Biomechanics of corneal wound healing

James Charles Ramier

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Biomechanics of Corneal Wound Healing

James Charles Ramier

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of MASTERS OF SCIENCE in Mechanical Engineering

August 1992

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Date: 8/13/92
ABSTRACT

Biomechanics of Corneal Wound Healing

The re-establishment of tissue continuity, function, and mechanical properties after a through-and-through incision is an important issue in corneal surgery and trauma. Variations in healing can affect vision and patient activity during the recovery period, which may last up to five years.

Growth factors introduced into the open wound have the potential to speed up the wound-healing process. The current investigation is concerned with quantitatively characterizing the mechanical properties of corneal tissue and evaluating the effects of epidermal growth factor (EGF), insulin-like growth factor (IGF), and platelet-derived growth factor (PDGF) on accelerating the wound-healing process as compared to a control group treated with phosphate-buffered saline (PBS).

A 30 day topical application period was employed to assess the influence of EGF, IGF, and PDGF versus PBS treated corneas following through-and-through incisions. Corneal test specimens were harvested at 3, 10, and 30 days postoperatively and subjected to uniaxial tension loading (perpendicular to the wound) with load and displacement values recorded. Gross strength properties of fracture toughness, peak tangent modulus, and peak stress were calculated and the stiffness characteristics of the test specimens were obtained through curve fitting of the stress-stretch ratio profile.

Comparison of strength properties indicated that IGF application enhanced peak stress, tangent modulus, and fracture toughness at 3 and 10 days while EGF application produced dominant responses at 30 days. Stiffness characterization revealed enhanced stiffness with EGF at 3 and 30 days, while PDGF treatment showed dominant stiffness characteristics at 10 days. The results suggest that select growth factors may preferentially augment the time course of corneal wound healing.
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<tr>
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<td>Adenosine Diphosphate</td>
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<td>BID</td>
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<td>ECM</td>
<td>Extra Cellular Matrix</td>
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<td>EGF</td>
<td>Epidermal Growth Factor</td>
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<td>Platelet-Derived Growth Factor</td>
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<td>TGF</td>
<td>Transforming Growth Factor</td>
</tr>
<tr>
<td>TID</td>
<td>Three times daily</td>
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1. INTRODUCTION

1.1 Overview

Wound healing is an important issue in corneal surgery and trauma where the reestablishment of tissue continuity and function is of central importance. Variations in healing can affect the refractive characteristics of the cornea (27). The loss of strength at the wound and its progressive return will affect patient activity during the recovery period (27). The effect of growth factors in speeding up the healing process in corneas subject to through and through incisions is the topic of this report.

The time course of skin wound healing has been documented (4,21,23) along with the general phases of healing involved in the process (4). However, documentation regarding the time course of corneal wound healing and the influence of growth factors in augmenting this time course is only lightly covered in the open literature.

A quantitative characterization of corneal tissue from an engineering standpoint was necessary for qualitative comparisons of the growth factor applications. The idea of pseudoelasticity was utilized to describe the stress stretch ratio curve obtained from uniaxial tension tests of cornea specimens, which likewise quantify the stiffness characteristics of the wound.

An acceleration in the return of normal mechanical properties to corneal wounds through the use of growth factors would be a breakthrough of major importance. Research of this kind may be very beneficial in the field of medicine with effects reaching beyond that of corneal wound healing. The remainder of this paper is dedicated to detailing the wound-healing process, use of growth factors, and the quantitative characterization of wound healing.
1.2 Literature Review

Gasset and Dohlman (13) document the time course (up to 100 days) of the tensile strength of several different types of corneal wounds. The tensile strength of cornea is attributed to the length and the organization of polymerized collagen within the fibrils, with the cross-linking mechanisms and architectural pattern of the fibers being more important than the amount of collagen present (4,6,9,13,20). The first three weeks post injury is the time of greatest histochemical and histological activity (20,28). Gasset and Dohlman (13) demonstrate that by 100 days the strength of a corneal wound was only 50% that of a normal cornea.

Augmentation of the time course in corneal wound healing has been lightly investigated previously. Epidermal growth factor has been conclusively shown to accelerate corneal wound healing in rabbits (17,18,19,22,25,29). Maurice (20), noting corneas lack of blood vessels and the multitude of growth factors released by platelets, speculates on the potential positive effects of blood elements introduced into an open wound. However, the quantitative assessment of platelet-derived growth factor and other growth factors has not yet appeared in the open literature. Further discussion of wound healing from general and cornea specific aspects is conducted in the next Section.
2. INTRODUCTION TO WOUND HEALING

2.1 General Wound Healing

Wound healing has been the subject of much research, particularly documenting the time course of wound strength from histological and strength perspectives.

The wound healing process has been described, in a simplified form, as possessing three main overlapping phases (4) (see Figure 1):

1.) Inflammation (early and late)
2.) Granulation tissue production
3.) Matrix formation and remodeling

The following will briefly describe each phase. A flow-chart of significant healing phases and subphases is depicted in Figure 2.

2.1.1 INFLAMMATION

The early inflammatory response is characterized by a series of events starting with blood vessel disruption which leads to extravasation\(^1\) of blood constituents and concomitant platelet\(^2\) aggregation, blood coagulation, generation of Bradykinin\(^3\) and complement\(^4\) derived anaphylatoxins\(^5\) (4). The activated platelets release numerous biologically active substances that promote cell migration and growth into the site of injury. Intrinsic activities of blood vessel endothelium\(^6\) limit the extent of platelet aggregation and blood coagulation to the wounded area.

---

\(^1\) The escape of fluids into the surrounding tissue.
\(^2\) Small, anuclear blood cells.
\(^3\) Plasma polypeptides possessing considerable biological activity, capable of increasing blood flow in, and the permeability of, small blood capillaries.
\(^4\) The term complement encompasses a group of plasma proteins that, when appropriately activated, bring about responses that defend the body against injury.
\(^5\) Anaphylatoxins increase vascular permeability.
\(^6\) A form of squamous (flat, scaly cells) epithelium consisting of flat cells which line the blood and lymphatic vessels, the heart, and various other body cavities.
Phases of wound repair. Healing of a wound has been arbitrarily divided into three phases: (A) inflammation (early and late), (B) granulation tissue formation, and (C) matrix formation and remodeling. These phases overlap considerably with one another and are plotted along the abscissa as a logarithmic function of time. Inflammation is divided into early and late phases denoting neutrophil-rich and mononuclear cell-rich infiltrates, respectively. The magnitude of wound contraction parallels granulation tissue formation, as indicated. Collagen accumulation actually begins shortly after the onset of granulation tissue formation, continuing throughout the phase of matrix formation and remodeling.

Figure 1: Time Course of Skin Wound Healing (4)
Figure 2: The Wound Healing Process. (Based on information from (4,16))
Cell activation or death at a wound site induces an influx of inflammatory leukocytes\(^7\) and increases the permeability of undamaged vessels adjacent to the injured area resulting in more widespread leakage of plasma\(^8\) proteins. The first leukocytes observed infiltrating an area of inflammation and injury are neutrophils which are shortly followed by monocytes (4). Both are attracted by a variety of chemotactic factors. The function of neutrophils in an injury site is to rid the area of bacterial contamination, while the influx of monocytes and their conversion to macrophages is seen as critical to the initiation of tissue repair. Clark (4) makes reference to an arbitrary division made between the early and late phases of inflammation occurring as neutrophil infiltrate resolves and macrophage accumulation continues.

The late phase of inflammation is a continued period of macrophage action. Tissue macrophages, in addition to functioning like neutrophils, phagocytose effete neutrophils and also release biologically active substances. Peripheral blood monocytes continue to infiltrate the wound site progressively exhibiting the phenotype\(^9\) of macrophages. Platelets release adenosine diphosphate (ADP), facilitating coagulation, generating more thrombin, while ADP and thrombin recruit additional platelets. Several adhesive proteins are released when platelets discharge \(\alpha\) granules including:

1.) Fibrinogen  
2.) Fibronectin  
3.) Thrombospondin  
4.) Von Willebrand factor VIII

Growth factors are released concomitant with the release of \(\alpha\) granules. Platelet fibrinogen, once converted to fibrin by thrombin, adds to the fibrin clot and both fibrin and fibronectin act as a provisional matrix for the influx of monocytes (critical to initiation of tissue repair) and fibroblasts\(^10\), a major component of granulation tissue.

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\(^7\)White blood corpuscles.  
\(^8\)Blood plasma consists of water in which numerous chemical compounds, both solids and gases, are dissolved.  
\(^9\)The outward, visible expression of the hereditary constitution of an organism, traits.  
\(^10\)Cells from which connective tissue is developed.
Macrophages have a pivotal role in the transition between wound inflammation and wound repair. Macrophages and platelets produce growth factors and other substances crucial to initiation of granulation tissue formation.

Blood clotting may be considered a part of the inflammatory response, since Hageman factor (HF) activation leads to Bradykinin generation and initiation of a classic complement cascade\textsuperscript{11}. Blood clotting occurs through one of three major routes:

1.) HF is activated by adsorption onto fibrillar collagen or other suitable surfaces and induces an intrinsic (3,4) coagulation system.

2.) Factor VII, activated by tissue procoagulant factor found in interstitium and released from damaged cells induces an extrinsic (3,4) coagulation system.

3.) Platelets and endothelial cells activated by low levels of thrombin and platelets activated by contact with fibrillar collagen express coagulation factors and phospholipids that facilitate clotting.

The inciting event of clotting is the expression of a surface that promotes adsorption and activation of specific coagulation proenzymes.

**2.1.2 Granulation Tissue Production**

Granulation tissue consists of a dense population of macrophages, fibroblasts, and neovascularure\textsuperscript{12} embedded in a loose matrix of collagen\textsuperscript{13}, fibroneectin, and hyaluronic acid (HA). The process of formation of granulation tissue includes the accumulation of macrophages, ingrowth of fibroblasts, deposition of loose connective tissue, and angiogenesis\textsuperscript{14} (4).

\textsuperscript{11} The classic complement cascade is a chemical reaction involving distinct proteins.

\textsuperscript{12} New blood vessel formations.

\textsuperscript{13} A fibrous insoluble protein found in connective tissue. Collagen represents about 30% of the total body protein.

\textsuperscript{14} The development of new blood vessels.
Fibroblasts proliferate and migrate into the wound space, undergoing an alteration of their cell phenotype, thus permitting cell motility and concomitant deposition of of loose extracellular matrix (ECM). These fibroblasts also align themselves and newly-deposited matrix along the radial axes of the wound forming cell-to-cell and cell-to-matrix links. This linkage enables a tension resulting in wound contraction. This matrix also provides a substrate upon which macrophages, new blood vessels, and more fibroblasts can migrate into the wound.

It should be noted that macrophages, fibroblasts, and blood vessels move into the wound space as a unit affirming interdependence. As fibroblasts move into the wound space they deposit loose ECM initially composed of large quantities of fibronectin. Fibronectin has several properties that aid in the formation of granulation tissue including the capacity to bind connective tissue cells and ECM simultaneously. The ability for cells to rapidly adhere to, and detach from, fibronectin substratum and the fibronectin matrix allows for fibroblast movement over a surface. Wound contraction has been ascribed to actin-rich myofibroblasts which are the most numerous cells in granulation tissue. These myofibroblasts are typically found aligned along the lines of contraction.

Peripheral blood monocytes activated by inflammatory macrophages establish a situation for continual synthesis and release of growth factors. Platelets are the first cell component to release substantial amounts of preformed growth factors into the wounded area including Platelet-derived growth factor (PDGF), Epidermal growth factor (EGF), Fibroblast growth factor (FGF), and Transforming-growth factor type α or type β (TGF - α, TGF - β) (more information on growth factors is introduced in Section 2.3).

Neovascularization occurs simultaneously with fibroplasia through capillary buds that sprout from blood vessels adjacent to the wound and extend into the wound space. Blood vessel growth is suspected to depend on the

---

15 Formation of fibrous tissue.
following phenomena (4):

1.) Phenotype alteration (endothelial cell)  
2.) Stimulated migration  
3.) Mitogenic stimuli  
4.) An appropriate extra cellular matrix  
5.) A free edge defect

Endothelial cells adhere avidly to fibronectin, therefore blood vessel fibronectin may act as a contact guidance system for endothelial cell movement at the tip of capillary buds (4).

Epithelial reformation re-establishes the protective barrier of the tissue to withstand infection and dehydration. Re-epithelialization is considered with granulation tissue development since both processes represent new tissue generation and since re-epithelialization and granulation tissue growth occur synchronously. Re-epithelialization begins with the movement of epithelial cells from the free edge of the wound across the defect. These cells have previously undergone marked phenotypic alteration allowing for mobility and the motor apparatus for motility. It is interesting to note that in cutaneous\textsuperscript{16}, cells move in a leapfrog fashion while in the cornea, cells move in a single file with the lead cells remaining in the lead (4).

2.1.3 MATRIX FORMATION AND REMODELING

Remodeling is the replacement of temporary fibrils with new fibrils that correspond more closely with the diameter and organization of normal tissue. Structural support and strength in new tissue is provided by collagen. Collagens are a family of closely related triple-chain glycoproteins that are found in ECM. There are at least three types of collagen found in connective tissue:

1.) Fibrillar, which have uninterrupted triple helices (type I, type II, and type III).

\textsuperscript{16} Skin.
2.) Basement membrane collagen which has an uninterrupted triple helix and forms a meshwork in the lamina densa of membranes (type IV).

3.) Other interstitial collagens with interrupted helices (type V).

The process of matrix formation and remodeling occurs and begins simultaneously with granulation tissue formation. Remodeling is the term used to describe the period when the matrix is altered with rapid elimination of fibronectin and the slow accumulation of large fibrous bundles of collagen (type I) which provides the residual scar with increasing tensile strength (4).

The geometry of the collagen network will determine the mechanical properties of the wound. Collagen fiber diameter increases with time post wounding and is related to tensile strength. It should be noted that for cutaneous tissue the maximum strength of the scar tissue is only 70% as strong as intact skin (4).

Extra cellular matrix is deposited first at the wound margin along with the initial development of granulation tissue and more centrally as granulation tissue grows into the wound space. The composition and structure of granulation tissue ECM depends on the elapsed time since injury and the distance from the wound edge.

The initial deposits of ECM contain large quantities of fibronectin. Fibronectin plays a critical role during the formation of granulation tissue, also in matrix formation and remodeling. Fibronectin may serve as a template for fibrillar collagen deposition (4). Fibrillar collagens form fibrous bundles that greatly enhance tissue tensile strength.

Hyaluronic acid is a linear polymer occurring in early granulation tissues playing a critical role in fibroblast proliferation (4). HA appears, during morphogenesis, at times of cell movement and mitosis and disappears at the onset of differentiation. As granulation tissue matures, HA is replaced by sulfated glycosaminoglycans (GAGs, proteoglycans) that provide the tissue
with more resilience but accommodate cell movement and proliferation less well. GAGs are a prominent component of the interface between epithelium and mesenchyme\textsuperscript{17}, which play a critical function during wound repair with regard to re-epithelialization and angiogenesis. Therefore the early matrix consisting of fibronectin and HA, which was conducive to cell migration and proliferation, is converted to a collagen and proteoglycan matrix that increases the tissue tensile strength and resilience.

### 2.2 Corneal Wound Healing

Figure 3 is a cross section of an eyeball. The first step in sight is when light enters the eye through the cornea which is subsequently focused by the lens onto the retina. Light is able to pass through the cornea, in part, because of its avascularity\textsuperscript{18}.

The cornea consists of three principal layers (see Figure 4):

1.) Epithelium
2.) Stroma
   a. Anterior lamina
   b. Substantia propria corneae
   c. Posterior elastic lamina
3.) Endothelium

The overall anatomy of the cornea is depicted in Figure 4 for a specimen taken near the margin, or juncture tissue between cornea and sclera.

The epithelium is composed of stratified squamous cells and serves as a barrier covering the front of the cornea and functions to transport fluids into and out of the stroma.

Ordered from exterior to interior, the stroma, which makes up 85% to 95% of the corneal thickness, is further composed of three sub-layers. The anterior lamina, also known as Bowman’s membrane, consists of closely interwoven fibrils containing no corneal corpuscles (modified fibroblasts) and is

\textsuperscript{17} A diffuse network of cells giving rise to connective tissues.

\textsuperscript{18} Lack of blood vessels.
Figure 3: Cross Section of Eye. (Adapted from Gray's Anatomy, 29th American Edition, 1973, ed by Goss, C.M., Lea & Febiger, Philadelphia, page 1046)
Vertical section of human cornea from near the margin. (Waldeyer.) Magnified. 1. Epithelium. 2. Anterior lamina. 3. Substantia propria. 4. Posterior elastic lamina. 5. Endothelium of the anterior chamber. a, Oblique fibers in the anterior layer of the substantia propria. b, Lamellae, the fibers of which are cut across, producing a dotted appearance. c, Corneal corpuscles appearing fusiform in section. d, Lamellae, the fibers of which are cut longitudinally. e, Transition to the sclera, with more distinct fibrillation, and surmounted by a thicker epithelium. f, Small blood vessels cut across near the margin of the cornea.

**Figure 4**: Cross Section of Cornea. (Adapted from Gray’s Anatomy, 29th American Edition, 1973, ed by Goss, C.M., Lea & Febiger, Philadelphia, page 1051)
regarded as a condensed part of the substantia propria. The substantia propria corneae is a fibrous, tough, and unyielding transparent layer of roughly 60 flat superimposed lamellae made up of bundles of modified connective tissue. Corneal spaces contain corneal corpuscles. The posterior elastic lamina, also known as Descemets membrane, is an elastic, transparent, homogeneous membrane of extreme thinness covering the posterior surface of the substantia propria corneae.

**Endothelium** is a single mesothelial\(^{19}\) layer covering the posterior surface of the elastic lamina functioning both as a barrier (through the restriction of movement of fluid from the aqueous humor into the hydrophilic stroma) and as a pump (effecting ion-coupled fluid transport in a direction opposite to that of the fluid leak). Endothelium has little or no regenerative capability, therefore corneal transplantation is the only current method for replacement of damaged or diseased endothelium (14,18).

In the eye, wound closure and healing following corneal wounding involves a complex series of events in both the epithelium and the stroma. Maurice (20), in his review of skin wound healing, noted that many of the processes are common between the skin and the cornea. The roles of monocytes, their transformation into fibroblasts, and the lining of the wound edges with fibronectin are also of importance in the corneal wound-healing cycle.

The corneal wound healing phase analogous to granulation tissue production in the skin similarly consists of a dense population of macrophages and fibroblasts of which keratocytes\(^{20}\) are a major supplier (5,20). Damaged fibrils are digested and replaced with new less organized ones during this phase.

\(^{19}\) Forms the simple squamous epithelium that covers the surface of all true serous membranes.

\(^{20}\) The mesenchymal cells of the cornea.
2.2.1 **EPITHELIAL RESPONSE**

Epithelial wound closure occurs through the migration of the remaining epithelial cells surrounding the defect area and the formation of an epithelial plug (5). Two phases have been characterized in this closure, the latent and the healing. The latent phase is when the cellular and subcellular reorganization of cells occur, and the healing phase is when the closure of the wound occurs at a steady rate through a single layer of motile cells. These phases correspond to the inflammation and granulation-tissue-production phases of skin wound healing.

2.2.2 **STROMAL RESPONSE**

It is known that in the initial stages of healing, the epithelium migrates into the tract of the stromal wound space forming an epithelial plug which retracts as the stromal scar is formed. The role of stroma in the healing of a corneal wound has not been fully characterized in the open literature.

2.2.3 **ENDOTHELIAL RESPONSE**

The initial response of the endothelial monolayer to wounding is the activation of the surviving endothelial cells to surround the wound area, these cells then enlarge, flatten, and migrate to the damaged area (14). Three stages in endothelial wound healing have been identified (14). In stage 1 the wound site becomes covered by spindle shaped cells with the corneal thickness being at a maximum. Endothelial pump site density is at a minimum and the new endothelial barrier begins to form. Stage 2 shows the initiation of an intact and contact-inhibited (14) monolayer where cells flatten, become polygonal and form junctional complexes. Endothelial permeability and pump site density return to normal, resulting in corneal thickness experiencing its greatest decrease. Stage 3 is a continuation, despite prior normalization of endothelial function, of morphologic remodeling and decreasing corneal thickness.
2.3 Growth Factors

Traditional cell culture techniques continue to evolve with the discovery of the growth promoting effects of certain proteins on different cells. The growth of normal cells is typically controlled by a variety of substances, including a group of polypeptide growth factors which are hormone-like agents in both function and structure. Growth factors are all polypeptides that exert their biological information through specific membrane receptors\(^{21}\). Certain growth factors affect specific cells differently than others, and some growth factors are species specific, while others are not.

2.3.1 EPIDERMAL GROWTH FACTOR (EGF)

Epidermal growth factor can be obtained from mouse submaxillary glands and has been identified in various human tissues and fluids including urine, saliva, plasma, and breast milk. EGF can also be synthesized using recombinant DNA techniques.

Epidermal growth factor has been shown to enhance growth and maturation of organ cultures of epithelial tissues, accelerate the epithelial healing rate in non-penetrating scrape wounds and to increase wound strength after full-thickness stromal incisions (16,17,18,19,22,25,29). EGF has been observed as a potent mitogen for fibroblasts and can stimulate or inhibit proliferation or differentiation of a wide variety of cells. EGF enhances the wound strength of full-thickness corneal wounds in a dose-dependent manner (19).

Epidermal growth factor receptors are found on corneal epithelium, endothelium, conjunctiva\(^{22}\) and the posterior pigmented layer of the iris and the lens epithelium.

\(^{21}\) Receptors are groups of cells functioning in reception of stimuli.

\(^{22}\) The mucous membrane of the eye lining the inner surface of the eyelids, reflected over the anterior part of the sclera.
2.3.2 INSULIN-LIKE GROWTH FACTOR (IGF I, IGF II)

Insulin-like growth factors, so named for producing insulin-like actions, are polypeptides obtained from human plasma. IGF's link (without inducing) growth hormone and the stimulation of metabolic processes leading to cell proliferation. IGF's have been characterized as a mitogenic stimulus for multiple cell types (16,18).

2.3.3 PLATELET DERIVED GROWTH FACTOR (PDGF)

Platelet-derived growth factor plays a role in the normal tissue repair process. It has been shown to be chemotactically active for human monocytes, neutrophils, smooth muscle cells, and fibroblast activities related to tissue inflammation and repair following wounding (16).

2.4 Significance of Research

Rapid return of normal mechanical properties is one of the primary goals of wound repair. The acceleration of the naturally-occurring wound healing process is an important issue in corneal surgery, trauma, and infection. Variations in healing can affect ocular refractive characteristics, and the strength of the wound will have an effect on patient activity during the recovery period. Under normal circumstances, human corneal wound healing is considerably slower than in some animals.

Growth factors play important roles in inflammation and wound healing in cutaneous tissue repair. It is therefore logical to investigate the potential of growth factors in promoting corneal wound healing. The focus of the research documented herein is to quantify the biomechanical properties of corneal tissue during healing following a through-and-through incision. The biomechanical properties will be used to assess whether growth factors such as EGF, IGF, and PDGF can accelerate corneal wound healing versus normal controls treated with phosphate buffered saline (PBS).
3. MECHANICAL CHARACTERIZATION OVERVIEW

It is well known that many biological tissues, including cornea, exhibit hysteresis when subjected to cyclic loading and unloading (10). When held at a constant strain (defined as the ratio of the total deformation over the original length), stress relaxation in the tissue occurs (decreasing reaction load) and when held at a constant stress (defined as the force per unit area, in axial loading the magnitude of the applied force divided by the cross sectional area), the tissue exhibits creep (continued elongation stress). Strain relationships for biological tissues are generally nonlinear with the material properties exhibiting directional sensitivity (anisotropy).

3.1 Pseudoelasticity

For many problems involving finite deformation of biological tissue it is convenient to use constitutive equations relating stresses to finite strains. It is also convenient to compare material constants involved in the constitutive equations in order to assess changes in mechanical properties.

For preconditioned tissues (tissues subjected to the loading - unloading cycle until the stress - strain relationship is well defined, repeatable, and predictable) the stress - strain relationship is unique for the loading branch and the unloading branch separately. Thus the material can be treated as one elastic material in loading and another elastic material in unloading, and the theory of elasticity can be employed to handle each material during its respective load/unload regime. This analysis approach is termed “pseudoelasticity”. Pseudoelasticity, while not an intrinsic property of the material, is a convenient description of the stress - strain relationship during loading or unloading (12).

Fung (12) elaborates on the usefulness of the concept of pseudoelasticity extolling its virtue of relative strain rate insensitivity. For our study, a constant strain rate was used throughout the experiments to limit rate-dependent effects, such as dissipation.
3.2 Postulated Analytical Model

Several mathematical formulas were reviewed to characterize the stress-stretch profile of our specimens subject to uniaxial extension. Previous work (7,8,15,24) on highly elastic material suggest that the strain energy, hence stress-strain relation, depends on the strain invariants $I_1$, $I_2$, and $I_3$ which are defined through material geometric stretch ratios. Under the assumptions of isotropy, homogeneity, and incompressibility, the following strain invariants for soft biological tissues were defined by Demiray (7,8) for uniaxial extension:

\begin{align*}
I_1 &= \lambda^2 + 2/\lambda \\
I_2 &= 1/\lambda^2 + 2\lambda \\
I_3 &= 1
\end{align*} \hspace{1cm} (1.)

where $\lambda$ is the stretch ratio (stretch ratio = strain + 1) in the axial direction ($\lambda_1$), defined as the deformed length divided by the original length (see Figure 5). $I_3 = 1$ reflects the incompressibility assumption for the tissue. This formulation corresponds with that of Rivlin and Saunders (24), and Kao and Razgunas (15). It should be noted that in the undeformed state $\lambda_1 = \lambda_2 = \lambda_3 = 1$ such that $I_1 = I_2 = 3$.

After review and trial and error it was decided that the following quantification would best suit our needs. This decision was based on factors discussed in Sections 4.2.2 and 5.1. Fung (10) postulates a uniaxial stress-stretch ratio relation as:

$$ \sigma = C(\exp(B\lambda)) - A $$ \hspace{1cm} (2.)

where $\sigma$ is stress, $\lambda$ is the stretch ratio in the axial direction, with parameters $A$, $B$, and $C$ determined experimentally.
Taking this a step further, C can be determined by finding a reference point on the curve, say $\sigma = \sigma^*$ when $\lambda = \lambda^*$. Therefore (10):

$$\sigma = (\sigma^* + A)\exp(B(\lambda - \lambda^*)) - A \quad (3.)$$

Note: if $\lambda$ is referred to the natural state, we must have by definition, $\sigma = 0$ when $\lambda = 1$. This is only possible if:

$$A = \sigma^*\exp(B(1 - \lambda^*))/\{1 - \exp(B(1 - \lambda^*))\} \quad (4.)$$

The slope of the stress - stretch ratio curve defined in equation (3.) can be examined by differentiating (3.) with respect to $\lambda$. This yields, after manipulation:

$$\frac{\partial \sigma}{\partial \lambda} = D(\exp(B\lambda)) \quad (5.)$$

where

$$D = (\sigma^* + A)B/\exp(B\lambda^*) \quad (6.)$$

Note that $\sigma^*$ and $\lambda^*$ are reference parameters and hence variations in material parameters $A$ and $B$ are incorporated in the definition of $D$ and equation (6.). The rate of load bearing with increments in stretch ratio can be assessed through comparison of both $B$ and $D$ material parameters.
\[ \lambda_1 = \frac{L}{L_0} \]
\[ \lambda_2 = \frac{d}{d_0} \]
\[ \lambda_3 = \frac{w}{w_0} \]

Figure 5: Stretch Ratio Definition
4. METHODOLOGY

4.1 Experimental Protocol

An in vivo animal model was determined to be the appropriate approach for this research since it would allow the knowledge gained to be applied to humans in the clinical situation. All animals used in this study were treated in accordance with the ARVO Resolution on the Use of Animals in Research. The surgical and specimen preparation described in Sections 4.1.1 and 4.1.2 were conducted by research colleagues at the University of Rochester Cornea Research Laboratory (27).

4.1.1 Wounding Procedure (27)

White New Zealand rabbits were put under general anesthesia using intravenous sodium pentobarbital (30 mg / kg) with local reinforcement administered preoperatively (Topical Proparacaine drops). After the animals were found to be appropriately anesthetized, nictating membranes were cut bilaterally and with the aid of a Zeiss operating microscope (Carl Zeiss, Inc., Thornwood, NY) the rabbits received full thickness incisions, made with a saphire knife, on both corneas in an approximately six mm straight line running horizontally. Collagen shields were placed on both eyes with the right eye shield presoaked in growth hormone and the left eye shield presoaked in saline. Collagen shields were removed after 24 hours. Each day animals received one drop of gentamycin in both eyes, phosphate buffered saline (PBS) drops in the left eye (control) and the proper growth hormone in the right, all given three times daily (TID). Growth hormones (EGF, IGF, PDGF) were administered in concentrations of 100 mg / kg.

Precautions were taken to minimize wound contamination and postoperative infection (including conjunctival infection and exudate) including cleansing the periorbital skin around the eyes with betadine solution preoperatively and the administration of bentamycin eye drops, one drop twice daily (BID) in both eyes beginning immediately after the procedure and continuing until seven postoperative days. Animals were killed with an intra-
Missing Page
Figure 6: Sketch of Mounted Specimen
Figure 7: Sketch of Test Set-Up (27)
Voltage was easily converted to force (kg) using the formula:

\[ \text{volts} \times \text{full scale range (}.1, .2, \text{or} .5 \text{volts)} / 10 \quad (7.) \]

A simultaneous videotape record of the deformation time course was made with the load cell output voltage superimposed. The data from the strip chart and videotape were used to calculate tissue geometry, maximum wound strength, peak tissue stiffness, and tissue fracture toughness (the test set-up is shown in Figure 7). Video data were digitized and used to determine tissue load - deflection diagrams suitable for curve-fitting to the analytical model outlined in Section 3.3

### 4.2 Analysis Protocol

The videotaped record of the deformation time course in combination with the strip chart data provided sufficient information for analysis.

#### 4.2.1 VIDEO DIGITIZING

The main tool used for video digitizing and analysis was the JAVA™ version 1.20 (Jandel Scientific, Corte Madera, CA) software operating on an IBM PS/2 Model 30-286 personal computer, JVC HR - D67OU Video Cassette Recorder, a Panasonic WV - 3240 Video Camera, and a Panasonic CT - 1330 M monitor. The image analysis system contained in this software had several features ideally suited for our purposes including The Frame Grabber, Morphometric Measurement, and The Data Worksheet.

The **Frame Grabber** (video digitizing) creates a junction among the hardware elements of JAVA: the camera (or VCR), the video monitor, and the computer. The ability of this software to freeze (grab and store) an image from a stream of images by converting the frame to a set of digital values lends itself to manipulation and measurement.

The **Morphometric Measurement** system utilizes the x and y locations of the video monitor pixels (discreet cells) to form the basis for geometric mea-
Missing Page
Figure 8: Typical Load-Deflection Curve (27)

Figure 9: Typical Data Segment for Curve Fitting Prior to Conversion to Stress Along Ordinate and Stretch Ratio Along Abscissa
4.2.2 STRESS-STRETCH RATIO CHARACTERIZATION

Another software package, ASYSTANT 1.1 (Asyst Software Technologies Inc., Rochester, NY) operating on an IBM PS/2 Model 30-286, was used exclusively for compliance characterization via curve-fitting stress-stretch ratio data obtained when each specimen was loaded.

Curve fitting is simply the application of a quantitative relationship containing a number of parameters to a set of paired x and y (y depending on x) data in order to represent that data at infinite x locations within the range of the original x data. The experimental stress-stretch ratio data is specified as $x_i$ (stretch ratio) and $y_i$ (stress) data pairs at the $i$th location over $n$ discreet values of stretch ratio. A typical data segment used in this phase is shown in Figure 9 which is recognized as a subset of the data depicted in Figure 8. The two main steps involved in curve fitting are the choice of the quantitative relation and the adjusting of the fit parameters until a "best fit" is obtained. The goodness of the fit can be determined from three values computed by ASYSTANT: the sum of the squared residuals ($\text{Err}^2$), the square of the multiple correlation coefficient ($R^2$), and the significance of fit (Sig) which is computed by applying an F-test to the multiple correlation coefficient. Mathematical definitions of these coefficients obtained from the 1988 ASYSTANT, Student Version, Analysis Reference manual (Asyst Software Technologies, Inc., Rochester, NY) are shown below.

$$\text{Err}^2 = \sum_{i=1}^{n} (y_i - F[x_i])^2 \quad F = \text{user function} \quad (8.)$$

$$R^2 = 1 - \text{Err}^2/\text{Res}^2 \quad \text{Res}^2 = \sum_{i=1}^{n} |y_i|^2 \quad (9.)$$

$$\text{Sig} = P_{F[n-m,m]}(F > F) \quad F = R^2(n-m)/(1 - R^2)m \quad (10.)$$
Note that Err² is merely the result of a least squares fit of a user-defined approximation F(x) to the dependent data set yᵢ at each discreet xᵢ.

ASYSTANT consists of a Desk Calculator that provides much of the raw-calculating capabilities of the system, and a number of Main Menu Options. Data is passed between the options through the Desk Calculator Parameters and Variables, which provide storage of numbers and arrays, and are always available for computation.

The Main Menu Option used for the stress-stretch ratio characterization was the Curve Fit option with a user-supplied fitting function. Data points for the stress-stretch ratio curves were input into ASYSTANT's desk calculator storage locations manually along with a curve-fitting function. The Gauss-Newton algorithm contained within ASYSTANT was used to adjust the parameters until the disparity between successive parameter modifications was small enough to satisfy the convergence criterion which was chosen as no change in the fifth decimal place of the R² value.

4.2.3 DATA REDUCTION

In order to compare various experimental specimen data sets using plots of stress versus stretch ratio, it was necessary to modify the original data values of force and displacement. Stress was obtained by dividing force by the undeformed cross sectional area at the wound site, this is termed the engineering stress as it takes the geometric factor of original cross sectional area into consideration. The stretch ratio is defined simply as the deformed length divided by the original length. The stretch ratio is a dimensionless number and is equivalent to strain + 1.
5. RESULTS

In order to successfully complete this project it was necessary to:

- Characterize the stress - stretch ratio curve of corneal tissue for comparative purposes
- Evaluate the effects of the growth factors on the mechanical properties of corneal tissue

5.1 Analytical Formulation

Based on the literature review it was expected that the stress - stretch ratio curve would take on an exponential form (7,8,10,12,15,24,26). This was confirmed through observation of our recorded data. As mentioned in Section 3.3, it was decided that Fung’s (10) expression:

\[ \sigma = (\sigma^* + A)\exp(B(\lambda - \lambda^*)) - A \]  

(3.)

would best suit our needs. This decision was based on the ease of application, fit error, and the ease in comparison of the fitting parameters. Note that \( \sigma^* \) and \( \lambda^* \) represent a reference point in the data set and are constant parameters for a given specimen, A and B are determined from experiment, and \( \lambda \) and \( \sigma \) are known values in the data set.

From equation (3.) it was expected that the B parameter would control the rate of curvature of the stress - stretch ratio relation (i.e., as B increases so would the rate of curvature) and that the A parameter would dictate the initial slope of the curve (i.e., as A increases so would the initial slope). This appeared to be true in our results.

5.2 Application of Analytical Formulation

As mentioned in Section 4.2.2, ASYSTANT 1.1 was used to curve fit the raw data. Individual specimen data sets were approximated using equation (3.) and groups of specimens based on growth factor and time period were also approximated using equation (3.). These specimen groups allowed for a pooling of experimental data according to time period and
applied growth factor such that a subsequent curve fit to these multiple data sets produced a "composite" fitted curve (hereafter referred to as "the composite"). Results of the composite curve fitting are contained in Appendices A - C. A best fit for the data was chosen on the basis of change in fit parameters and change in fit error as mentioned in Section 4.2.2. Following Fung’s protocol, $\sigma^*$ and $\lambda^*$ were chosen at the upper end of the stress - stretch ratio curve (large stress in the physiological range) and varied with specimen (10). On occasion, when fitting a data set, the fit parameters forced the origin of the curve to a negative stress level. This, not justified based on the experimental protocol, necessitated the use of the equation:

$$A = \sigma^* \exp(B(1 - \lambda^*)) / (1 - \exp(B(1 - \lambda^*))) \quad (4.)$$

to adjust the value of equation (3.) at the origin (controlled by the A parameter) to an acceptable value. It was determined by data comparisons before and after implementation of equation (4.) that this modification impacted the curve-fit results (material parameters A and B) minimally.

### 5.3 Fit Parameter Comparison

Stress and stretch ratio data were recorded for two different sets of fiduciary markers, from crosshead grip to crosshead grip and from bead to bead (see Figure 6). Limitations in the optical resolution of the video and morphometric measuring system made measuring the crosshead grip (referred to as simply "the crosshead") stretch ratio considerably easier and less error prone than that of the bead stretch ratio.

To compare fit parameters from each composite curve in a meaningful way it was required that a "baseline" value for either $\sigma^*$ or $\lambda^*$ be chosen (see Figure 10). The baseline parameter chosen for our experimental data was a common (to all curves) stress level, $\sigma^*$. Once $\sigma^*$ was set, it was possible to directly read from each trace respective $\lambda^*$ values (for each composite data set). The data set could then be re-curve-fit using $\sigma^*$ and $\lambda^*$ to
obtain comparable A and B parameter values (see Tables 1 and 2). Once comparable data was derived, the time dependency of the parameters and the influence of the growth factors at individual time periods were inspected.

Review of our data indicated that the material parameters are inversely proportional to each other; a high A value being associated with a low B value and vice-versa.

5.3.1 Time Dependency of Curve-Fit Material Parameters

As time progressed from three to thirty days, the material constants A, B, and D for both crosshead and bead data (for all growth factors and controls) increased in value (see Tables 1 and 2), signifying increasing stiffness (decreasing compliance). There was a deviation from this trend however, for EGF crosshead data which showed a decrease in the B parameter at each time period yet the D value continuously increased. In all other crosshead cases the values of A and B at thirty days were greater than respective values at three days. PBS crosshead and bead data and PDGF crosshead data showed an increase in A and B values at ten days from three days, but at thirty days both growth factors showed a decrease in values of A and B from ten days. The values at thirty days were greater than those at three days. The D value obtained from equation (6.) was useful in helping to quantify the overall stiffness of the composite curve in cases like these when the A and B values didn’t follow the trends. Graphical representations of these trends are depicted in plots contained in Appendix B.

5.3.2 Influence of Growth Factors at Time Period

Comparison of the material parameters at each time period shows that certain growth factors enhance the speed of the healing process. Appendix C contains graphical representations of this data. Agreement between the fit parameters (Tables 1 and 2) and the tensiometry data (see below) corroborate this observation. At 3 days EGF was shown to be the stiffest, at 10 days PDGF was the stiffest, and at 30 days EGF was the stiffest.
3 DAY BEAD COMPOSITE DATA

\[ \sigma^* = 0.0049 \]

\[ \lambda^*_\text{EGF} = 1.094 \]

\[ \lambda^*_\text{IGF} = 1.105 \]

\[ \lambda^*_\text{PBS} = 1.153 \]

\[ \lambda^*_\text{PDGF} = 1.22 \]

**Figure 10**: Sample Curve Fitting From Baseline Parameters
<table>
<thead>
<tr>
<th>Time Period</th>
<th>Growth Factor</th>
<th>$\sigma^*$ (MPa)</th>
<th>$\lambda^*$</th>
<th>$A$ (MPa)</th>
<th>$B$</th>
<th>$D$ (MPa)</th>
</tr>
</thead>
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<tr>
<td>3 Day</td>
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<td>.0049</td>
<td>1.07</td>
<td>1.2598E-3</td>
<td>8.23868</td>
<td>7.53E-6</td>
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<tr>
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<td>IGF</td>
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<td></td>
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<td>-1.0552E-4</td>
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<td>8.92E-9</td>
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<td>1.01</td>
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<td>2.43E-4</td>
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<td>.0408</td>
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<td>1.77E-6</td>
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<td>PBS</td>
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<td>.075283</td>
<td>6.80393</td>
<td>5.69E-4</td>
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<td>1.003</td>
<td>.44705</td>
<td>5.49399</td>
<td>.01</td>
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<td>7.89E-8</td>
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**Table 1:** Curve Parameters - Crosshead - Common $\sigma^*$

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<th>Time Period</th>
<th>Growth Factor</th>
<th>$\sigma^*$ (MPa)</th>
<th>$\lambda^*$</th>
<th>$A$ (MPa)</th>
<th>$B$</th>
<th>$D$ (MPa)</th>
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</thead>
<tbody>
<tr>
<td>3 Day</td>
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<td>1.094</td>
<td>.05811</td>
<td>.583753</td>
<td>.019</td>
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<tr>
<td></td>
<td>IGF</td>
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<td>1.105</td>
<td>7.8125E-3</td>
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<td>5.83E-4</td>
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<td>PBS</td>
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<td>1.153</td>
<td>.02445</td>
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<td></td>
<td>PDGF</td>
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<td>1.22</td>
<td>1.4E-4</td>
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<tr>
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<td>1.005</td>
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<td>8.81756</td>
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</tr>
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</table>

**Table 2:** Curve Parameters - Bead - Common $\sigma^*$
5.4 Strength Parameter Comparison

Progression of time from 3 to 30 days showed all specimens treated with growth factors to possess increasing values of the mechanical properties of fracture toughness, peak tangent modulus, and peak stress. Appendix D contains graphical representations of the tensiometry results. At 3 days IGF had the largest tangent modulus, fracture toughness, and peak stress; at 10 days IGF had consistently higher values than the control group for the tangent modulus, fracture toughness, and peak stress; and at 30 days EGF had the highest values for the tangent modulus, fracture toughness, and peak stress.
6. DISCUSSION

The combined data of the control specimens and those specimens with applied growth factors suggest increased stiffness and enhanced mechanical load bearing with time, indicative of enhanced collagen fiber formation and cross-linking (27).

Previous histological examination (27) revealed that at 3 days the wound is still mostly open, at 10 days the wound is completely closed and appeared to be bridged by collagen fibrils in a mostly disorganized pattern, and at 30 days the wound was more organized with collagen fibrils falling into the regular lamellar pattern. A triphasic time history was observed possibly reflecting distinct phases of corneal tissue repair analogous to cutaneous tissue repair (27). A correlation between the histological examination and tensiometry results may exist and may be explained through wound-healing biology.

It is important to keep in mind that while the biomechanical effects of the growth factors may be observed at 30 days, the growth factor may have been biologically active at 8 days; therefore, histological examination and a fundamental understanding of histochemical proceedings in the field of wound healing is necessary.

Platelets play an active role in the inflammatory phase of cutaneous wound healing, adhering to collagen and releasing adhesive proteins and growth factors. These substances aid in clotting and are instrumental in the initiation of granulation tissue formation. PDGF is one substance released during this early phase in wound repair and may explain the response (stiffness and strength) of the avascular corneal tissue to PDGF at the 10 day time point. Following this line of reasoning, IGF, obtained from plasma, might also have its response (stiffness and strength) explained in this way. Such events would correspond with the hypothesis of Maurice (20) that introduction of blood constituents may induce positive enhancement of overall corneal wound healing.
Fibroblast activity and epithelial healing rate have been observed to be affected by EGF (17,18,19,22,25,29). Granulation tissue production and matrix formation and remodeling are the last two phases of cutaneous wound healing occurring synchronously and for an indefinite time course starting soon after day 2 (see Figure 1), analogous trends are expected in corneal wound healing. Granulation tissue production and matrix formation and remodeling are characterized by a dense population of fibroblasts and epithelial reformation and may be the reason for the effectiveness of EGF at the 30-day time period in our study.

6.1 Summary

Our observations suggest a triphasic time course in wound healing for corneal tissue based on biomechanical testing and histological observation. The apparent response of wound healing to different growth factors also suggests that different growth factors may ‘target’ specific healing phases or sub-phases, thereby augmenting the wound healing process.

6.2 Recommendations

There were limitations in our experiment which may have affected the results:

For our experiment there was a relatively small sample size. This may bias any statistical analysis and affect statistical relevance. Sample sizes are noted on the tables in the Appendices.

As can be seen in Figure 6, the location of the beads is critical for proper measurement of the stretch ratio. A template to help locate the beads in the same location from specimen to specimen would have simplified the preparation of specimens and may have had an effect on the variability of the stretch ratio of different specimens.

The morphometric measurement system is based on measuring from pixel
to pixel. Between beads an extraneously measured pixel may bias the measurement by nearly 3%, while between the crosshead grips measurement bias was only .2%.

The specimen thickness measurement was based on a calculation utilizing the planform area, specimen weight, and tissue density:

\[ t_{\text{mean}} = \frac{(\text{wt}/A_{\text{p}})}{(0.00101(\text{g/mm}^3))} \]  

(11.)

as opposed to direct measurement. This was necessary due to the flexibility of the material. However, this also introduced a possible source of error. The stress measurement calculation utilized the original cross sectional area of the specimen which was partially determined from this thickness measurement (cross sectional area = width \times thickness).

Specimen size and material also indicated that strict climate control may have been necessary to limit the effects of temperature and humidity, however, a climate controlled room was not available.

The hourglass shape of the specimens also introduced error into the evaluated material properties. Appendix E contains finite element analyses of both rectangular and hourglass planformed specimens showing stress contours. The hourglass specimens clearly exhibit stress concentrations in the wound location, therefore, the gross strength properties of fracture toughness, peak stress, and tangent modulus may all be underestimated by as much as 15% (27), the stiffness characterization may also have been affected. In all tensile tests the specimen fracture initiated at the edge of the wound, propagating across, as opposed to occurring uniformly across the wound. The time course data and subsequent rise in wound strength properties postoperatively remains qualitatively correct if hourglass planform stress concentrations are considered. A device to prepare rectangular specimens prior to the removal of the inherent concavity of cornea would be beneficial to this type of research.
The disparity between individual data sets within a composite set may have been related to the fact that the tissue was not preconditioned. For our testing it was necessary to destroy the specimens in order to obtain peak strength values, however, in the future it may be possible to precondition the specimens utilizing known peak strengths of the tissue before recording data to create stress - stretch ratio plots.
APPENDIX A

Growth Factor Raw Data
EGF 10 DAY CROSSHEAD DATA

Stress (MPa) vs. Stretch Ratio

EGF 10 DAY BEAD DATA

Stress (MPa) vs. Stretch Ratio
EGF 30 DAY CROSSHEAD DATA

EGF 30 DAY BEAD DATA
EGF Composite Curve-Fit Parameters

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Location</th>
<th>$\sigma^*$ (MPa)</th>
<th>$\lambda^*$</th>
<th>$A$ (MPa)</th>
<th>$B$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Day [4]</td>
<td>Crosshead</td>
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<td>1.25396E-3</td>
<td>8.29314</td>
</tr>
<tr>
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<td>Bead</td>
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<td>1.39</td>
<td>0.097534</td>
<td>0.364868</td>
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<td>10 Day [3]</td>
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</tr>
<tr>
<td>30 Day [3]</td>
<td>Crosshead</td>
<td>0.9806</td>
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<td>0.5278</td>
<td>6.42935</td>
</tr>
<tr>
<td></td>
<td>Bead</td>
<td>0.5884</td>
<td>1.23</td>
<td>0.79575</td>
<td>2.44145</td>
</tr>
</tbody>
</table>

$[n] = \text{number of specimens}$

Table 3: Curve Parameters - EGF - Composite Fitting
IGF 10 DAY CROSSHEAD DATA

Stress (MPa)

0.000
0.040
0.080
0.120
0.160
0.200
0.240

Stretch Ratio

1.00 1.02 1.04 1.06 1.08 1.10 1.12

IGF 10 DAY BEAD DATA

Stress (MPa)

0.000
0.040
0.080
0.120
0.160
0.200
0.240

Stretch Ratio

1.00 1.04 1.08 1.12 1.16 1.20 1.24
IGF 30 DAY CROSSHEAD DATA

**Stress (MPa)**

<table>
<thead>
<tr>
<th>Stretch Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
</tr>
<tr>
<td>1.04</td>
</tr>
<tr>
<td>1.08</td>
</tr>
<tr>
<td>1.12</td>
</tr>
<tr>
<td>1.16</td>
</tr>
<tr>
<td>1.20</td>
</tr>
</tbody>
</table>

IGF 30 DAY BEAD DATA

**Stress (MPa)**

<table>
<thead>
<tr>
<th>Stretch Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
</tr>
<tr>
<td>1.10</td>
</tr>
<tr>
<td>1.20</td>
</tr>
<tr>
<td>1.30</td>
</tr>
<tr>
<td>1.40</td>
</tr>
<tr>
<td>1.50</td>
</tr>
<tr>
<td>1.60</td>
</tr>
<tr>
<td>1.70</td>
</tr>
</tbody>
</table>
IGF Composite Curve-Fit Parameters

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Location</th>
<th>σ* (MPa)</th>
<th>λ*</th>
<th>A (MPa)</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bead</td>
<td>.01373</td>
<td>1.235</td>
<td>7.2254E-3</td>
<td>4.25481</td>
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<tr>
<td></td>
<td>Bead</td>
<td>.1079</td>
<td>1.16</td>
<td>.03558</td>
<td>7.92239</td>
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<tr>
<td></td>
<td>Bead</td>
<td>.8335</td>
<td>1.68</td>
<td>.1811</td>
<td>2.53419</td>
</tr>
</tbody>
</table>

[n] = number of specimens

Table 4: Curve Parameters - IGF - Composite Fitting
PBS 3 DAY CROSSHEAD DATA

PBS 3 DAY BEAD DATA
PBS 10 DAY CROSSHEAD DATA

\[ \text{Stress (MPa)} \]

\[
\begin{array}{cccc}
1.00 & 1.04 & 1.08 & 1.12 \\
1.16 & 1.20 & 1.24 \\
\end{array}
\]

\[ \text{Stretch Ratio} \]

PBS 10 DAY BEAD DATA

\[ \text{Stress (MPa)} \]

\[
\begin{array}{cccc}
1.00 & 1.10 & 1.20 & 1.30 \\
1.40 & 1.50 \\
\end{array}
\]

\[ \text{Stretch Ratio} \]
### PBS Composite Curve-Fit Parameters

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Location</th>
<th>( \sigma^* ) (MPa)</th>
<th>( \lambda^* )</th>
<th>( A ) (MPa)</th>
<th>( B )</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Day [4]</td>
<td>Crosshead</td>
<td>0.01765</td>
<td>1.168</td>
<td>4.6589E-3</td>
<td>8.5002</td>
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<tr>
<td></td>
<td>Bead</td>
<td>0.01393</td>
<td>1.38</td>
<td>0.02408</td>
<td>1.20069</td>
</tr>
<tr>
<td>10 Day [3]</td>
<td>Crosshead</td>
<td>0.1814</td>
<td>1.18</td>
<td>0.07584</td>
<td>6.78586</td>
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<td></td>
<td>Bead</td>
<td>0.1667</td>
<td>1.28</td>
<td>0.11751</td>
<td>3.15427</td>
</tr>
<tr>
<td>30 Day [3]</td>
<td>Crosshead</td>
<td>0.5884</td>
<td>1.166</td>
<td>0.05716</td>
<td>14.775</td>
</tr>
<tr>
<td></td>
<td>Bead</td>
<td>0.5884</td>
<td>1.13</td>
<td>0.1319</td>
<td>13.0601</td>
</tr>
</tbody>
</table>

\([n]\) = number of specimens

---

**Table 5**: Curve Parameters - PBS - Composite Fitting
PDGF 10 DAY CROSSHEAD DATA

Stress (MPa)

Stretch Ratio

PDGF 10 DAY BEAD DATA

Stress (MPa)

Stretch Ratio
## PDGF Composite Curve-Fit Parameters

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Location</th>
<th>$\sigma^*$ (MPa)</th>
<th>$\lambda^*$</th>
<th>$A$ (MPa)</th>
<th>$B$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bead</td>
<td>0.00686</td>
<td>1.275</td>
<td>-4.8244E-5</td>
<td>6.05766</td>
</tr>
<tr>
<td>10 Day [2]</td>
<td>Crosshead</td>
<td>0.4413</td>
<td>1.126</td>
<td>0.44737</td>
<td>5.49189</td>
</tr>
<tr>
<td></td>
<td>Bead</td>
<td>0.5394</td>
<td>1.36</td>
<td>0.88743</td>
<td>1.32735</td>
</tr>
<tr>
<td>30 Day [2]</td>
<td>Crosshead</td>
<td>0.3923</td>
<td>1.122</td>
<td>0.06064</td>
<td>16.3725</td>
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<td></td>
<td>Bead</td>
<td>0.2942</td>
<td>1.198</td>
<td>0.05951</td>
<td>8.82164</td>
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</tbody>
</table>

[n] = number of specimens

**Table 6:** Curve Parameters - PDGF Composite Fitting
APPENDIX B

Time Course Composite Data For Growth Factors
<table>
<thead>
<tr>
<th>Stretch Ratio</th>
<th>1.00</th>
<th>1.10</th>
<th>1.20</th>
<th>1.30</th>
<th>1.40</th>
<th>1.50</th>
<th>1.60</th>
<th>1.70</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 DAY</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>10 DAY</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>30 DAY</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
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<td>0.60</td>
<td>0.60</td>
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<tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Stretch Ratio

Day 0: 0.70
Day 10: 0.60
Day 30: 0.50

The graph shows the stretch ratio over time with PDGF bead composite data.
APPENDIX C

Composite Data Per Time Period
10 DAY CROSSHEAD COMPOSITE DATA

- EGF
- IGF
- PBS
- PDGF

Stress (MPa)

Stretch Ratio

10 DAY BEAD COMPOSITE DATA

- EGF
- IGF
- PBS
- PDGF

Stress (MPa)

Stretch Ratio
<table>
<thead>
<tr>
<th>Stretch Ratio</th>
<th>0.80</th>
<th>0.60</th>
<th>0.40</th>
<th>0.20</th>
<th>0.00</th>
</tr>
</thead>
<tbody>
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<td>EGF</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>0.20</td>
<td>0.00</td>
</tr>
<tr>
<td>IGF</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>0.20</td>
<td>0.00</td>
</tr>
<tr>
<td>PBS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.20</td>
<td>0.00</td>
</tr>
<tr>
<td>PDGF</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.20</td>
<td>0.00</td>
</tr>
<tr>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>0.20</td>
<td>0.00</td>
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</tbody>
</table>
APPENDIX D

Tensiometry Results
Peak Tangent Modulus (MPa) $=\pm$ Standard Error of the Mean

<table>
<thead>
<tr>
<th>Growth Factor</th>
<th>3 Days</th>
<th>10 Days</th>
<th>30 Days</th>
</tr>
</thead>
</table>

[n] = number of specimens

Table 7: Peak Tangent Modulus (MPa) $=\pm$ Standard Error of the Mean
**Peak Stress (kPa) +/- Standard Error of the Mean**

<table>
<thead>
<tr>
<th>Growth Factor</th>
<th>3 Days</th>
<th>10 Days</th>
<th>30 Days</th>
</tr>
</thead>
</table>

[n] = number of specimens

**Table 8**: Peak Stress (kPa) +/- Standard Error of the Mean
Fracture Toughness (kPa) ± Standard of the Mean

<table>
<thead>
<tr>
<th>Growth Factor</th>
<th>3 Days</th>
<th>10 Days</th>
<th>30 Days</th>
</tr>
</thead>
</table>

[n] = number of specimens

Table 9: Fracture Toughness (kPa) ± Standard Error of the Mean
APPENDIX E

Finite Element Analysis of Specimen Shape
In summary from reference (27): Figures 11 and 12 depict contours in load-directed stress for each specimen planform as determined from finite element analysis. The contour legend is depicted in each figure and has units of grams per square millimeter. The numerical finite element analysis employs a nominal load applied to each specimen so that a stress level of 2 g/mm² should occur at midlength. Figure 11 clearly shows that rectangular specimens possess uniform stress levels in the wound region. Hourglass stress distribution is not uniform, as seen in Figure 12, and possesses a distinct stress concentration at the lateral edges of the wound.
Figure 11: Stress Contours of Rectangular Planform Specimen
Figure 12: Stress Contours of Hourglass Planform Specimen
REFERENCES


