428a Physical Properties of the Native and Engineered Vitreous Humor, Cornea, and Sclera

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Physical properties of tissue are closely related to tissue function. Many diseases are associated with altered chemical and mechanical states of tissue and a resulting loss of functionality. Proper vision relies heavily on the eye's ability to maintain optical clarity and structural integrity under daily fluctuations in pressure, variations in humidity and temperature, constant muscular strain and sudden movements. Diseases that cause visual changes, such as diabetic retinopathy, myopia, and keratoconus, may be treatable by engineering the vitreous, sclera, and cornea.

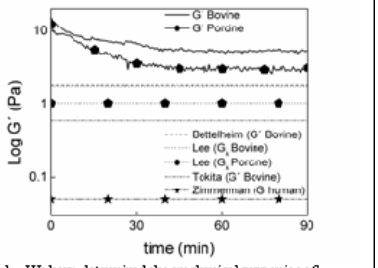

Figure 1 – We have determined the mechanical properties of vitreous samples from fresh porcine, and bovine eyes. 2.0-3.0 g samples were gently removed from freshly enucleated eyes and loaded immediately on a Rheometrics RFS II fluids rheometer which had been modified to overcome wall slip by adding cleats to a parallel-plate geometry. Shear moduli were monitored as samples were subjected to oscillatory strain (amplitude = 3%) at a fixed frequency (10 rad/sec) in a hydrated atmosphere at 20°C. For comparison, reported values from the prior literature[2-5] are shown; they are indicated by horizontal lines because transient behavior was not mentioned.

Understanding the physical structure and properties of the vitreous is of fundamental and therapeutic interest, providing insight into transport of molecules to the retina and defining the ideal properties of vitreous replacement materials. However, the mechanical properties of the vitreous humor remain elusive despite attempts to characterize them due to the lubricating properties of its constituent molecules and its fragile network structure. We have overcome these difficulties using a novel "cleat" tool[1] for dynamic shear rheometry which suppresses slip and permits sample loading with minimal disturbance of tissue structure and properties.

The elastic character of the tissue is manifested in the storage modulus, G', which reached a steady-state value after an initial decay (Figure 1). The initial value may be closer to the properties in vivo, however it was difficult to assign a quantitative value due to sensitivity to the precise time from dissection to acquisition of the first modulus measurement. Nevertheless, even the steady-state values were much higher than reported in the prior literature[2-5] (Figure 1). For the central portion of the vitreous (the bulk of the tissue), the steady-state modulus of porcine specimens (G'= 2.6 Pa +/- 0.9) was significantly less than that of bovine specimens (G' = 6.5 +/- 2.0 Pa). Shear moduli of samples taken from the anterior portion of the vitreous were as much as 3 times higher.

After rheological characterization, two obvious changes in the tissue suggested that the steady-state moduli we measured were lower than the moduli of the vitreous in vivo. First, the post-measurement vitreous appeared to sag, elongating far more when lifted with forceps than it did when first removed from the globe. Second, a small puddle of liquid was left behind on the instrument where aqueous material – rich in hyaluronic acid but without detectable protein – had seeped out of the vitreous.

The molecular-level interactions responsible for the structure and viscoelastic properties of the vitreous are of fundamental and therapeutic interest, providing insight into natural liquefaction and directions for tissue engineering. The primary goal of engineering the vitreous is to destabilize the native network to facilitate clinical removal of the tissue. To explore the connection between rheological changes and loss of fluid, we quantified the change in vitreous weight under various conditions. We examined the relative importance of covalent bonds, hydrogen bonds, hydrophobic interactions and electrostatic interactions by performing incubations in: collagenase and hyaluronidase, urea, Triton®-X100, buffers with a variety of pH values, and salts with monovalent or divalent cations (NaCl and $MgCl_2$).

Treatment	Maximum weight change (Mf/Mo)	Concentration
NaCl	0.45	5M
MgCl2	0.4	1M
10mM citrate / phosphate buffer	0	pH=2
Collagenase	0	<1000 units
Hyaluronidase	0.45	100 units
Urea	0	5M
Triton® X-100	0.3	0.3M
Mechanical	0.52	2 hrs.

Table 1 – Freshly extracted vitreous was incubated in the following agents for up to 24 hrs, to determine the chemical nature of the most structural significant intermolecular interactions: collagenase and hyaluronidase to cleave covalent bonds in the two predominant network components, urea to disrupt H-bonds, citrate and phosphate buffers with a variety of pH values to alter H-bonding schemes and charge profiles, Triton®-X100 to interfere with hydrophobic interactions, and NaCl and $MgCl_2$ to screen electrostatic interactions.

Collagenase digestion destroyed the vitreous, leaving a viscous liquid. In contrast, hyaluronidase digestion merely softened and reduced the weight (~40%) of the vitreous body. As a function of pH there was a sharp transition from moderate weight loss (~50% or less for pH from 3 to 10) to dramatic weight loss (98% or more, "catastrophic collapse" for pH = 1.5 or 12.5). A similarly drastic transition occured with increasing urea concentration: moderate weight loss for urea <5M; catastrophic collapse for urea ≥5M. Concentrations of Triton (≤ 10%) that are usually adequate to denature proeteins caused only a moderate weight loss (~30%). For all salt concentrations examined ([NaCl] ≤ 5M, $[MgCl_2] \leq 1M$) the weight loss was ~60% or less. The effect of 1M $MgCl_2$ was equivalent to the effect seen in 5M NaCl.

Thus, retention of covalent collagen bonds is necessary, but not sufficient to maintain vitreous structure. In addition, H-bonding (disrupted by carbamide) appears to play an important structural role in the vitreous while hydrophobic and electrostatic forces appear to have a less significant role (Table 1). Thus, the most promising non-covalent interactions to target for tissue engineering agents are H-bonds.

In contrast to the vitreous, our goal in the cornea and sclera is to reinforce the existing structures. The cornea and sclera are important in maintaining the geometry of the eye for good vision, therefore, alterations in the mechanical properties can have detrimental effects on eyesight. Myopia is associated with an elongation, weakening, and thinning of the sclera, and keratoconus is associated with pathological changes in the collagen structure. Fortifying the diseased tissue by crosslinking native proteins or adding an artificial interpenetrating network may lead to successful treatments that diminish or eliminate future problems.

Mechanical properties of 8mm tissue sections from the cornea and posterior sclera have been measured using dynamic shear rheometry with a cleat tool modified from the vitreous work. Because of the gentle nature of these measurements, samples could be loaded, measured, taken off, treated, and measured on the rheometer again. Treatment with a crosslinking agent (2% w/w glyceraldehyde for 24 hrs.) significantly increased the modulus of human and porcine cornea and sclera, by as much 10 times, which is in qualitative agreement with prior literature[6].

Evaluations of other treatments involving the integration of a polymer network with the tissue also yielded promising results. After initial modulus measurements on the rheometer, tissue sections were treated with a mixture of poly(ethylene glycol) dimethacrylate (PEGDM) and photoinitiator in Dulbecco's phosphate buffered saline. After irradiation with ~4mW/cm2 365nm UV irradiation for 30 minutes, the modulus was measured again. The effect of different concentrations of PEGDM (0-25% w/w) was investigated, as well as the effect of oxygen presence during irradiation.

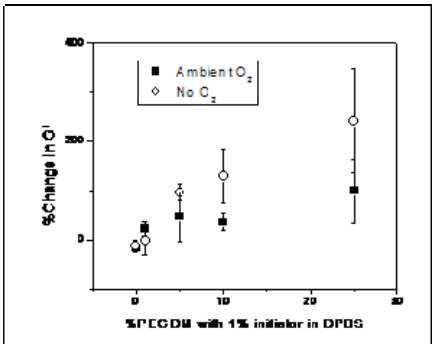

Figure 2 – We have determined the mechanical properties of sclera samples from fresh porcine eyes. 8mm samples were removed from freshly enucleated eyes and loaded on a TA Instruments AR1000 rheometer which had been modified to overcome wall slip by adding cleats to a parallel-plate geometry. Shear moduli were measured while the sample was subjected to oscillatory stress (amplitude = 5 Pa) at a fixed frequency (1 rad/sec) both before and after samples were treated with 550Mw PEGDM mixtures in the presence or absence of oxygen.

In the sclera, higher PEGDM concentrations caused a greater increase in modulus (Figure 2). Increased oxygen concentration reduced modulus changes. Preliminary tests on the cornea have shown similar modulus increases with retention of optical transparency.

Photoactivated treatments like PEGDM mixtures may prove more useful in clinical practice than chemical crosslinking with glyceraldehyde. While both treatments could potentially achieve the same change in modulus, photoactivated treatments can be localized to specific target regions and avoid areas where treatment would be deleterious. In addition, oxygen inhibition of the reaction could provide a method of avoiding reaction in sensitive areas like the vasculature.

References:

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