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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 microfibrilis and hyaluronan network. Consequently, we were able to differentiate the role of each main component from the overall viscoelastic properties.

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/XI 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 rheometery (Nickerson et al., 2005a, b, 2008; Lee et al., 1992a, b, 1994; Tokita, 1984; Bettelheim 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).

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We studied the viscoelastic properties of vitreous humor *in vitro* using a stressed-control 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 stressed-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 trap sealed with vacuum oil was used to enclose the sample. All the experiments were done with zero normal force on samples to minimize the damage to the sample and at the elevated temperature of 37 °C to mimic in-vivo conditions.

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 $\dot{\gamma}=0.01, 0.1,$ and $1\ s^{-1}$ were applied and shear stress was monitored as function of strain %. 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 moduli 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 $\tau_0=0.5$, 1, and 2 Pa. The constant shear stress was held until the sample reached the linear steady state condition. Creep compliance, $J(t)$, was calculated as $J(t)=\gamma(t)/\tau_0$, where $\gamma(t)$ is a deformation and τ_0 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 ($\omega=0.1$–10 rad/s) for the vitreous gel. The averages for the storage and loss modulus are $G'=1.08\pm0.22$ Pa and $G''=0.25\pm0.07$, respectively. Both G' and G'' sharply increase at frequencies greater than approximately 7 rad/s (1 Hz). Frequencies below $\sim$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 $\gamma=3\%$ are in a linear viscoelastic region.

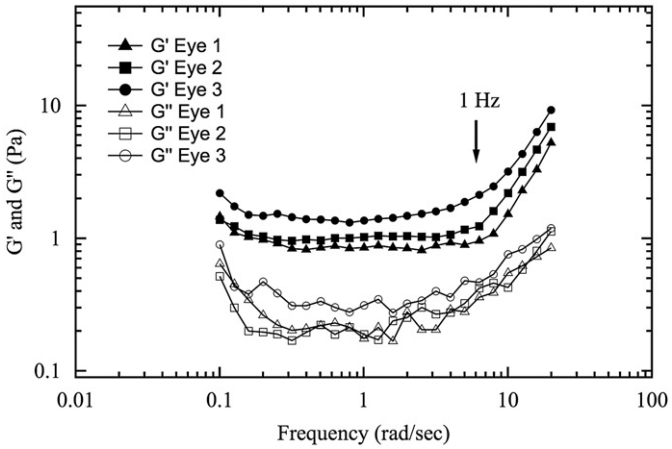

Fig. 1. Storage modulus, solid symbols, and loss modulus, hollow symbols, are plotted as function of frequency for $\gamma=3\%$ for three different eyes. A sharp increase was observed for both G' and G'' at frequencies greater than approximately 1 Hz.

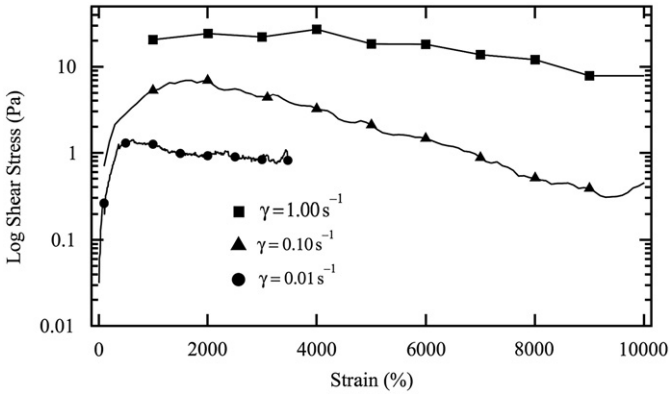

Fig. 2. Failure analysis of vitreous (shear stress–strain) at different shear rates. For $\dot{\gamma}=0.01\ s^{-1}$, $\tau_{max}=1.42\pm0.21$ Pa and $\gamma_{fail}=610.13\pm0.44\%$. For $\dot{\gamma}=0.10\ s^{-1}$, $\tau_{max}=7.01\pm1.73$ Pa and $\gamma_{fail}=1599.53\pm1.25\%$. For $\dot{\gamma}=1.00\ s^{-1}$, $\tau_{max}=27.36\pm6.90$ Pa and $\gamma_{fail}=4001.17\pm12.27\%$.

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 $\gamma_{fail}=0.01\ s^{-1}$, $\tau_{max}=1.42\pm0.21$ Pa and $\gamma_{fail}=610.13\pm0.44\%$ and for $\dot{\gamma}=1\ s^{-1}$, $\tau_{max}=27.36\pm6.90$ Pa and $\gamma_{fail}=4001.17\pm12.27\%$.

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, where as 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 rheometery. Three distinct regions were observed from the creep experiment (Fig. 3). The first region, lasting approximately 1 s, is the elastic region