Biogel Release to the Ocular Surface of Epithelial Growth Factors

Mid-Semester Report
March 5, 2010

Team Members
John Byce: Co-Leader
Sarah Reichert: Co-Leader
Anthony Sprangers: Communicator
Jeff Hlinka: BWIG
Alex Johnson: BSAC

Client
Dr. Neal Barney

Advisor
Professor Christopher Brace

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 have chosen to pursue the poly(ethylene glycol) hydrogel for our final design.

Table of Contents

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. 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, the membrane that lines the eyelids [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, resulting from both classes of dry eye, induce 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. Topical pharmacological agents that have been shown to secrete mucus include diquafosol, rebamipide, gefarnate, ecanet sodium and 15(S)-HETE. Diquafosol is the most researched agent of the five 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. While not all of the agents have been extensively researched and proven to work in humans, they may become useful as clinical therapeutics in the near future [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 proven 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 factors. These growth factors 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 living 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 human subjects. 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 time. 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 biogel must be exact and consistent in both its degradation and growth factor release rate. As

growth factors stimulate cell proliferation, failure to precisely control release rate could be extremely harmful to the patient. 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 biogel. Premature degradation is problematic, as it would result in a large, undesirable release of growth factors.

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 ten 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. 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 seven to seven and a half. 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 two by five millimeters 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

Poly(ethylene glycol) Hydrogel

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].

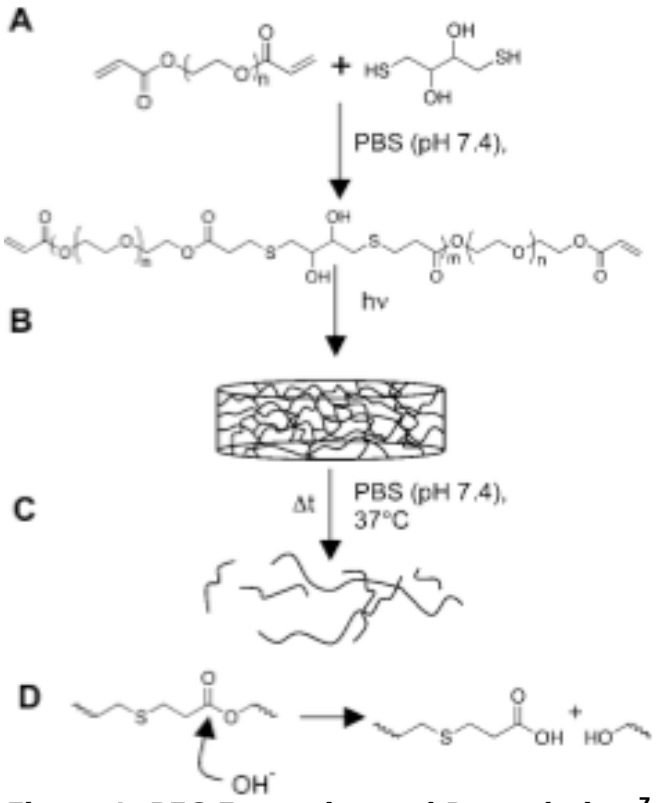

Figure 1: PEG Formation and Degradation [7]
(A) Addition reaction between poly(ethylene glycol) diacrylate and dithiothreitol.
(B) Photocross-linking of acrylate-terminated polymers.
(C) Degradation of hydrogel over time.

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 dithiol 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 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.

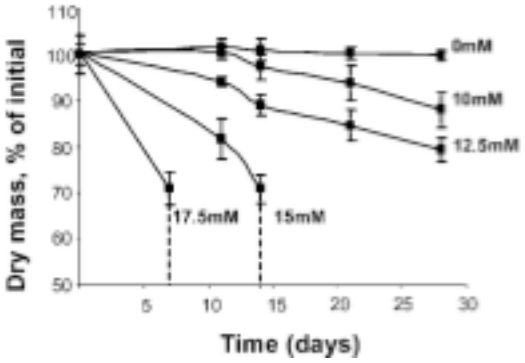

Figure 2: Hydrogel Degradation Over Time [7]
Hydrogels were prepared with 8 kiloDaltons PEG-diacrylate chains and the indicated concentrations of DTT. Dashed lines indicate complete degradation of the hydrogel.

Once the hydrogel network is established, the treatment that is to be administered to the patient can be incorporated into it 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 looked into 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].

Originally, collagen shields were developed as a bandage for corneal wounds to protect the healing ocular surface from damage caused by blinking. They started 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].

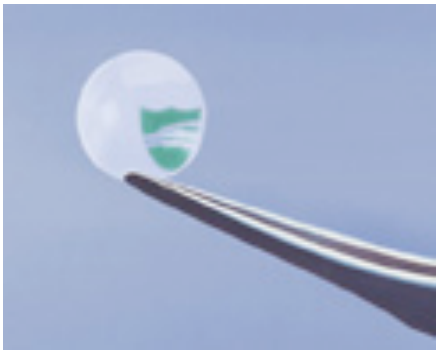

Figure 3: Oasis Collagen Shield [10]
The collagen shield fits onto the corneal surface of the eye in the same was as a contact lens.

Degradation rates for current collagen shields exceed three days and have been developed to dissolve over a period as long as 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. 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 when 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].

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 ^{o}C [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 ^{o}C, 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.

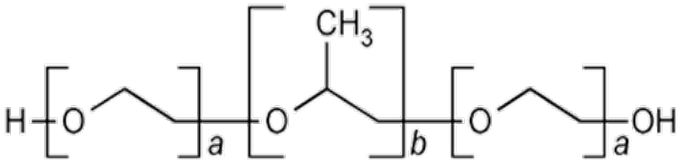

Figure 4: Poloxamer [12]
The monomer sequence [-H(C_2H_4O)(C_3H_6O)(C_2H_4O)OH-]of all poloxamers, the number of units gives the poloxamer varying properties.

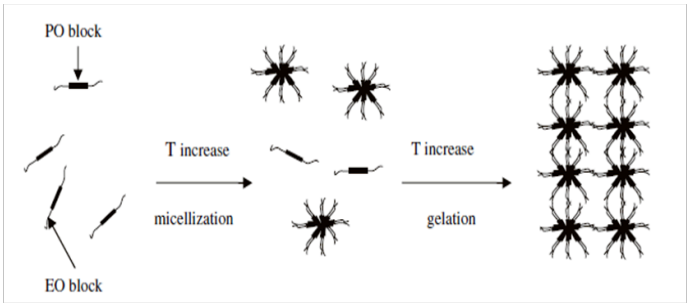

Figure 5: Schematic of Poloxamer 407 gelation as temperature increases [13]
The micelles contain a hydrophobic, PO block core with a hydrophilic, EO block exterior.

Along with thermo-reversibility, the Poloxamer 407 gel is non-toxic and allows for incorporation of both hydrophobic and hydrophilic drugs [13, 14]. Incorporating growth factors into the hydrogel can be done by soaking the gel in solution. Soaking the gel allows 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 concentration. 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 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 remainder of the semester. 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 chosen 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.

Desgin	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 ten 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 dollars. 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.

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 [18].

Epidermal growth factor (EGF) is the most promising growth factor for the repair of corneal epithelial cells, as it mainly functions to differentiate and proliferate epithelial cells. However, our final design may need to involve the use of more than one type of growth factor. This is because EGF is a relatively small growth factor and will likely entirely diffuse out of the biogel in less than the desired seven to ten day treatment period. A study evaluated by our client demonstrated how the corneal wound of an elderly woman was healed via exposure to insulin-like growth factor 1 (IGF-1) and substance P (SP). In the study, the patient was treated with

drops of IGF-1 and SP, administered in fifteen-minute time intervals over a two-hour period. After a week of treatment, the epithelial wound was fully healed [18]. Although the healing of corneal wounding is not the same as renewing epithelial cells under constant attack from inflammation, it is possible that these growth factors may possess the ability to carry out our desired function.

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, 6000 Daltons, in comparison with other growth factors [19]. This factor may cause an undesirable rapid diffusion, which would result in the gel releasing all of the growth factors encapsulated within its networks 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. Again, this results in a gel that releases all of its growth factors before full degradation. Incorporating multiple growth factors that all function to proliferate epithelial cells but vary in size could potentially solve this problem. Growth factors with various molecular weights and structures would begin diffusing out of the gel at different intervals in accordance to their size variation. A feasible addition, IGF-1, has a molecular weight of 7649 Daltons [20]. As a biogel containing both IFG-1 and EGF began to swell and degrade, EGF would begin diffusing first, while the larger IGF-1 would still be encapsulated in the network pores. IGF-1 would only be able to diffuse out of the gel upon further swelling. Multiple growth factors incorporated in this manor would allow for a sustained release for the entirety of the gel's degradation.

Future Works

Testing

Two key factors of the gel, degradation and drug release, will need to be tested. Initial testing will be administered to many gels of various concentrations in order to allow for altering the gel concentration to meet design specifications. The intended test for gel degradation is similar to the tests used in previous hydrogel studies. A hydrogel will be placed in a solution, then periodically taken out, blotted dry, and weighed [13]. Once gel degradation is determined, a gel of the same concentration will be soaked in a solution containing a known concentration of methyl blue. The absorbed concentration within the gel can be determined by comparing the concentration of methyl blue that remains in the solution after the gel is removed to the value prior to soaking. The gel will then be placed in a known volume of deionized water. Methyl blue has a maximum absorbing wavelength of 608 nanometers, so spectrophotometry can be used to test methyl blue concentration within the deionized water at periodic time intervals [17, 21].

Once initial testing is completed, the gel concentration that yields the optimal results for our purposes will be selected and undergo further testing. To better determine the exact degradation rate of the gel, gravimetrical analysis of dry mass will be used. Several gels of the same concentration will be placed in a solution, and each gel will be taken out at different time intervals to be freeze-dried and weighed [7]. For further testing of drug release, the selected gel will be placed in a known concentration of EGF solution, EGF uptake with be calculated in the same way as methyl blue. The gel will then be applied to a SV40-human corneal epithelial cell (HCEC) culture. This will allow for in vitro testing of EGF release on an epithelial culture medium [22]. If all of these tests prove to be successful, the product will need to undergo clinical trials to ensure its in vivo efficacy.

General

In the future, there is the possibility of incorporation of cell adhesion molecules to improve drug efficiency. Epithelial cell surfaces contain integrin proteins, which function to adhere other proteins and interact in cell-to-cell and cell-to-extracellular-matrix contacts. The integrin proteins of the epithelial cells in the eye are particularly good at adhering laminin, collagen, and fibronectin. They are partially responsible for cell migration, which is a key component in the treatment of the ocular surface [22]. Addition of a ligand into the hydrogel that would stimulate integrin protein function may increase epithelial cell proliferation, thereby increasing efficacy of the hydrogel.

In order to better control drug release rates, a drug delivery vehicle may also be integrated into the design of the hydrogel. A main necessity of the drug delivery vehicle would be incorporation of EGF. Drug delivery vehicles are advantageous for hydrogels applied to the ocular surface because they prevent the lacrimal enzymes from degrading the EGF molecule while it is still encapsulated within the gel, and would therefore increase drug efficacy [23]. Alginate microspheres are a conceivable option for a drug delivery vehicle because they have been previously tested in collagen hydrogels and are known to be able to encase growth factors[24].

Projected Costs

We are planning on creating and testing the PEG gel with the assistance of Dr. William Murphy, a faculty member of the College of Engineering at the University of Wisconsin-Madison. As the materials needed are present in his laboratory, they will not need to be purchased. Therefore, our primary expenses will be for purchasing the growth factors. Epidermal growth factor costs $180 per milligram [25]. If we decide to incorporate multiple growth factors to extend the time of release, we will purchase IGF-1, which costs $150 per milligram, or

SP, which costs $75 per milligram [26, 27]. The total projected cost of our product will be between $180 and $405. However, these prices are subject to change based on the possibility of receiving discounted prices through Dr. Murphy's laboratory.

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. 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
19. *Epidermal growth factor (EGF)*. Retrieved 2/12, 2010, from http://www.curehunter.com/public/keywordSummaryD004815.do
20. *IGF-1 (mouse, rat) ELISA*. Retrieved 2/15, 2010, from http://www.alpco.com/single.asp?CatNumber=22-IG1MS-E01
21. http://omlc.ogi.edu/spectra/mb/index.html
22. Elner, S. G., & Elner, V. M. (1996). The integrin superfamily and the eye. *Investigative Ophthalmology Visual Science, 37*(5), 696-701.
23. Gaudana, R., Jwala, J., Boddu, S., & Mitra, A. (2009). Recent perspectives in ocular drug delivery. *Pharmaceutical Research, 26*(5), 1197-1216.
24. Liu, W., Griffith, M., & LI, F. (2008). Alginate microsphere-collagen composite hydrogel for ocular drug delivery and implantation. *Journal of Materials Science: Materials in Medicine, 19*(11), 3365-3371.
25. Retrieved 3/3, 2010, from http://www.genscript.com/product_001/rec_protein/code/Z00333/category/protein/Epidermal_Growth_Factor_EGF_human.html
26. Retrieved 3/1, 2010, from http://www.steroidsfinder.com/buy-IGF1-L3R-1mg.htm
27. Retrieved 3/3, 2010, from http://www.anaspec.com/products/product.asp?id=30909&productid=15331

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

Project Design Specifications
March 10, 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 10 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 10 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.