ENGINEERING THE MECHANICAL PROPERTIES OF OCULAR TISSUES

Thesis by
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ABSTRACT

The mechanical properties of the structural tissues of the eye (cornea, sclera, and vitreous) are critical for vision. Age and disease can cause changes in their physical properties and compromise visual acuity; in the extreme, such changes can lead to blindness. Thus, there is great interest in understanding the mechanical properties of ocular tissues and in developing appropriate therapeutic strategies.

The goal of this thesis is to discover and manipulate the molecular mechanisms that determine the bulk physical properties of the vitreous and the cornea. These tissues are both ordered biocomposites of fibrous collagen embedded in soft matrices of proteoglycans (PGs) and glycosaminoglycans (GAGs). The hydration state, mole fraction, and particularly the organization of these components determine the mechanical properties of the respective tissues. Whereas the mechanical strength of these tissues has traditionally been attributed to their collagenous components, we present evidence that the PGs and GAGs also make significant contributions. We also suggest hypotheses regarding the mechanisms by which the carbohydrate components contribute and how they can be utilized for therapeutic purposes.

In order to study the unique physical properties of the vitreous, novel instrumentation was developed. We describe the use of cleated surfaces on parallel disk tools to quantitatively measure the rheological properties of diverse slip-prone fluids and soft materials. Densely-packed protrusions (0.45mm x 0.45mm cross section x 0.6mm length, 0.9mm apart) penetrate the slip layer, preventing significant flow between cleats. This creates a no-slip boundary ~ 0.16mm below their tips, which serves as the sample gap boundary, in direct analogy to the parallel plate geometry. This “cleat” geometry suppresses slip without application of significant normal force, it imposes well-defined shear to enable absolute measurements, and is compatible with small sample volumes. The geometry was validated in steady and oscillatory shear using a series of materials not prone to slip (Newtonian oils and an entangled polymer melt). The advantage of cleated tools over other slip-prevention
methods was demonstrated using slip-prone materials, including an emulsion, a suspension, and porcine vitreous humor.

The vitreous humor is a transparent gel comprised of a delicate, swollen double network of 10 – 20 nm collagen type II fibrils and charged GAG chains (hyaluronic acid). While extensive progress has been made in identifying the components and biochemistry of the vitreous, prior to the “cleat geometry” experimental limitations hampered quantitative determination of its mechanical properties. With cleated tools we overcame wall slip and avoided tissue compression during measurements of the dynamic moduli of fresh porcine and bovine vitreous. Shear moduli decreased five-fold from initial to steady-state values in the first hour after dissection. Steady-state values (Porcine: $G' = 2.6 \pm 0.9$ Pa and $G'' = 0.6 \pm 0.4$ Pa, $n = 9$; Bovine: $G' = 6.5 \pm 2.0$ Pa and $G'' = 2.0 \pm 0.6$ Pa, $n = 17$) are significantly greater than previously reported. The decrease in modulus after removal from the eye correlates with a decrease in mass: porcine vitreous expels ~5% of its mass within 5 minutes and continues to decay to a steady-state mass ~10% lower than its initial mass in the absence of external driving forces. The expelled fluid has a substantial hyaluronan concentration but a very low protein content. These results indicate that the vitreous network is under tension at its native volume, and its high initial modulus results from this state of tension. We hypothesize that hyaluronan plays a role in sustaining the “internal tension” by Donnan swelling.

The therapeutic goal in vitreous engineering is liquefaction: we seek pharmacological agents capable of gently separating the vitreous from the retina and destabilizing the network without damaging the adjacent tissues (retina and lens). We measured the stability of the vitreous against agents designed to target covalent bonds, hydrogen bonds, electrostatic attractions, and hydrophobic interactions using a simple weighing procedure. We found that in addition to covalent bonds, hydrogen bonds appear to play a particularly important role in stabilizing the vitreous network. This is in agreement with clinical observations that treating eyes with urea prior to vitrectomy provided a significant therapeutic benefit. We found that treating porcine vitreous with therapeutic doses of urea in vitro reduced the shear modulus by ~ 30%. Limited in vivo animal studies measured no
softening effect and indicated that the therapeutic benefit of urea may be a reduction of vitreoretinal adhesion.

The cornea is also composed of collagen fibrils embedded in a PG/GAG matrix. The cornea, however, contains far more collagen, PG, and GAG than vitreous, and its components are also more ordered: the collagen (type I) is in the form of 30 nm fibrils, precisely arranged lamellae and evenly spaced in a keratin sulfate-rich matrix. Our therapeutic goal in the cornea is to stabilize its nanostructure and mechanical properties against keratoconus, a degenerative disease in which the cornea softens and bows outward under the force of intraocular pressure.

We present coordinated biomechanical and biochemical analyses of corneal tissue that has been crosslinked using glycation. Non-enzymatic crosslinking alters the viscoelastic properties of protein-rich tissues, but a quantitative correlation between the formation of specific advanced glycation end products (AGEs) and physiologically relevant mechanical property changes has not previously been established. We report that corneas treated with 1% and 2% glyceraldehyde solutions produce a 300% and 600% rise in shear modulus, respectively, which strongly and linearly correlates with increased fluorescence and the formation of the AGEs argpyrimidine, lys-hydroxy-triosidine, and arg-hydroxy-triosidine ($R^2 = 0.999, 0.970, \text{ and } 0.890$ respectively). NMR studies are used to demonstrate that enzymatic digestion does not alter AGEs and has some advantages over acid hydrolysis. The level of mechanical reinforcement observed in these studies is probably sufficient to stabilize keratoconus corneas, based upon successful treatments with other crosslinking strategies.

Comparing quantitative correlations between modulus and AGE accumulation in corneas with analyses of collagen fibers isolated from mouse tail tendons suggests that glycation-induced corneal stiffening cannot be attributed solely to changes in collagen. We present a novel hypothesis that the mechanically-relevant AGE crosslinks are those that change the properties of the soft PG/GAG matrix and its coupling to the collagen fibrils, rather than the much more numerous AGEs that crosslink amino acids within fibrils.
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SYMBOLS AND ABBREVIATIONS

AGE  Advanced Glycation Endproduct
δ   Penetration depth / Phase angle
G'  Storage Modulus
G'' Loss Modulus
GA  Glyceraldehyde
GAG Glycosaminoglycan
G-3-phosphate Glyceraldehyde-3-phosphate
HA  Hyaluronic acid
HPLC High Performance Liquid Chromatography
Lc  Cleat length
MGO Methylglyoxal
MW  Molecular Weight
NMR Nuclear Magnetic Resonance
PBS Phosphate-buffered Saline
PG  Proteoglycan
PVD Posterior vitreous detachment
TBT Tendon breaking time
η   Viscosity
η* Complex Viscosity
σ   Shear stress
γ   Shear Strain
γ' Strain Rate
1.1 Background

The ability to create and maintain fixed spatial relationships between cells and organs is vitally important for higher organisms. Residing in fixed locations allows cells and tissues to work cooperatively through specialization and division of labor. One illustration of the importance of precise physical properties and arrangements is mammalian vision, which relies on the precise geometry of the cornea, the mechanical strength of the sclera to support the retina, and the orbital ligaments to control the line of sight.

The mechanical properties of structural tissues such as these are derived from the nanoscale architecture and properties of their constituent molecules. Most structural tissues are biocomposites of fibrous proteins embedded in soft carbohydrate matrices. Collagen is the primary fibrous component; proteoglycans and glycosaminoglycans act as the matrix. The hydration state, mole fraction, and organization of these components vary between tissues and species, but the basic structure—high tensile-strength fibrils organized in soft matrices—is highly preserved. Rare genetic mutations that weaken collagen fibrils or
disrupt other aspects of this molecular pattern lead to devastating systemic diseases.\textsuperscript{3-6} A number of more common diseases, such as arthritis and diabetes, are also associated with degeneration of collagenous tissues.

The debilitating nature and prevalence of heritable and degenerative disorders that affect connective-tissues has stimulated considerable biochemical and biomechanical research. Unfortunately, the molecular (biochemical) and biomechanical aspects of this important field have been investigated independently rather than in concert. We will present significant advancements that have come as a result of combining biochemical analyses with novel bulk characterization techniques.

Broadly stated, the goal of the present research is to discover and manipulate the molecular mechanisms that determine the bulk physical properties of the cornea and vitreous humor (Figure 1). This goal can be divided into three specific objectives:

1) To quantitatively determine the mechanical properties of connective tissues

2) To understand the molecular basis of these mechanical properties and their implications for disease and tissue engineering

3) To create therapeutic changes in the mechanical properties of the cornea and vitreous
Our approach to these objectives is to combine analytical chemistry, rheology, and polymer physics with \textit{in vivo} animal studies and the clinical experience of collaborators from industry and medicine. Biochemical and biomechanical investigations were conducted in parallel with drug discovery and clinical research, providing feedback between clinical and laboratory work. Clinical research identified potential therapeutics and evaluations of efficacy, while laboratory research addressed fundamental questions regarding the basis of the mechanical properties of collagenous tissues and how they can be engineered. The success of this approach in exploring potential therapeutics for the vitreous humor and cornea demonstrates the utility of an integrated approach to understanding and engineering connective tissues in general.
1.2 The Vitreous Humor

The vitreous is a transparent, collagenous gel that fills the posterior chamber of the eye. It is more than 98% water, avascular, and nearly acellular; thus, the vitreous was historically considered an inert space-filler. However, over the past few decades it has become clear that the vitreous plays an essential structural role in the development, maintenance, and pathologies of vision. Sebag has summarized the functions of the vitreous as developmental—mediating proper growth of the eye; optical—maintaining a clear path to the retina; mechanical—supporting the various ocular tissues during physical activity; and metabolic—providing a repository of various small molecules for the retina. Proper performance of these functions depends upon the unique physical properties of the vitreous.

The vitreous is thought to derive its physical properties from its hydrated double network of collagen type II fibrils and high molecular-weight, polyanionic hyaluronan macromolecules (Figure 2). Heterotypic collagen fibrils (10 – 20 nm diameter) are composed of a small, collagen type V/XI core surrounded by collagen type II. Human vitreous hyaluronan (HA) is polydisperse with an average molecular weight that is estimated to be ~ 5,000,000. Prior literature indicates that the vitreous completely liquefies when digested with collagenase enzyme, whereas it only shrinks when digested with hyaluronidase. On this basis it has been presumed that the network of collagen fibrils provides mechanical strength, and the swollen HA macromolecules simply fill the space between fibrils to prevent aggregation. In Chapter 3 we will discuss the collagen-HA double network in greater depth and present rheological and biochemical evidence that hyaluronan does contribute
profoundly to the elastic character of the vitreous. This realization changes the way we view the network, particularly in the context of vitreous degeneration and engineering.

Figure 2. Schematic depiction of the network structure of the vitreous. The vitreous is composed of a highly-swollen double network of collagen type II fibrils (≈ 15 nm in diameter) and hyaluronic acid (≈ 5M MW).
With age the collagen-HA network degrades and loses mechanical integrity: pockets of fluid (lacunae) form near the retina as the components of the vitreous network aggregate and pull away from the retina. Posterior vitreous detachment (PVD) is normally inhomogeneous, leaving points of adhesion that cause localized traction on the retina. Incomplete PVD and the resultant vitreoretinal traction are thought to play a role in a number of diseases, including macular holes, macular edema, vitreous hemorrhage, retinal tears, and retinal detachment. The only treatment currently available for alleviating vitreoretinal traction is surgical removal of the vitreous (vitrectomy). Motivated by the need for a less invasive and traumatic treatment, efforts have been made to find “pharmacological vitrectomy agents” capable of inducing PVD and liquefying or significantly softening the vitreous, thereby alleviating traction without surgery. Proposed therapeutics from the literature will be discussed in detail in Chapter 4, but they generally consist of enzymes designed to cleave the proteins responsible for the mechanical integrity of the vitreous. Little attention has been given to the possibility of targeting noncovalent intermolecular interactions. We present results that indicate that disruption of hydrogen bonds strongly destabilizes the vitreous network, whereas disruption of electrostatic or hydrophobic interactions has a much weaker effect.

In addition to collagen type II and HA, 15 “minor” proteins and proteoglycans have been identified in the vitreous (Table 1). These components are minor in terms of mass, but may be crucial for the structure and stability of the vitreous, much as nails are a “minor” component of a wood-framed house. A number of these components, including link protein, fibronectin, and vitronectin, are known to connect proteins with polysaccharides in
other tissues. They may perform a similar function in the vitreous, stabilizing the collagen-HA network and linking it to other structures in the eye; however, little is known about the role of minor components in the molecular architecture of the vitreous network.

Given the importance of the viscoelastic properties of the vitreous to its function and to pathology, it is striking that there is no consensus on the value of its modulus in the prior literature. This is due in part to a lack of sufficient experimental methods for quantitatively measuring the mechanical properties of the vitreous and how they change as a result of various treatments. To address this need we developed a novel rheological tool that enabled us to make the first quantitative measurements of the mechanical properties of the vitreous. We discovered that the modulus of the vitreous is significantly higher in situ than after removal from the eye. Further exploration of this discovery led us to a novel hypothesis regarding the mechanical properties of the vitreous: that HA increases the modulus of the vitreous by swelling the collagen network to a state of tension.

The novel tool also allowed us to measure modulus changes that resulted from treating the vitreous with a particular proposed pharmacological vitrectomy agent—urea. Clinical observations that urea may facilitate vitreous removal led us to investigate its influence on the mechanical properties of the vitreous in vitro and in vivo. Slit lamp observations of urea-treated vitreous, together with reduced surgical time during vitrectomy, suggested to the clinicians that urea “liquefied” the vitreous. By quantitatively characterizing the modulus of the vitreous, our work showed that treatment did not liquefy vitreous in vitro or in vivo. By working side-by-side with a team of eye surgeons working under the direction
of Professor Hugo Quiroz-Mercedo at the Hospital “Dr. Luis Sánchez Bulnes” de la APEC in Mexico, we were able to reconcile clinical observations with rheological measurements. The clinical benefit was more likely the result of reduced vitreoretinal adhesion and phase separation as the collagen network contracted away from the retina to relieve tension. We also explored the effects of other agents on vitreous and found that hydrogen bonding plays a more significant role in stabilizing the vitreous network than electrostatic or hydrophobic effects. Taken together, these results provide a basis for rational design of future pharmacological vitrectomy agents.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration Human/Pig [μg/ml]</th>
<th>Location</th>
<th>Proposed functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>&gt;980,000⁹/same</td>
<td>Throughout</td>
<td>Maintains vitreous mechanical properties and facilitates transport⁷</td>
</tr>
<tr>
<td>Salts (NaCl, KCl, CaCl₂, and MgCl₂)</td>
<td>~9,000⁹/same</td>
<td>Throughout</td>
<td>Global charge balance, Donnan swelling; vitreous is isotonic with blood and most other tissues⁷</td>
</tr>
<tr>
<td>Total Protein</td>
<td>800⁷/700¹⁷</td>
<td>Throughout</td>
<td>—</td>
</tr>
<tr>
<td>Total polysaccharide</td>
<td>240¹⁸/~250¹⁷</td>
<td>Throughout</td>
<td>—</td>
</tr>
<tr>
<td>Collagen type II</td>
<td>~225⁹/150¹⁷</td>
<td>Throughout</td>
<td>Resist elongation of the eye and provide structural framework for the vitreous body⁷</td>
</tr>
<tr>
<td>Hyaluronic acid</td>
<td>65-400⁹/165¹⁷</td>
<td>Throughout</td>
<td>Resist compression of the eye, hydrate tissue, space collagen fibrils⁷</td>
</tr>
<tr>
<td>Protein Name</td>
<td>% Protein</td>
<td>Location</td>
<td>Description</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-----------</td>
<td>---------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Albumin</td>
<td>293°/</td>
<td>Throughout</td>
<td>Soluble protein, no known structural role</td>
</tr>
<tr>
<td>Link protein</td>
<td>0.6(bovine)°/</td>
<td>Unknown</td>
<td>1:1 with versican; it may be there to link versican to HA°/</td>
</tr>
<tr>
<td>Collagen V/XI</td>
<td>~30°/</td>
<td>Throughout</td>
<td>Form the core of heterotypic collagen II fibrils°/</td>
</tr>
<tr>
<td>Collagen IX</td>
<td>&lt;30°/</td>
<td>Throughout</td>
<td>Decorate surface of heterotypic collagen II fibrils, prevent fibril aggregation, possibly link fibrils to noncollagenous components°/</td>
</tr>
<tr>
<td>Collagen VI°/</td>
<td>Unknown</td>
<td>Concentrated on the zonular fibers</td>
<td>Bind collagen fibrils to HA and other species (has been shown to bind von Willebrand factor, collagen II fibrils, decorin and HA)°/</td>
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<tr>
<td>Collagen XVIII°/</td>
<td>Unknown</td>
<td>Vitreoretinal interface</td>
<td>Vitreoretinal adhesion; has been co-localized with opticin at vitreoretinal interface; contains endostatin as a non-collagenous domain°/</td>
</tr>
<tr>
<td>Cartilage oligomeric matrix protein (COMP)°/</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown, but also found in cartilage and tendon; contains von Willebrand factor domains (see collagen VI)°/</td>
</tr>
<tr>
<td>Microfibril-associated glycoprotein-1 (MAGP1)°/</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Decorate exterior of zonular fibers°/</td>
</tr>
<tr>
<td>Opticin°/</td>
<td>Unknown</td>
<td>Vitreous base and lamina cribrosa</td>
<td>Acts in conjunction with collagen XVIII to mediate vitreoretinal adhesion°/</td>
</tr>
<tr>
<td>Fibrillin°/</td>
<td>Minor but probably &gt; [coll VI]°/</td>
<td>Attached to lens capsule</td>
<td>Structural fibrils for lens capsule anchoring &amp; articulation°/</td>
</tr>
<tr>
<td>Fibronectin°/</td>
<td>6°/</td>
<td>Throughout</td>
<td>Mediate binding between collagen and polysaccharides°/</td>
</tr>
<tr>
<td>Protein</td>
<td>Concentration</td>
<td>Distribution</td>
<td>Proposed Function</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------</td>
<td>--------------</td>
<td>-------------------------------------------------------</td>
</tr>
<tr>
<td>Vitronectin</td>
<td>4²⁸/ Unknown</td>
<td>Unknown</td>
<td>Mediate collagen-polysaccharide binding; sensitive to denaturation²⁵</td>
</tr>
<tr>
<td>Versican</td>
<td>60²⁹/ 22(bovine)²⁰</td>
<td>Unknown</td>
<td>1 per 150 moles of HA; possible link between HA and collagen and has been show to dissociate (if it was associated) in 4M guanidinium HCL; HA binding has been demonstrated¹⁷,²⁵</td>
</tr>
<tr>
<td>VIT1³⁰</td>
<td>Unknown</td>
<td>Unknown</td>
<td>May have structural role³⁰</td>
</tr>
<tr>
<td>Laminin/ Collagen type IV³¹</td>
<td>Unknown</td>
<td>Inner limiting membrane surrounding vitreous</td>
<td>While not components of the vitreous proper, they may participate in peripheral vitreous adhesion</td>
</tr>
</tbody>
</table>

Table 1. Known components of the vitreous humor listed with available information regarding concentration (µg / mL), distribution, and proposed function.
1.3 The Cornea

Like the vitreous, the cornea is composed of collagen fibrils embedded in a proteoglycan (PG) and glycosaminoglycan (GAG) matrix; however, unlike the vitreous, the cornea has a highly-ordered structure. The major structural element of the cornea (~ 90% of its thickness) is the stroma, which is composed of approximately 200 lamellae of oriented collagen type I fibrils embedded in a hydrated PG/GAG\textsuperscript{12} (Figure 3). The precise arrangement of collagen fibrils allows the cornea to retain optical clarity in spite of the relatively high density of collagen fibrils (30 nm diameter) required to retain the shape of the cornea.

![Figure 3. Stroma microstructure.](image)

[A] Represents the rigid collagen type I fibrils and smaller strands of proteoglycan that compose the lamellae of the corneal stroma. [B] Shows an enlargement of part of one of the fibrils, displaying the collagen triple helices aligned within a fibril. [C] Depicts the protein core of a proteoglycan non-covalently associated with the surface of a collagen fibril and decorated with polysaccharide chains. Micrograph was used by permission from Prof. K. Kadler, U. Manchester.
Whereas a primary therapeutic objective in the vitreous is softening and inducing PVD to alleviate vitreoretinal traction, a major, unmet clinical need in the cornea is enhancing its mechanical stability to prevent the progression of keratoconus. Keratoconus ("cone-shaped cornea") is a condition in which the cornea softens and slowly begins to protrude outward under the force of intraocular pressure. It affects roughly 1 in 2,000 people, normally beginning in the teens or early twenties, and causes progressive loss of visual acuity, eventually leading to blindness. In early stages, keratoconus is treated by application of hard contact lenses that correct vision and help maintain the shape of the cornea. If keratoconus progresses further, cornea transplantation is the only known treatment. The expense and difficulty of obtaining transplant tissue and the invasive nature of the surgery motivate our efforts to find a chemical treatment for keratoconus.

Collaborators at ISTA Pharmaceuticals, Inc. (Irvine, CA) developed a non-toxic, glycation-based crosslinking strategy to stabilize the cornea against keratoconus using glyceraldehyde (GA). Glyceraldehyde reacts with primary amines to form several known advanced glycation endproducts (AGEs), including two crosslinks and three AGEs that are also formed in reactions with methylglyoxal (MGO), another species investigated in this work (Figure 4). We have demonstrated that therapeutic (nontoxic) doses of glyceraldehyde are capable of significantly increasing the shear modulus of porcine corneas. Equivalent increases in modulus, achieved through alternative crosslinking strategies, have been shown to stabilize keratoconus eyes in clinical trials.
Biochemical analyses of GA-treated corneas revealed an additional protective effect of GA treatment: they are far less susceptible to proteolytic degradation (Chapter 5). This is particularly significant in light of the current hypothesis that keratoconus-induced softening comes as a result of overactive proteases in the cornea. The enzyme protective effect also indicates that GA may be a suitable treatment for corneal ulcers, which have also been
linked to increased proteolytic activity and treated with crosslinking strategies.\textsuperscript{36} The effect of GA on corneal ulcers has not yet been addressed.

Glycation-induced changes in enzyme resistance and modulus also correlate with increased fluorescence and AGE accumulation. We were able to isolate and quantify specific AGES from glycated corneas and demonstrate that modulus increases linearly with accumulation of each of them, including argpyrimidine, a pendent adduct. Thus, it appears that crosslinking and noncrosslinking AGES rise together and that various individual AGES could serve as a surrogate to track tissue stiffening, whether or not the individual surrogate AGE is a crosslink. It may be possible to use an equivalent empirical relation to noninvasively measure (e.g., by fluorescence) the degree of tissue stiffening in clinical practice.

Quantitative correlations between the chemical and mechanical impact of glycation on corneal tissue also yield new insight into the molecular mechanisms of AGE-related tissue stiffening. The literature holds that glycation stiffens collagenous tissues by changing the properties of the constituent collagen fibrils;\textsuperscript{37, 38} however, our results demonstrate that glycation-induced corneal stiffening cannot be attributed solely to changes in the properties of the collagen fibrils. We present a novel hypothesis that the mechanically relevant AGE crosslinks are those that change the properties of the soft PG/GAG matrix and its coupling to the collagen fibrils, rather than the much more numerous AGES that crosslink amino acids within fibrils.
New insights into the increase in modulus associated with AGEs may also be broadly relevant to aging, diabetes, and tissue engineering research. The mechanisms by which glycation stiffens tissues \textit{in vitro} may be relevant to certain pathologies of aging and diabetes. When properly understood, glycation has the potential to be turned from a pathologic process to a therapeutic strategy. The cornea is a good example, but it is merely a case-in-point. This strategy can be applied to a number of areas, from wound healing to bioadhesion to improving the mechanical properties of protein-based and polyamide synthetic tissues. Imparting strength to weakened connective tissue through glycation may provide an alternative to tissue transplants in diseases such as keratoconus.

\subsection*{1.4 Broader Implications}

A unifying theme that emerges from both the vitreous and cornea work is that collagenous tissues depend integrally on the contributions of their carbohydrate components for mechanical strength. We hope that future efforts to engineer the mechanical properties of collagenous tissues will recognize the important mechanical role of carbohydrate components and apply this knowledge in the design of therapeutics.

The overarching goal of this thesis is to bridge the gap between the chemical, biomechanical, and clinical aspects of tissue engineering. Working closely with physicians to focus on these three aspects in parallel has allowed developments from the lab to rapidly influence therapeutic formulations for clinical trial (e.g., optimal pH of urea treatment), and feedback on the \textit{in vivo} relevance of \textit{in vitro} discoveries allowed us to rapidly verify the significance of new findings. We hope that the success we have had in elucidating the
molecular interactions that play a significant role in biomechanics will provide a model for productive cross-field collaborations.

1.5 Organization of Thesis

There were no rheological methods suitable for quantitative characterization of the vitreous prior to this work. Chapter 2 presents the novel “cleat geometry” developed specifically for this purpose.

Chapters 3 and 4 address the properties and network structure of the vitreous. In Chapter 3 the mechanical properties of the vitreous are defined. A novel hypothesis regarding a direct contribution of hyaluronic acid to the mechanical stiffness of the vitreous is also presented. In Chapter 4 the stability of the vitreous network in various chemical environments is examined as a basis for selecting potential pharmacological vitrectomy agents. Hydrogen bonding is shown to play a key role in stabilizing the vitreous network and urea is examined as a potential therapeutic for softening the vitreous.

Chapters 5 and 6 address glycation in the cornea. In Chapter 5 the chemical and mechanical impact of glycating corneal tissue with glyceraldehyde is examined. In Chapter 6 mechanical measurements of glycated collagen fibers from mouse tail tendons are used to demonstrate that the enhanced mechanical strength of glycated collagenous tissues cannot be attributed solely to the stiffening of collagen fibrils – the surrounding matrix (presumably proteoglycans) must also play a role.
17. Noulas AV, Theocharis AD, Feretis E, Papageorgakopoulou N, Karamanos NK, Theocharis DA. Pig vitreous gel: macromolecular composition with


