponds to a lower cross linking density and faster degradation, the larger the ratio of DTT:PEGDA the quicker the hydrogel is expected to degrade.

In accordance with this relationship, we conducted our degradation testing with molar ratios of 1:2, 1:3, 1:4, and 1:5 DTT:PEGDA. Although a 1:1 ratio would theoretically degrade the fastest, it is not feasible in practice as long polymer chains and extremely low cross-linking density resist the formation of a gel during UV exposure. To achieve accurate ratios of DTT:PEGDA , a 1 M stock solution of DTT was created and various volumes of it were added to solutions of PEGDA and buffer via micropipette in proportions leading to the appropriate molar ratios.

To experimentally determine the DTT:PEGDA ratio that best fit the design specifications, we conducted a 13 day degradation test. Thirty-six total gels, nine of each ratio, were made and submerged in buffer solution. The 9 gels at each ratio correspond to days 0, 1, 2, 3, 4, 6, 7, 9, and 13 of the degradation test. These intervals were chosen based on availability of access of the laboratory over our 13-day test period. On each day of testing, both a wet and dry mass were recorded for one gel of each DTT:PEGDA ratio. Wet masses were obtained by blotting the gels with weighing paper and then using an analytical balance. Dry masses were obtained through a freeze-drying process. Gel samples were removed from buffer solution and soaked in deionized water for two hours while being agitated by an orbital shaker. The deionized water was changed after one hour of agitation to discard the salts that were rinsed from the gel network. After being fully cleansed with deionized water, the gels were placed into marked vials,

frozen for ten minutes in a conventional freezer, and placed in a vacuum freeze-drying chamber for 24 hours. Masses were then obtained by using an analytical balance.

The first time this test was conducted, we were unaware of the correct rinsing process and simply washed with gels with deionized water before placing them into the freeze dryer. Insufficient cleansing resulted in the presence of salts within the gel network after freeze drying, and resulted in an increase in measured dry masses. This data has been included in the appendix for completeness and means of comparison with the corrected data obtained during the second round of testing, also in the appendix.

Gel degradation is characterized by the mass erosion percentage, percentage of dry mass to initial wet mass. Chart 3 shows a representation of this trend over time. This value decreases with time for all of the ratios tested, with the 1:2 DTT:PEGDA ratio showing the most rapid

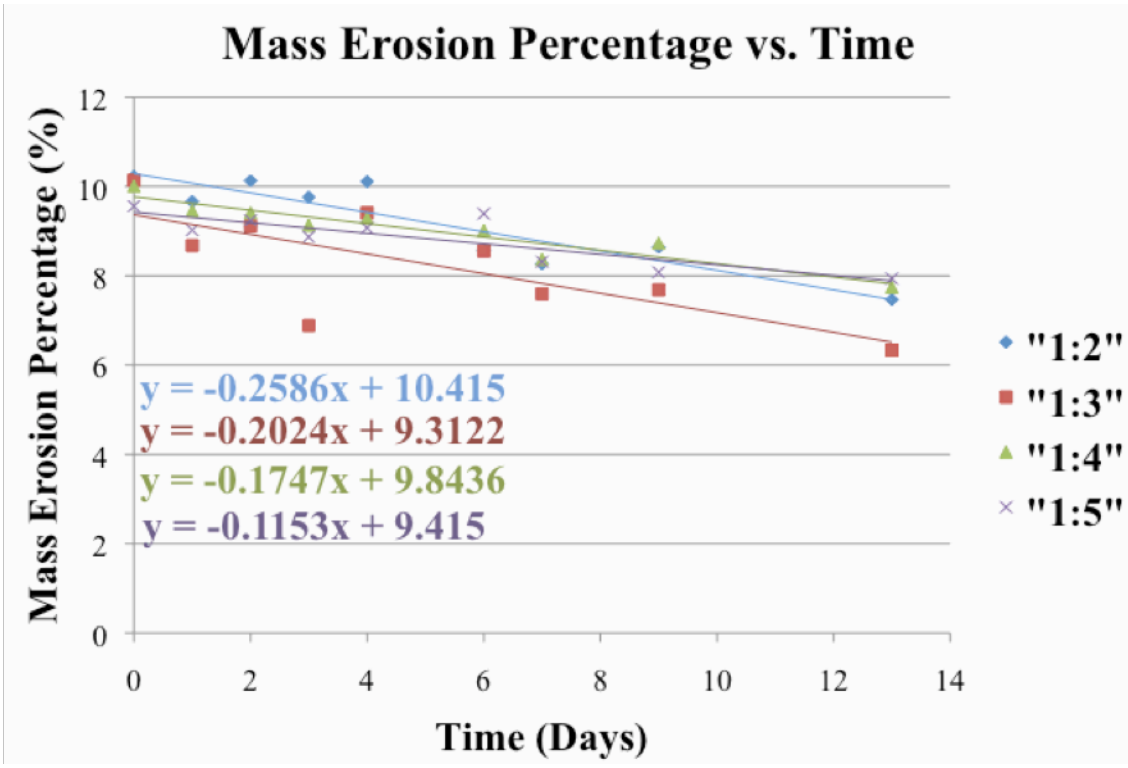

Chart 3: Degradation Results
The 1:2 hydrogel degrades the fastest. By extrapolation this gel is expected to fully degrade in 40 days. A gel with more DTT must be used to meet the design criteria.

degradation. This is in accordance with what was expected, as the higher the ratio the lower the cross-linking density. Extrapolation of the data sample for 1:2 DTT:PEGDA predicts full degradation in 40 days, which is largely greater than the desired window specified in our design criteria. However, testing was conducted at standard room temperature, 20 °C, and gels were not exposed to any mechanical stimuli. As our product is intended to be used at the slightly elevated temperature of the eye, 32 to 34 °C, and exposure to the constant mechanical stimulus of blinking, actual degradation time is expected to decrease rapidly. Further experimentation with gels placed in an incubation chamber during degradation along with agitation to simulate blinking will be conducted to solidify a more accurate representation of degradation time of our hydrogel on the ocular surface.

Live/Dead Assay

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. Dr. Ellen Cook and Dr. Jim Stahl, both of the Department of Medicine – Ocular Immunology, assisted us in performing a live/dead assay to determine whether or not the hydrogels we made would induce cytotoxicity, which means to be poisonous to the cells. To obtain the most accurate data possible, we used a cell line derived from normal human conjunctiva, as this will be the intended area of application in patients[22].

In four wells of a tissue culture plate, we grew the cells in 2 mL of a protein media that contained the necessary components to sustain life. On top of the media we placed a Cyclopore® membrane, sterilized through gamma irradiation. With pore sizes of 3.0 μm, this enabled the 1:2 DTT:PEGDA gels that were placed on top of the membrane inserts to evenly diffuse into the cell cultures upon degradation. Another 1 mL of the protein media was aliquoted on top of the membrane to provide an environment in which the gels would not dry out and could degrade.

This media was changed in all of the remaining wells after each day of testing. In order to accurately determine the effect of the gels on the cells, a reference was needed. This was accomplished through creating comparable controls. Four more wells were set up in the same way outlined for the gels, except that gels were not included on top of the membrane inserts. Finally, to ensure that the gels would be the only potential cause for cell death other than natural causes, all equipment and materials used were sterilized and testing was carried out under a sterile fume hood[23].

As the intended length of application of the product is around one week, we chose to take measurements 1, 2, 5, and 7 days after the gel and control wells had been established. On the indicated testing day, the inserts and gel were removed from one of each of the conditions. The remaining protein media was then aspirated off the cells, which were adhered to the surface of the culture plates. 2 mL of the chemical trypson was alliquoted into each via a micropipette. Plates were then placed on an agitator in an incubator that maintained a temperature of 37 °C for approximately ten minutes. This process caused the cells to detach from the surface of the wells. Next, 1 mL of the chemical known as RPMI was pipetted into each well to neutralize the trypson. Cells and fluids were transferred to test tubes with sterile transfer pipettes and placed in a centrifuge for three minutes. This caused the cells to collect on the bottoms of the test tubes so that the majority of the supernatant could be aspirated off and they would be present in a higher concentration in the remaining solution[23].

In order to make a representative count of the number of live and dead cells present, 25 μL of the remaining solutions were transferred to new test tubes using a micropipette. Then, and again with a micropipette, 25 μL of trypan blue was added to each. This chemical functioned to stain the cells for easier viewing. It causes live cells to appear yellow or beige in color, while

dead cells turn blue. The resulting solutions were pipetted into a hemocytometer, a specialized microscope slide that contains a grid pattern that assists with cell counting. To determine cell viability, 100 cells for each condition were counted. During this count, tallies were kept on the number of live and dead cells[23].

As seen in figure 8, the results of the first two days of testing were very favorable. With the majority of cells surviving and the count between the gel and control conditions only being off by one cell of the hundred for each day, it can be concluded that there is no statistical difference between the conditions, and therefore the gels do not cause cytotoxicity. Unfortunately, the media that was exchanged into the wells after this time unknowingly introduced both a fungus and bacteria. This caused extensive cell death and prevented further testing from occurring as the number of surviving cells in both conditions were too few to count and the bacterial and fungal cells created an interference.

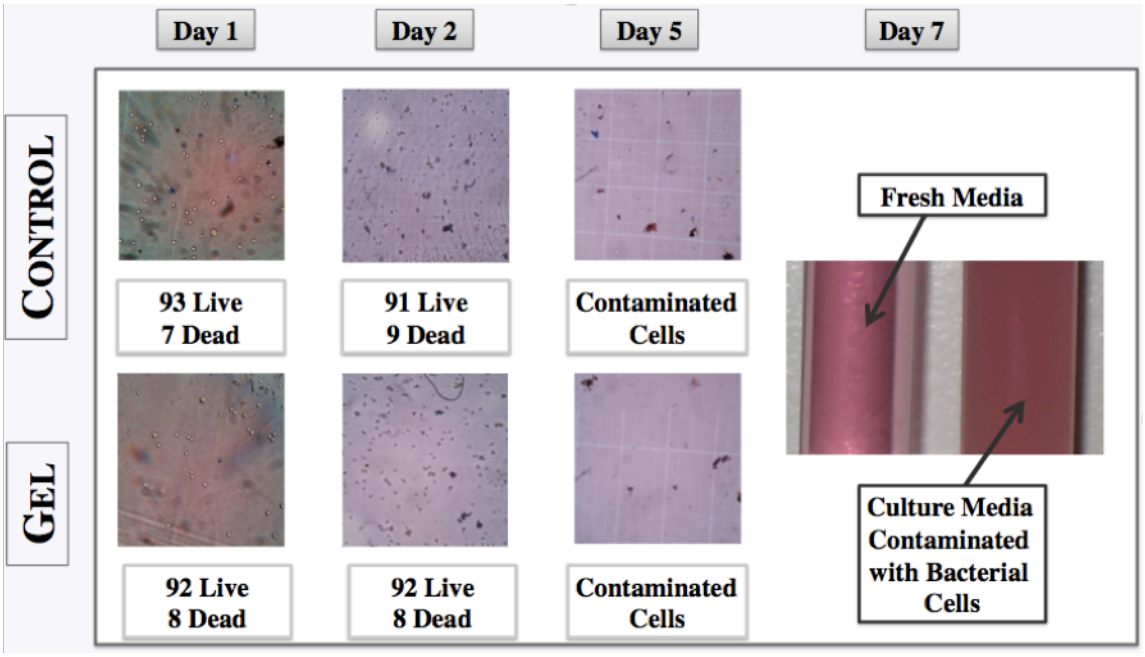

Figure 8: Live/Dead Assay Results
As demonstrated by the pictures, there was no statistical significance in the survival of the cells in the gel and control during the first two days of testing. However, both conditions became infected after this time, and further results could not be obtained.

Costs

Money Spent this Semester

We initially had a $400 budget for purchasing materials. A summary of the funds spent to complete the project is listed in table 4. The materials needed for making the hydrogels cost a total of $229.65, and were all purchased from Sigma-Aldrich®. The high cost of shipping and handling was due to the necessity for the some of the chemicals to be ground shipped in a refrigerated truck because of their corrosive and flammable properties prior to reaction. We were fortunate enough to have Dr. William Murphy and Dr. Michael Toepke of the University of Wisconsin-Madison donate their time, lab materials, and the necessary equipment to perform our swelling and degradation testing. Likewise, Dr. Jim Stahl and Dr. Ellen Cook of the Department of Medicine - Ocular Immunology at the UW Hospital allowed us to use their lab and assisted us in performing the live/dead assay free of charge.

Table 4: Summary of Costs

We were able to stay well under budget because of the donations of lab materials, equipment, and time by many of the people whom we worked with.

Item	Quantity	Cost
Poly(ethylene glycol)	250 g	$27.80
Triethylamine	100 mL	$9.30
Acryloyl Chloride	100 g	$88.00
DL-Dithiothreitol Solution	10 mL	$20.20
Poster	1	$43.75
Taxes/Shipping/Handling	N/A	$84.35
Total	N/A	**$273.40**

Projected Costs of Treatment

Our design could potentially be the cheapest treatment for dry eye when compared to the current treatments available. Restasis®, the only current FDA-approved treatment for chronic dry eye, runs between $100 and $200 for a monthly prescription[4]. TheraTears® seems relatively cheap, costing around $15 per bottle. However, depending on the severity of the dry eye, a

patient could potentially need up to four bottles a month, resulting in a total cost of $50 to $60[4]. Punctal occlusion and surgery are more advanced treatments and can cost thousands of dollars[5].

Our design has the potential to cost around $20 per month, with possible fluctuations arising due to the additional costs of commercialization and the time and effort expended on clinical trials. This amount is derived from the costs of supplies, which when the purchase of growth factors is included, would be around $500. With this amount of materials, a minimum of 50 gels could be created. If the degradation time is corrected to the intended seven to fourteen day period, patients would only need to insert two to four gels per month, resulting in a cost of around $20. If commercialized, the gel could possibly be a top competitor because it would be economically friendly and an effective, reliable treatment.

Time Management

The majority of the time spent on our project was divided between research and testing. A complete summary of all of our activities can be seen in table 5. Research consisted of finding a biogel that either met or could be altered to meet our design criteria. We also looked into different types of growth factors, ways they could be incorporated into the gel, and how to test the rate at which they would diffuse out. We were unable to find a commercial hydrogel that met the needs of this application, so we synthesized one in the lab of Dr. William Murphy, a faculty member of the Biomedical Engineering Department at the University of Wisconsin-Madison. Dr. Michael Toepke, a post-doctorate from the lab, taught us how to make and test the swelling and degradation of PEGDA hydrogels. We spent a considerable amount of time in the lab, as testing required measurements to be made almost every day over the course of two weeks. The other component of our testing, the live/dead assay, was performed at the Ocular Immunology lab at the UW Hospital. With the assistance of Dr. Ellen Cook and Dr. Jim Stahl, we prepared and

counted the cells for four days during a one-week period. Due to the extensive time needed to perform these tests accurately, it was necessary for us to divide up the workload between group members.

Table 5: Summary of Semester Timeline

The two activities that required the most time throughout the semester were research and testing. Although the research was spread out over many weeks, the testing was compacted into the last few weeks and required daily measurements.

Tasks	January		February				March				April				May
	22	29	5	12	19	26	5	12	19	26	9	16	23	30	7
Meetings															
Advisor															
Client															
Team															
Product Development															
Research															
Brainstorming															
Design Matrix															
Design Prototype															
Order Materials															
Fabricate Prototype															
Testing															
Deliverables															
Progress Reports															
PDS															
Mid Semester PPT															
Mid Semester Report															
Final Poster															
Final Report															
Website															

Future Works

Physiologic Conditions

Further degradation testing must be done. The results of the previously conducted tests were not as expected, as it was determined that the fastest degrading gel would take 40 days to completely disintegrate. This is likely due to the fact that the environment the gels were exposed to during testing was comparable to the ocular surface only by pH, which was 7.4. It is expected that if the gels are exposed to a more exact physiological environment they will degrade in a shorter period of time. Further degradation testing will be conducted with gels submerged in the

7.4 pH buffer at 32 to 34 ^{o}C, the temperature of the ocular surface. This is expected to increase reaction rates by increasing the thermal energy in the testing environment. The solutions will also be continuously agitated to simulate blinking and ocular surface movement. This is expected to increase the reaction rates by applying a continuous mechanical stress on the gel. When allowed to degrade in this replicated physiological environment, it is expected that the 1:2 DTT:PEGDA gel will degrade in a much shorter time period, possibly even within the 7 to 14 days desired to meet the design criteria.

Diffusion Testing

The testing of drug diffusion rates will be conducted with the 1:2 DTT:PEGDA gel. Because of the high cost of epidermal growth factor (EGF), initial tests will be conducted with a model protein. To ensure reliable results the model protein must have similar characteristics to EGF, such as molecular weight and isoelectric point. Several such proteins have been looked into already. Of these, insulin has seemed to be the best candidate. EGF has a molecular weight of 6,100 Da and an isoelectric point, pH at which the protein has no charge, of 4.6. Insulin has a molecular weight of 5,800 Da and an isoelectric point of 5.3[24]. These values are close enough to use insulin as a representative of EGF to determine the rate at which it will diffuse out of the gel at an ocular pH, which ranges from 7.0 to 7.5.

The model protein will be labeled with an Atto Protein Labeling Kit available through Sigma-Aldrich®. The recommended use for these kits is a pH of 7.0, which is within the pH range on the ocular surface[25]. Following instructions included in the labeling kit, the proteins will be labeled and included in the gel solution prior to photo cross-linking. The diffusion rate will then be determined by the release of the fluorescence-labeled protein. This will be measured using fluorescence spectrometry with the excitation and emission wavelengths specified by the

protein labeling kit. A calibration curve must then be constructed using solutions of known concentrations to determine the spectrometer response. This calibration curve will result in a linear, $y = mx + b$, equation where x is the concentration and y is the spectrometer response. Because this linear fit is inaccurate for very low concentrations, the gel will be allowed to degrade in a solution with a known concentration ($[X]_{initial}$) of labeled protein. This solution will be analyzed at 12 hour intervals, and the change in concentration ($\Delta[X] = [X]_{final} - [X]_{initial}$) will be plotted versus time in hours over the gel degradation period[25].

Drug Delivery Vehicle

The diffusion of EGF is expected to be relatively quick because of its low molecular weight. If this occurs during the diffusion testing, a drug delivery vehicle will have to be incorporated into the gel to meet the specified design criteria. The same chemistry used to make the gels can be used on a much smaller scale to produce PEGDA microspheres. EGF will be encapsulated within the microspheres as seen in figure 9. Although 8 kDa PEGDA was used to produce the hydrogel, varying molecular weights of PEG are commercially available. By varying the molecular weight of the PEG in the microsphere, diffusion rates can be altered from one day to several months[26].

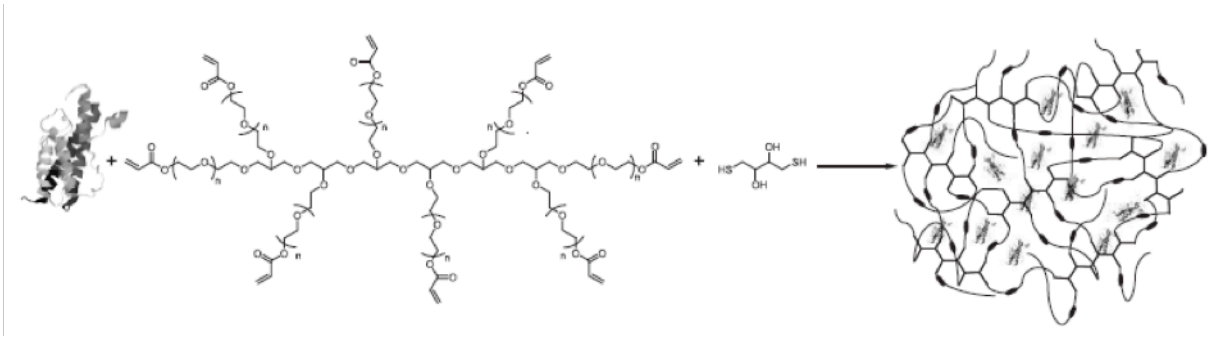

Figure 9: Protein Encapsulation[26]
Epidermal growth factor will be encapsulated within the PEGDA microspheres. These will then be integrated into the hydrogel to have a more controlled drug release rate.

Clinical Testing

The first step in clinical testing will be to obtain accurate, reliable, and reproducible data in the degradation and drug diffusion testing. After degradation and diffusion rates have been established the hydrogel must then be thoroughly tested for toxicity. Although PEG has been approved by the FDA for drug delivery, both diacrylate (DA) and dithothreitol (DDT) have not. Because of this, toxicity testing must be conducted on two species with complete eye and systematic evaluation[27]. The duration of this testing must be at least equal to or exceed the expected duration of the application time during clinical trials[28]. Regulations outlined in the FDA handbooks "Guidance for Industry: Immunotoxicology Evaluation of investigational New Drugs" and "Guidance for Industry: CGMP (Current Good Manufacturing Practice) for Phase 1 Investigation Drugs" will be followed to ensure the proper analysis of immune response and that all ethical matters are taken into consideration.

The final step prior to manufacturing the product is clinical trials. All human subjects will be informed of the relative risks and benefits of the clinical tests. The ethical principles that follow will be established and maintained through clinical trials:

> The National Commission for the Protection of Human Subjects in Biomedical and Behavioral Research's Three Ethical Principles[29]:
>
> > **Beneficence:** Maximizing good outcomes for science, humanity, and individual research participants, while avoiding or minimizing unnecessary risk, harm, or wrong.
> > **Respect:** Protecting the autonomy of autonomous persons and treating all, including the nonautonomous, with courtesy and respect.
> > **Justice:** Ensuring reasonable, nonexplorative, and carefully considered procedures and their fair administration, with fair distribution of costs and benefits among person and groups.

A yet to be determined number of human subjects diagnosed with dry eye will be instructed to administer the hydrogel for a specified period of time. Throughout this time period efficacy of the hydrogel will be determined.

References

1. Djalilian, A., Hamrah, P., & Pflugfelder, S. (2005). Disorders of tear production and the lacrimal unit. In J. Krachmer, M. Mannis & J. Hollan (Eds.), *Cornea: Fundamentals, diagnosis and management* (Second ed., pp. 521) Elsevier Mosby.
2. Lemp, M. L., MD, & Foulks, G. N., MD. (2008). The definition & classification of dry eye disease. *Guidlines from the 2007 International Dry Eye Workshop,* (April 2008), 1-6.
3. *Sjögren's syndrome foundation.* Retrieved 2/27, 2010, from http://www.sjogrens.org/
4. Herskowitz, R., O.D. (2009). *Dry eye medications.* Retrieved 3/1, 2010, from http://www.eyecaresource.com/conditions/dry-eyes/medications.html
5. Management and treatment of dry eye disease: Report of the management and therapy subcommittee of the international dry eye workshop (2007). *The Ocular Surface, 5*(2), 163-171. doi:April 2007
6. Haines, C. (2007). *Dry eyes.* Retrieved 03/01, 2010, from http://www.medicinenet.com/dry_eyes/article.htm
7. 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.
8. University of Illinois at Chicago: Department of Ophthalmology and Visual Sciences. *Collagen corneal shields.* Retrieved 03/01, 2010, from http://www.uic.edu/com/eye/LearningAboutVision/EyeFacts/CollagenCornealShields
9. Chi H. Lee, Anuj Singla, & Yugyung Lee. (2001). Biomedical applications of collagen. *International Journal of Pharmaceutics,* (221), 1-22.
10. Oasis Medical. (2004). Retrieved 03/01, 2010, from http://www.oasismedical.com/Products_Node_View.asp?id=23
11. Singh-Joy, S. D., & McLain, V. C. (2008). Safety assessment of poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402, 403, and 407, poloxamer 105 benzoate, and poloxamer 182 dibenzoate as used in cosmetics. *International Journal of Toxicology, 27 Suppl 2*, 93-128.
12. Sheehan, Catherine. *Poloxamer, chemical structure, molecular formula, reference standards.* Retrieved February 25, 2010, from http://www.uspbpep.com/usp28/v28230/usp28nf23s0_m66210.htm
13. Dumortier, G., Grossiord, J., Agnely, F., & Chaumeil, J. (2006). A review of poloxamer 407 pharmaceutical and pharmacological characteristics. *Pharmaceutical Research, 23*(12), 2709-2728.
14. Stratton, L. P., Dong, A., Manning, M. C., & Carpenter, J. F. (1997). Drug delivery matrix containing native protein precipitates suspended in a poloxamer gel. *Journal of Pharmaceutical Sciences, 86*(9), 1006-1010.
15. Kim, E., Gao, Z., Park, J., Li, H., & Han, K. (2002). rhEGF/HP-β-CD complex in poloxamer gel for ophthalmic delivery. *International Journal of Pharmaceutics, 233*(1-2), 159-167.
16. Fattal, E., De Rosa, G., & Bochot, A. (2004). Gel and solid matrix systems for the controlled delivery of drug carrier-associated nucleic acids. *International Journal of Pharmaceutics, 277*(1-2), 25-30.

17. Niu, G., Du, F., Song, L., Zhang, H., Yang, J., Cao, H., et al. (2009). Synthesis and characterization of reactive poloxamer 407s for biomedical applications. *Journal of Controlled Release, 138*(1), 49-56.
18. 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
19. Barney, N. P., MD. (2002). Substance P, insulinlike growth factor 1, and surface healing. *Clinicopathologic Reports, Case Reports, and Small Case Series, 120*(FEB 2002), 215-216. Retrieved from www.archophthalmol.com
20. *Epidermal growth factor (EGF).* Retrieved 2/12, 2010, from http://www.curehunter.com/public/keywordSummaryD004815.do
21. Parlato, M. (2010). *Personal communication.*
22. Diebold, Y., Calonge, M., Enriquez de Salamanca, A., Callejo, S., Corrales, R. M., Saez, V., et al. (2003). Characterization of a spontaneously immortalized cell line (IOBA-NHC) from normal human conjunctiva. *Investigative Ophthalmology & Visual Science, 44*(10), 4263-4274.
23. Cook, E. PhD, and Stahl, J. PhD. April 21, 2010. *Personal communication.*
24. Taylor, J. M., Mitchell, W. M., & Cohen, S. (1974). Characterization of the high molecular weight form of epidermal growth factor. *The Journal of Biological Chemistry, 249*(10), March 15, 2010.
25. Sigma-Aldrich Inc. Atto 665 protein labeling kit. Retrieved May 2, 2010, from http://www.sigmaaldrich.com/catalog/ProductDetail.do?lang=en&N4=73919|SIGMA&N5=SEARCH_CONCAT_PNO|BRAND_KEY&F=SPEC
26. Van de Wetering, P., Metters, A. T., Schoenmakers, R. G., & Hubbell, J. A. (2005). Poly(ethylene glycol) hydrogels formed by conjugate addition with controllable swelling, degradation, and release of pharmaceutically active proteins. *Journal of Controlled Release, 102*(3), 619-627.
27. U.S. Department of Health and Human Services, & Food and Drug Administration. (October 2002). Guidance for industry: Immunotoxicology evaluation of investigational new drugs. *Pharmacology and Toxicology.*
28. Z. Chen and R. Osterberg. (June 2008,). Considerations of preclinical toxicity studies for ocular drug development. http://www.accessdata.fda.gov/ScienceForums/forum02/AF-03.htm.
29. Sieber, J. E. (1993). Ethical considerations in planning and conducting research on human subjects. *Academic Medicine, 68*(9)

Appendix

Table 1: Measurements Obtained from Improper Rinsing Prior to Freeze Drying
As seen, the dry mass measurements do not conform to a decreasing trend as they should. This was due to salts encapsulated in the hydrogel networks not being properly rinsed, and therefore causing an apparent increase in dry mass.

Gel	Initial Mass (mg)	Wet Mass (mg)	Dry Mass (mg)
1:2 A	44.16	44.16	5.28
1:2 B	44.83	130.04	5.56
1:2 C	43.68	141.89	5.59
1:2 D	42.84	153.98	5.34
1:2 E	45.44	151.45	6.17
1:3 A	49.51	49.51	5.25
1:3 B	43.75	189.82	6.46
1:3 C	36.47	171.30	4.41
1:3 D	43.05	182.26	5.99
1:3 E	37.06	175.87	5.50
1:4 A	42.15	42.15	5.26
1:4 B	41.54	174.40	5.87
1:4 C	39.69	156.76	5.09
1:4 D	39.17	141.32	5.93
1:4 E	47.58	175.13	6.63
1:5 A	38.59	38.59	4.88
1:5 B	30.39	123.14	3.84
1:5 C	46.11	167.41	6.73
1:5 D	55.90	215.49	8.24
1:5 E	34.76	141.29	5.26

Table 2: Measurements Obtained from Proper Rinsing Prior to Freeze Drying
In this table, the dry mass measurements show a general decreasing trend for each ratio, which indicates that degradation is occurring.

Gel	Initial Mass (mg)	Wet Mass (mg)	Dry Mass (mg)
1:2 A	40.30	40.30	4.12
1:2 B	42.43	156.51	4.10
1:2 C	41.76	156.69	4.23
1:2 D	35.76	134.59	3.49
1:2 E	40.95	151.26	4.14
1:2 F	47.04	160.80	4.03
1:2 G	44.15	157.30	3.65
1:2 H	52.90	191.71	4.57
1:2 I	53.56	107.62	4.00
1:3 A	42.80	42.80	4.34
1:3 B	49.09	226.96	4.26
1:3 C	40.81	191.63	3.72
1:3 D	43.14	200.09	2.97
1:3 E	40.38	206.94	3.80
1:3 F	45.48	210.59	3.89
1:3 G	42.00	189.25	3.19
1:3 H	45.66	212.60	3.51
1:3 I	39.31	207.15	2.49
1:4 A	46.87	46.87	4.69
1:4 B	46.84	190.03	4.43
1:4 C	41.33	162.44	3.89
1:4 D	47.74	186.30	4.36
1:4 E	44.45	171.30	4.13
1:4 F	40.70	163.96	3.67
1:4 G	42.43	169.41	3.55
1:4 H	40.43	155.71	3.53
1:4 I	48.53	186.35	3.76
1:5 A	40.91	40.91	3.91
1:5 B	35.68	168.47	3.22
1:5 C	41.77	180.50	3.86
1:5 D	42.60	193.78	3.78
1:5 E	46.62	215.26	4.23
1:5 F	40.47	164.62	3.80
1:5 G	40.06	167.70	3.33
1:5 H	36.27	143.68	2.93
1:5 I	35.40	160.44	2.81

Biogel Release to the Ocular Surface of Epithelial Growth Factors (Ocular Biogel)

Project Design Specifications
May 5, 2010

Group Members: John Byce, Sarah Reichert, Anthony Sprangers, Alex Johnson, Jeff Hlinka

Advisor: Professor Brace

Function:

Significant dry eye is an affliction that affects up to ten million people in the United States. There are currently options available to treat the symptoms of dry eye, but a way to treat the causes and repair the damage has yet to be found. We aspire to design and fabricate a dissolving biogel that is capable of sustained release of epidermal growth factors that will work to maintain healthy epithelium and restore damaged tissue on the ocular surface.

Client Requirements:

- Design should incorporate a sustained release of growth factor.
- Product should dissolve in lacrimal fluid over a 7 to 14 day period.
- Must be harmless to the ocular surface of eye.
- Must hold up to the standards and regulations of the Food and Drug Administration.

1. Physical and Operational Characteristics

A. **Performance requirements**: The product will only be required to be used once, as it is intended to dissolve completely during use.
B. **Safety**: The product must not be harmful to the ocular surface of the human eye.
C. **Accuracy and Reliability**: The product must be extremely accurate in its sustained delivery as growth factors facilitate cell proliferation, which may be harmful to a user if the sustained delivery method fails. Along with this accuracy, there is a demand for complete reliability, as failure to function properly could be detrimental to the patient's health.
D. **Life in Service**: The ideal length of time that the product should be on the eye while dissolving and delivering medication is 7 to 14 days.
E. **Shelf Life**: The product should be capable of being stored in conditions similar to comparable products. This includes being stored at room temperature in a closed container for up to 24 months.
F. **Operating Environment**: The product design must be made to function on the ocular surface of a human patient. A typical ocular surface contains lacrimal fluid of pH range from 7 to 7.5. The normal temperature range of the eye is 32 to 34 °C.

G. **Ergonomics**: The final product must be easy to administer by an unqualified user. It must possess the ability to be quickly and efficiently placed, as many of its competing products are simple in terms of application. The product must also require minimal maintenance or re-application once it is applied.
H. **Size**: The product must either fit on the eye, or between the layers of conjunctiva on the surface of the eye and lower eyelid. The approximate area should be 2 mm by 5 mm. An estimate of about 3 to 5 mL in volume of biogel is expected to be sufficient for function, while maximizing comfort.
I. **Weight**: The product should be as lightweight as functionally possible, as it will be housed in the eyelid during use. A heavy product will cause discomfort and physical strain to the user.
J. **Materials**: All the materials used in this project must be compliant with the standards of the Food and Drug Administration, as it is designed for use on human subjects. Any materials that fit these criteria may be used.
K. **Aesthetics, Appearance, and Finish**: The product should not be distracting in appearance, as it should not be noticeable when placed on the eye.

2. Production Characteristics

A. **Quantity**: One biogel insert will be used per eye being treated at one time.
B. **Target Product Cost**: Similar products available on the market range from $100 to $120 for a one-month supply, so the entire product (biogel and growth factor) should be comparable in price.

3. Miscellaneous

A. **Standards and Specifications**: The final product will require the approval of the Food and Drug Administration.
B. **Customer**: Customers in search of a product to relieve dry eye desire ease of use and application, comfort and effective relief during use, and reasonable cost. All of these factors must be considered when designing a competing product for the market of dry eye relief.
C. **Patient-related Concerns**: As our design may eventually be commercially available for patient use, it must follow all restrictions enforced by the Food and Drug Administration. It must not cause harm to its users. The final product must also be ergonomically sound to ensure ease of use by an unqualified patient.
D. **Competition**: Restasis® is a prescription drug currently on the market that is used to treat chronic dry eye. It reduces inflammation and helps eyes increase tear production. There are also over-the-counter artificial tear lubricating drops, which are highly favorable for mild symptoms because of their price and ease of use.