Rheology of the vitreous gel: Effects of macromolecule organization on the viscoelastic properties

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ABSTRACT

The macromolecular organization of vitreous gel is responsible for its viscoelastic properties. Knowledge of this correlation enables us to relate the physical properties of vitreous to its pathology, as well as optimize surgical procedures such as vitrectomy. Herein, we studied the rheological properties (e.g. dynamic deformation, shear stress–strain flow, and creep compliance) of porcine vitreous humor using a stressed-control shear rheometer. All experiments were performed in a closed environment with the temperature set to that of the human body (i.e. 37 °C) to mimic in-vivo conditions. We modeled the creep deformation using the two-element retardation spectrum model. By associating each element of the model to an individual biopolymeric system in the vitreous gel, a distinct response to the applied stress was observed from each component. We hypothesized that the first viscoelastic response with the short time scale (~ 1 s) is associated with the collagen structure, while the second viscoelastic response with longer time scale (~ 100 s) is related to the microfibrils and hyaluronan network. Consequently, we were able to differentiate the role of each main component from the overall viscoelastic properties.

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1. Introduction

Vitreous Humor is a gel-like, complex, hydrated network, filling the posterior cavity of the eye located between the lens and the retina. Vitreous is composed of approximately 99 wt% water, 0.9 wt% salts, less than 0.1 wt% heterotypic collagen fibrils (type II, V/IX and IX), and a hyaluronan network. Despite the advances in understanding the molecular composition of vitreous, the reason for extreme heterogeneity of the vitreous structure is still unknown (Bishop, 2000; Swindle and Ravi, 2008).

The roles of the vitreous humor are numerous, mainly: developmental (Sebag, 1989), optical (Sebag, 1989); protective (Foulds, 1987; Jacobson, 1985; Sebag, 1989). Several ocular pathologies such as retinal tear, rhegmatogenous or tractional retinal detachment, retinal edema, choroidal detachment, vitreous hemorrhage, and glaucoma can arise as a result of vitreous related complications, which occur mostly due to the vitreous humor’s macromolecular organization and viscoelastic properties (Sebag, 1989; Stein, 2009). Furthermore, besides its viscoelastic nature, which renders its removal tricky, the tight adherence to the surrounding anatomical structures poses some challenges during vitrectomy. As such, understanding the correlation of the macromolecular organization of the vitreous gel, with its bulk viscoelastic properties, is essential to finding a relationship between physical properties and vitreous related pathology, as well as optimizing surgical procedures such as vitrectomy.

Currently, the interaction between vitreous components and their contribution to the overall viscoelastic properties are poorly understood. So far, most research has been limited by the fact that vitreous gel is highly fragile in nature and that measurement instruments have not been sensitive enough to adequately address its properties. Previous studies on the viscoelastic properties of vitreous have been pursued through various avenues. The first comprehensive rheological study was performed by Lee et al. (1992a, b, 1994) using magnetic bead microrheology. Although the results give several important insights into the properties of the vitreous, it is unclear whether the measured local properties report the bulk response. Several other attempts to measure bulk rheological properties of vitreous humor using methods such as shear rheometry (Nickerson et al., 2005a, b, 2008; Lee et al., 1992a, b, 1994; Tokita, 1984; Bettelehim and Wang, 1976) and indirect viscoelastic measurements (Zimmerman, 1980; Walton et al., 2002) exist. While these studies advanced the body of knowledge pertaining to the physical properties of vitreous, they are either incomplete from a rheological standpoint or have tested the fluid under conditions that do not resemble conditions in vivo. Additionally, the correlation between macromolecule organization and measured viscoelastic properties has not been thoroughly studied (Bishop, 2000).
We studied the viscoelastic properties of vitreous humor in vitro using a stress-controlled shear rheometer, which is sensitive and accurate enough for the characterization of an extremely delicate gel-like vitreous humor. In addition to other standard rheological tests, we performed creep deformation experiments by applying constant shear stresses in a finite time. The creep deformation is the most direct measurement of the material’s elasticity (Ferry, 1980). We further modeled the creep compliance and directly obtained two time scales from the viscoelastic response. By associating each element of the model to an individual component of the vitreous, we observed a separate response with a specific time scale from each component to the applied stress in a single rheology experiment. We further verified the results by comparing them with the viscoelastic properties of ultra-pure hyaluronic acid and collagen structures. This observation gives a new insight into the macromolecular mechanism responsible for the bulk behavior.

2. Materials and methods

Freshly harvested porcine eyes were purchased from Sierra Medical Supplies (Whittier, CA, USA). Ultra-pure hyaluronic acid with the molecular weight of 4 million Da dissolved in physiological sodium chloride phosphate (pH 7.0–7.5) was acquired from Advanced Medical Optics (Uppsala, Sweden). Eyes were acquired on the day of experimentation and the tests were performed within 10 h postmortem to ensure consistency in the results. After removal of the cornea and the lens, the whole vitreous was dissected in one piece from the eye. The anterior vitreous adheres to the ciliary body and the lens. When dissected, it usually contains some non-vitreous cells including retinal pigment epithelial cells. Therefore, the anterior vitreous was discarded, and the central vitreous was cut directly onto the rheometer plate. The vitreous was not exposed to air for more than 30 s before being enclosed in a solvent trap. A stress-controlled shear rheometer (AR-2000 TA Instruments) with 20 mm parallel disc geometry was used to obtain the rheological properties. The parallel discs were covered with 600-grit silicon carbide sandpaper to minimize the slippage of the sample and provide the effective zero-slip condition (Yoshimura and Prudhomme, 1988). In order to minimize the effect of water evaporation and liquid loss, a solvent disc was observed for both storage and loss modulus at frequencies greater than approximately 1 Hz.

Failure analyses were performed to determine the minimum shear stress and strain required to destroy the tissue. In a peak-hold experiment, constant shear rates of 0.01, 0.1, and 1 s⁻¹ were applied and shear stress was monitored as function of strain % for 30 s. The shear rates were selected in a manner that represents three different time scales, 1, 10, and 100 s, for the vitreous behavior. The onset of maximum stress was determined as the failure stress. Additionally, frequency tests were conducted to obtain storage and loss modulus as a function of frequency for the strain amplitude of 3%, which is in the linear viscoelastic region (i.e. plateau region in a strain-sweep test). Creep compliance experiments were performed on the vitreous humor and hyaluronic acid solutions (concentrations of 0.5, 1, 3, 6 mg/ml) for constant shear stresses of 0.5, 1, and 2 Pa. The constant shear stress was held until the sample reached the linear steady state condition. Creep compliance, G(t), was calculated as G(t) = τ(t)/σ⁰, where τ(t) is a deformation and σ⁰ is a constant shear stress.

3. Results

3.1. Dynamic deformation: storage and loss modulus

Storage modulus and loss modulus were obtained as a function of frequency for the strain amplitude of 3% (Fig. 1). We successfully captured a broad range of the plateau region (ω = 0.1–10 rad/s) for the vitreous gel. The averages for the storage and loss modulus are G’ = 1.08 ± 0.22 Pa and G” = 0.25 ± 0.07, respectively. Both G’ and G” sharply increase at frequencies greater than approximately 7 rad/s (1 Hz). Frequencies below ~0.1 rad/s are too small to capture meaningful data with this equipment. Our attempt to address the low frequency region using time-temperature superposition failed due to permanent changes in the structural composition of the vitreous gel at temperatures above 40 °C. We also repeated the oscillation experiments with strain amplitudes below 3%. Similarities in the results suggest that strain amplitudes below γ = 3% are in a linear viscoelastic region.

![Fig. 1. Storage modulus, solid symbols, and loss modulus, hollow symbols, are plotted as a function of frequency for γ = 3% for three different eyes. A sharp increase was observed for both G’ and G” at frequencies greater than approximately 1 Hz.](image1)

![Fig. 2. Failure analysis of vitreous (shear stress–strain) at different shear rates. For γ = 0.01 s⁻¹, τ_max = 1.42 ± 0.21 Pa and γ_max = 610.13 ± 0.44%. For γ = 0.10 s⁻¹, τ_max = 7.01 ± 1.73 Pa and γ_max = 1599.53 ± 1.25%. For γ = 1.00 s⁻¹, τ_max = 27.36 ± 6.50 Pa and γ_max = 4001.17 ± 12.27%.](image2)

3.2. Shear stress–strain flow: failure experiment

The onset of a rate-dependent maximum stress is the failure point (Fig. 2). After reaching the maximum, stress declines gradually with the increase of strain. Fig. 2 shows that the rate-dependent maximum increases as shear rate is increased. Shearing at lower rates allows the network to relax and therefore, fail at lower shear stress and strain (i.e. liquid-like behavior). At shear rate of 1 s⁻¹ the structure fails at much higher stress and strain (i.e. solid-like behavior). Quantitatively, for γ = 0.01 s⁻¹, τ_max = 1.42 ± 0.21 Pa and γ_max = 610.13 ± 0.44% and for γ = 1 s⁻¹, τ_max = 27.36 ± 6.50 Pa and γ_max = 4001.17 ± 12.27%.

In our analyses, the onset of failure in stress–strain tests was lower than the values reported by Nickerson et al. (2008). It should be noted that we performed our failure experiments on fresh vitreous gel without any pre-shear, whereas Nickerson et al. (2008) performed their tests after another time-dependent experiment where the modulus reached the steady state.

3.3. Creep experiment

We are reporting the results of the first creep compliance experiments of the vitreous humor using shear rheometry. Three distinct regions were observed from the creep experiment (Fig. 3). The first region, lasting approximately 1 s, is the elastic region...
Ferry (1980) in which vitreous elastically responds to sudden shear stress (i.e. solid-like behavior). The elastic region is quickly followed by retardation region ($\tau_{r1}$). Eventually, a steady state condition was attained (i.e. liquid-like behavior).

To our knowledge, the only previous creep experiments performed on vitreous were done by Lee et al. (1992a, b) using a micro-rheometer with an experimental duration of 3–12 s. In contrast to Lee’s work, we performed the creep experiments using a shear rheometer and increased the duration of the experiment in order to obtain additional time scales in response to the creep deformation.

3.4. Viscoelastic model

Assuming that the vitreous has two main polymeric components, we used a viscoelastic spectra model with two Voigt–Kelvin elements in series (Ferry, 1980) to model creep behavior of vitreous humor (Fig. 4). The mathematical expression of this discrete spectra model is

$$J(t) = \sum_{k} J_k \left(1 - e^{-t/\tau_k}\right) + \frac{t}{\eta_0}$$

where $k$ is a number of elements ($k=2$ for our model), $J_k$ is the compliance of each element, $\tau_k$ is defined as the retardation time and is a measure of the time required for the extension of the spring to its equilibrium length during retardation by a dashpot. We added the term $t/\eta_0$ to account for viscous behavior at the steady state region.

Fig. 5 shows the model applied to three shear stresses of $\tau_0 = 0.5, 1, \text{ and } 2 \text{ Pa}$. We found that only two discrete lines were needed, regardless of applied stress, to model the creep compliance. This indicates that vitreous fundamentally has two time scales in response to a constant stress in a creep experiment. The parameters of the models are provided in Table 1. The average of the first retardation time (elastic region) is $\tau_{r1} = 1.97 \pm 0.69$ s. The average of the second retardation time (retardation region) is $\tau_{r2} = 90.00 \pm 30.83$ s. The average storage modulus obtained from the first Voigt–Kelvin element is $G_1 = 1.66 \pm 0.96$ Pa, which is consistent with the modulus obtained from oscillation tests. The average elastic modulus obtained from the second Voigt element is $G_2 = 1.14 \pm 0.71$ Pa. Previously, Lee et al. (1992a, b) reported a three-parameter model for the microrheology creep experiments of the vitreous gel. However, their experiments’ run time was not long enough to capture the second time scale that we reported here. Therefore, only one Voigt–Kelvin element with one time scale was needed to model the creep compliance obtained from their experiment with a run time of around 10 s.

4. Discussion

The vitreous humor is a viscoelastic hydrogel with two main biopolymers. The first polymeric system is collagen structure. The second is hyaluronan network and dispersed microfibrils (Fig. 4). Because of the fundamental differences in the structures of these macromolecular networks, the time scales of their responses to constant stress are different. To capture all time scales reflecting the variety of macromolecular motions in the vitreous polymeric system, we performed an extended duration creep deformation test. Hence, we were able to resolve the effects of each component from the overall viscoelastic properties in a time-dependent analysis.

From the creep deformation, we obtained two time scales in three viscoelastic regions: (a) $\tau < \tau_{r1}$ (i.e. $\sim 1$ s), where gel shows solid-like
second Voigt–Kelvin element, is associated with the microfibrils and with the short time scale in creep compliance experiments. The results show considerable reduction of elasticity which is associated with collagenase to digest the collagen structure in the vitreous. Their structures in the overall viscoelasticity of the vitreous gel. The initial linked collagen gels support our hypothesis on the role of such associations (Almond et al., 1998a, b). Finally, the absence of an abrupt increase of shear stress. The module of elasticity obtained from the first Voigt–Kelvin element with the first time scale is \( G_1 = 1.66 \pm 0.96 \text{ Pa} \), which is within the range of the modulus obtained from the oscillatory test. Also, based on our model, collagen exerts a viscous behavior, which can be estimated as \( \eta_1 = \frac{t_1}{J_1} = 2.4 \text{ Pa} \). The viscous behavior of the collagen structure is possibly due to the mobility of the macromolecules and lightly cross-linked nature of the network. Previous studies on the viscoelastic properties of cross-linked collagen gels support our hypothesis on the role of such structures in the overall viscoelasticity of the vitreous gel. The initial response of cross-linked collagen structures to deformation is found to be elastic with a short time scale in the order of 1 s (Sheu et al., 2001; Lai et al., 2008). In a study on the vitreous humor, Sekuma et al. (2004) used collagenase to digest the collagen structure in the vitreous. Their results show considerable reduction of elasticity which is associated with the short time scale in creep compliance experiments.

We hypothesize that the second time scale obtained from the second Voigt–Kelvin element, is associated with the microfibrils and the hyaluronan network. Using the discrete retardation model, the average viscosity of the second polymer system is \( \eta_2 = 70 \text{ Pa} \). As expected, the majority of the viscous behavior of vitreous is from the hyaluronan network and random microfibrils (Almond et al., 1998a, b). From the second element, we obtained the average elastic modulus of \( G_2 = 1.14 \pm 0.71 \text{ Pa} \), which is smaller than \( G_1 \). Therefore, we believe that microfibril and hyaluronan networks always form viscoelastic solutions, further suggesting the presence of lateral aggregates of hyaluronan in the vitreous (Almond et al., 1998a, b; Brewton and Mayne, 1992; Bishop, 2000). In addition, such behavior indicates that hyaluronan chains do not form strong stable intermolecular associations (Almond et al., 1998a, b). Finally, the absence of an equilibrium compliance for creep deformation (Fig. 3) indicates that the vitreous is a lightly cross-linked polymer network whose thread-like macromolecules are not permanently attached to each other.

While the viscoelastic properties of collagen structures, especially creep compliance, have been extensively studied in the literature, availability of such data for rheological properties of hyaluronic acid (HA) solutions is limited. We performed similar creep experiments on HA solutions with different concentrations to compare their time scales with that of the HA network in the vitreous. The HA compliance was successfully modeled using one Voigt–Kelvin element (Ferry, 1980) with one time scale. The results show that the HA solution is a viscoelastic liquid with a large time

**Table 1**

Parameters of discrete retardation model for creep test at different shear stresses and eyes.

<table>
<thead>
<tr>
<th>Shear stress, ( \tau_0 ) (Pa)</th>
<th>Sample</th>
<th>( t_1 ) (s)</th>
<th>( J_1 ) (Pa(^{-1}))</th>
<th>( t_2 ) (s)</th>
<th>( J_2 ) (Pa(^{-1}))</th>
<th>( \eta_m ) (Pa s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>Eye 1</td>
<td>1.77 ± 0.05</td>
<td>1.36 ± 0.02</td>
<td>74.15 ± 1.36</td>
<td>2.64 ± 0.02</td>
<td>1332.0 ± 23.4</td>
</tr>
<tr>
<td></td>
<td>Eye 2</td>
<td>2.30 ± 0.06</td>
<td>1.90 ± 0.02</td>
<td>70.91 ± 5.12</td>
<td>0.85 ± 0.02</td>
<td>1263.0 ± 44.6</td>
</tr>
<tr>
<td></td>
<td>Eye 3</td>
<td>3.01 ± 0.06</td>
<td>0.70 ± 0.01</td>
<td>124.09 ± 3.48</td>
<td>1.89 ± 0.04</td>
<td>493.6 ± 19.4</td>
</tr>
<tr>
<td>1</td>
<td>Eye 1</td>
<td>2.00 ± 0.05</td>
<td>0.90 ± 0.01</td>
<td>127.16 ± 6.56</td>
<td>2.16 ± 0.09</td>
<td>1543.4 ± 417</td>
</tr>
<tr>
<td></td>
<td>Eye 2</td>
<td>1.85 ± 0.05</td>
<td>0.77 ± 0.01</td>
<td>51.77 ± 3.00</td>
<td>0.71 ± 0.01</td>
<td>1255.1 ± 77.2</td>
</tr>
<tr>
<td></td>
<td>Eye 3</td>
<td>3.01 ± 0.06</td>
<td>0.70 ± 0.01</td>
<td>123.91 ± 3.47</td>
<td>1.89 ± 0.03</td>
<td>492.7 ± 19.3</td>
</tr>
<tr>
<td>2</td>
<td>Eye 1</td>
<td>1.15 ± 0.03</td>
<td>0.28 ± 0.01</td>
<td>49.24 ± 2.58</td>
<td>0.48 ± 0.01</td>
<td>600.6 ± 26.4</td>
</tr>
<tr>
<td></td>
<td>Eye 2</td>
<td>1.42 ± 0.04</td>
<td>0.45 ± 0.01</td>
<td>102.70 ± 17.00</td>
<td>0.68 ± 0.12</td>
<td>1239.2 ± 548.0</td>
</tr>
<tr>
<td></td>
<td>Eye 3</td>
<td>1.22 ± 0.04</td>
<td>0.38 ± 0.01</td>
<td>86.02 ± 14.70</td>
<td>0.45 ± 0.07</td>
<td>1293.0 ± 413.0</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>1.97 ± 0.69</td>
<td>0.83 ± 0.50</td>
<td>90.00 ± 30.83</td>
<td>1.30 ± 0.83</td>
<td>1057.0 ± 407.3</td>
</tr>
</tbody>
</table>

**Fig. 6.** Comparison of the time scales obtained from the vitreous gel and hyaluronic acid (HA) solutions. HA time scales are in the same order of magnitude with the second vitreous time scale.
scale; generally on the same order of magnitude with the second time scale observed in the vitreous humor tests (Fig. 6). These results imply that the pure HA solution does not present a solid-like response to the abrupt increase of the shear stress. In other words, the large time scale obtained from the pure HA solution represents the transition from viscoelastic behavior to liquid behavior.

We also observed the same time scales in the creep tests, as in the failure analyses experiments. For example: \( \gamma = 1 \text{s}^{-1} \) represents the time scale of 1 s where vitreous shows solid-like behavior in a failure test (Fig. 2); \( \gamma = 0.01 \text{s}^{-1} \) represents time scale of 100 s where the gel shows liquid-like behavior (Fig. 2). A similar pattern is seen regarding the results of frequency sweep tests. For frequencies less than 1 Hz (time scale of 1 s), the storage modulus sharply increases and resembles solid-like behavior. Moreover, the frequency sweep results show that the loss modulus also increases at frequencies below 1 Hz. This reflects the relative motion of the chain segments between the entanglement coupling loci. Additionally, there are micromolecules in the solution that possibly contribute to the viscous behavior of the vitreous in this time scale.

The time scales obtained from the creep deformation are both quantitative and reproducible. In addition to resolving the effects of each vitreous component from the overall properties, these time scales may be useful in quantifying the effects of vitreous related pathology, as well as the short-term and long-term effects of pharmacological agents on the properties of the vitreous humor.

**Conflict of interest statement**

The authors confirm that there is no conflict of interest in this paper.

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**Appendix A. Supplementary material**

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jbiomech.2010.10.002.

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