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Rheology of Vitreous Gel

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Master of Science in Mechanical Engineering

by

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Abstract of the Thesis

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The viscoelastic properties of the vitreous gel, major component in human eye, has not been investigated thoroughly so far. Despite our recent findings on the link between vitreous complications and several ocular diseases such as tractional retinal detachment, retinal tear, vitreous hemorrhage and glaucoma, there are still some unknown aspects that require more study to be done. Here, the emphasis is on characterization of the recent experimental tools invented at UCLA for analyzing rheology of the vitreous gel. We were able to present similar results in agreement with previous work. In addition to that, the relationship between the viscoelastic behavior patterns in a pair of eyes was investigated in different species. While the creep compliance behavior was different among species, we found a clear similarity pattern in creep compliance behavior within pairs in all species. This finding will significantly advance the future studies of biomechanics of vitreous gel such as more investigation on correlation between vitreous component interactions and vitreous related pathologies or surgeries.

The thesis of Rommina Vedadghavami is approved.

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To two strong women in my life, my mother, Farzaneh and my aunt, Faranak.

To my mom for all the sacrifices she made for me, her love has liberated me...

To my aunt, for being there for me every step of the way. I am lucky to have you in my life.

I also dedicate this thesis to my favourite uncle in the world. Without his help, I couldn't have accomplished what I have so far...

I am grateful for your love and care.

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CHAPTER 1

Introduction

1.1 Structural Macromolecules in Vitreous Gel

The vitreous humor is a transparent delicate gel that is approximately 99% water and 0.9% salts. This gel is comprised of a highly swollen double netwrok of protein fibrils (primarily collagen *II*) and charged polysaccharide chains that is primarily hyaluronan[5]. The bulk of extracellular matrices of the vitreous gel is a natural composite structures containing network forming macromolecules. The vitreous is a water based structure reinforced by a low concentration of fibrillar proteins that are resistant to tractional forces. In addition, there are charged carbohydrates, i.e. glycosaminoglycans (GAGs), which absorb water, thereby providing a swelling pressure that spaces apart the fibrillar proteins in order to inflate the tissue to resist compressive forces[24].

1.1.1 Vitreous Collagen Structure

The vitreous contains a low concentration of collagen, about 60 $\mu g/ml$ in bovine eyes and 300 $\mu g/ml$ in human eyes[19].

As reported by previous studies, the concentration of collagen does not change throughout life, i.e, it is suggested that there is no after birth synthesis of vitreous collagen. Fig.1.1 shows the vitreous in eye[28].

Collagen structure in vitreous is mainly composed of type *II* collagen (60-70%), type *IX* collagen (25%), and type *V/XI* collagen (10-25%)[6]. Collagen type *II* is a fibril-forming collagen that is composed of three α-chains with different amino acid composition with length of 11.2 nm. Fibrils are made from the supermacromolecule organization of collagen

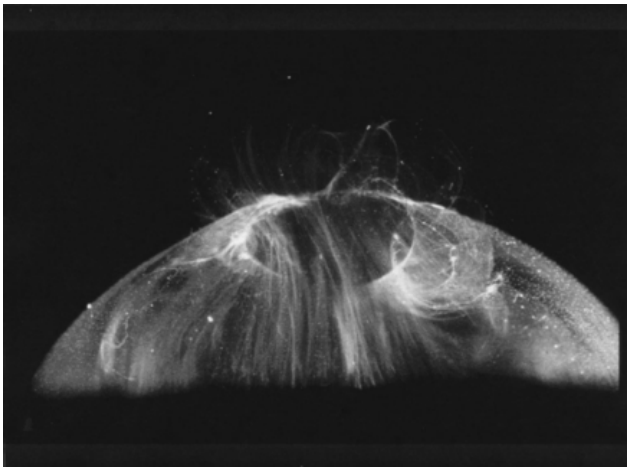


Figure 1.1: Image of vitreous gel taken using slit microscopy [31]

with a diameter of approximately 10-500 nm. When thiner collagen fibrils fuse together, thick collagen *II* could be formed. In general, collagen fibrils have (a) uniform thickness, (b) do not branch, (c) run from the posterior to interior eye[3]. Collagen*IX* consists a smaller fraction of the collagen in vitreous, but it cannot form fibrils in isolation[42]. Collagen *V/XI* is a minor component of the collagen network and they usually co-assemble with collagen*II* to form the the core of the heterotypic fibrils. It has been presented that collagen type *V/XI* can play an important role in the initiation of collagen fibril formation[44]. The network of collagen seems essential to the gel state of the vitreous. If collagen is removed from the vitreous, the gel simply becomes a viscous liquid.

1.1.2 Vitreous Hyaluronan Network

Hyaluronan or hyaluronic acid (HA) is a glycosaminoglycan in which N-acetylglucosamine and glucuronic acid are linked alternately by glycoside bonds to form a long chain. The chain has molecular weight of 1 to 10 million Da and the diameter of 100-500 nm. The concentration of hyaluronan in human eyes is 65-400 $\mu g/ml$[2]. The long chain of hyaluronan coils up forming a sponge-like random molecular network filled with water. The most significant characteristics of the hyaluronan are that (a) comparing to its molecular weight it contains large amount of water, (b) it has negatively charged molecule chains that easily interact with

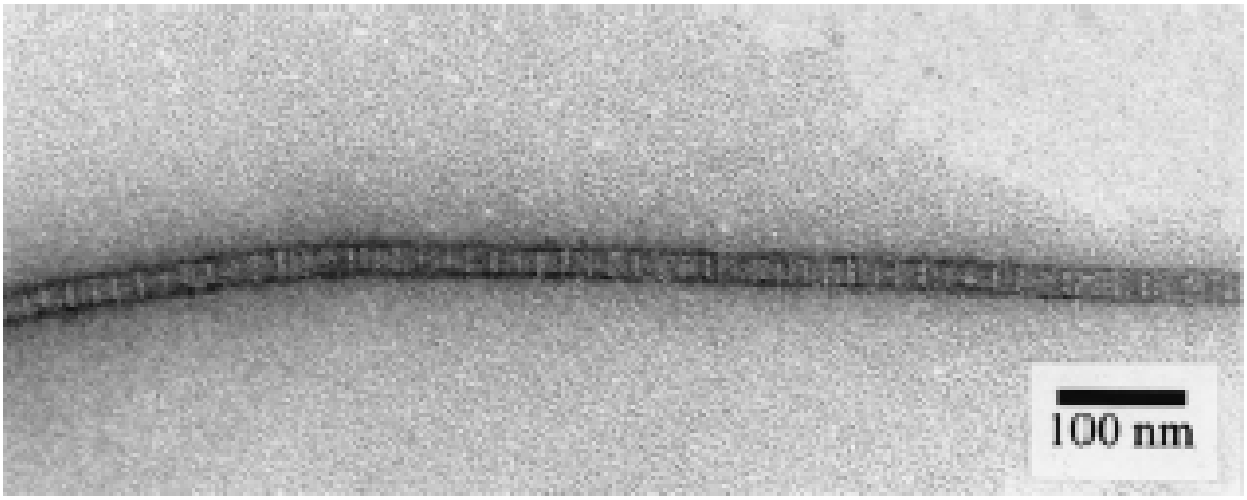


Figure 1.2: Negatively stained image of an isolated heterotypic collagen fibril from bovine vitreous[6]

other macromolecules, and (c) a small shear can cause a large network deformation [1].
HA fills the space between the fibrils and this interaction plays an important role in the mechanical properties of the vitreous gel [1]. Furthermore, the viscoelastic properties of the HA prevent the fibrils from aggregating under vibration and shear stresses. One can figure out the distance between collagen structure by the volume of the individual HA coil and its state of hydration.
Although the composition of extracellular matrix elements of the vitreous gel is similar in all species, the concentration of the components such as HA and Collagen varies in different species as summerized in Table1.1.This table presents the composition of the major components of vitreous gel for several species[30],[25].

1.2 Different Functions of Vitreous Gel

The vitreous gel is a transparent media that supports the structures within the eye. Its viscoelastic properties allow eye to return to its normal shape under applied stress[46]. The roles of the vitreous humor are numerous, mainly: developmental, optical, protective [29],[14],[16]. However, opthalmologists are likely to consider the vitreous as merely the clear gel filling the eye cavity and believe besides its development role, the vitreous has no significant function

Table 1.1: Collagen and HA composition in vitreous gel[30].[25]

Adult	Gel	Liquid	HA (μg/ml)	Collagen (μg/ml)
Human	80-40	20-60	100-400	280-1360
Rhesus monkey	60	40	100	113-139
Owl monkey	2	98	300-600	66-77
Porcine	100	0	80	150
Cow	100	0	800-900	684
Rabbit	100	0	20-40	385
Dog	100	0	40-60	144
Sheep	100	0	100-1070	384

in eye health and therefore, removing vitreous from eye does not have any adverse effect[43]. Nonetheless, a new understanding of the vitreous gel is emerging that believes the structure and function of an intact gel vitrous is important to the health of the human eye. Age-related vitreous degeneration, the process of liquefaction of the vitreous gel, may be the starting pathogenic step to many ocular disorders ranging from retinal detachment (RD) to nuclear sclerotic cataract[15].

1.2.1 Developmental Function

The eye geometry depends upon the vitreous size, growth, and tangential forces generated during development process[10]. It was demonstrated that changes in HA volume during development influence the volume of the vitreous and consequently affect the growth of eye to exact specification[23].

1.2.2 Optical Function

The index of refraction of the vitreous is 1.33, similar to that of water.The low concentration of macromolecule solutes permits the transmission of 90 % of light between 300-1400 nm

range[7]. It is believed that the primary function of the vitreous is to transmit the unhindered light to the retina[38]. The presence of large HA molecule keeps the collagen filaments widely dispersed and therefore, minimizes the light-scattering. In addition, the vitreous must serve as a barrier to the influx of cells and macromolecules from surrounding tissues. Furthermore, the vitreous inhibits the proliferation of cells involved in inflammatory, and nonvascular responses[13].

1.2.3 Mechanical Function

Vitreous gel is a viscoelastic substance that protects eye tissues from high-frequency stresses. The vitreous has solid-like response to mechanical stresses with frequency above 0.4 cycle/s[1]. It is shown that vitreous plays an indirect role in accomodation, the process by which changing lens curvature increases the reflective power of the eye for seeing near objects. The mechanism behind this process is based upon the existance of pressure and elasticity between the vitreous body, lens and anterior chamber[9].

1.2.4 Intraocular Oxygen Regulation

Recent studies showed that vitreous gel regulates intraocular oxygen tension. While the vascularized retina remains highly oxygenated, the vitreous gel serves as a barrier to protect the tissues such as the lens and trabecular meshwork that are most sensitive to oxidative stress. The vitreous gel consumes molecular oxygen diffusing into the vitreous from the retinal vasculature before it reaches the anterior segment. The oxygen consumption rate is much faster for the vitreous gel than liquid vitreous (e.g vitreous gel with age-related liquefaction or surgical removal) [35]. Therefore, the gel state of the vitreous could be critical for proper oxygen regulation. This new understanding of vitreous function links its structure to some ocular pathologies such as nuclear sclerotic cataract, apoptotic neuronal cell death, the trabecular meshwork to vitreous liquefaction in which excessive oxygen in the anterior segment causes oxidative stress and tissue damage[41].

1.3 Viscoelastic Propeties of Vitreous Gel

The vitreous gel is a solid-like substance that has both viscous and elastic properties. The physical properties of the vitreous gel are derived mainly from the collagen and hyaluronan components[4]. Therefore, the core of vitreous gel stability is the interaction between networks of HA molecules and collagen fibrils. Previous studies has shown that the collagen fibrils maintain a solid structure that is inflated by the hydrophilic hyaluronam. If hyaluronan is removed, the fibers collapse and gel shrinks; when collagen is removed, the remaining hyaluronan forms a viscous liquid[40].

However, some studies show that the network of collagen fibrils has been presumed to serve the load-bearing function since the vitreous does not collapse during enzymatic removal of hyaluronan but dissolves completely after digestion with collagenase Fig. 1.3[24].
It has been suggested that swollen hyaluronan macromolecules simply hydrate the network and fill the space between the fibrils to prevent aggregation. In addition, investigations suggested that hyaluronan-collagen interaction might be mediated by a third molecule that serves as the cross-linking element in this bipolymer system. The molecular weight of such mediator is reported to be around 10^6 Da which corresponds to the molecular weight of hyaluronan. Further studies must be performed to identify the nature of HA-collagen interactions and their relation to the overall physical properties of the vitreous gel [5].

1.4 Pathologies and Links to Vitreous

There are several ocular pathologies such as retinal tear, retinal edema, choroidal detachment, vitreous hemorrhage, tractional retina detachment and glaucoma that can arise as a result of vitreous related complications[29],[37]. Aging casuses the human vitreous to experience a process of liquefaction in which the gel volume decreases while the liquid volume increases. This process is evident after the age of 4 years and by the time the eye reaches full size, which is at 14-18 years of age, approximately 20 % of the vitreous cavity is liquid. By the age of between 80 and 90 years, more than 50 % of the vitreous is liquified Fig. 1.4[5].

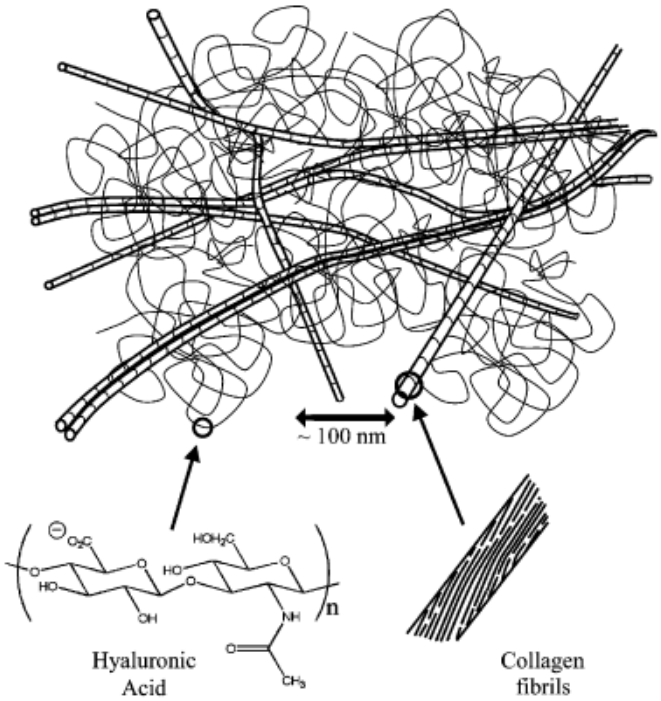


Figure 1.3: Schematic depiction of the network structure of the vitreous. The vitreous is composed of a highly swollen double network of heterotypic collagen fibrils (primarily type II) and hyaluronic acid[24]

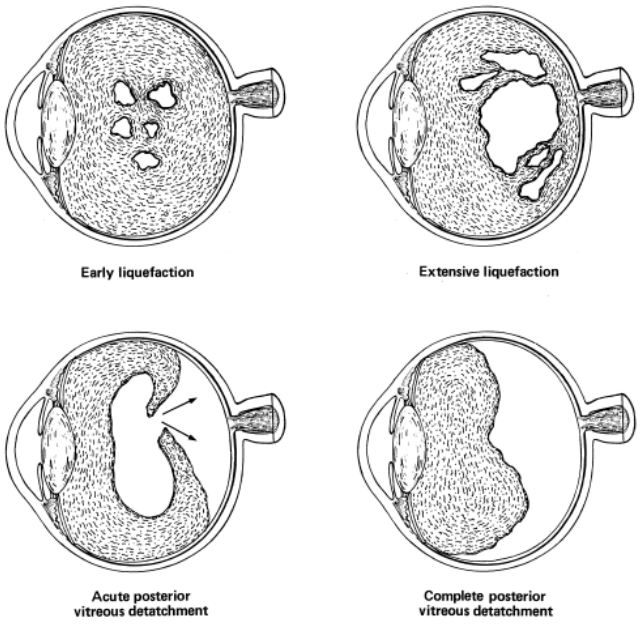


Figure 1.4: Age-related degeneration of the human vitreous body, In 25-30% of the population these degenerative changes eventually result in posterior vitreous detachment[6]

This process can lead to several complications such as posterior vitreous detachment.

1.5 Vitrectomy

Vitrectomy is a surgical procedure to remove the vitreous gel from the eye in order to decrease the vitreous related complications[22],[8]. Some challenging can occur while vitrectomy is conducted thus removing a viscoelastic matter like vitreous gel can be tricky. Therefore, the mechanism of removing is by cutting small segment of the vitreous and then collecting the segment by sucking it using a vacuum pump through the tubing. A small gauge cutters (usually 20, 23 and 35 gauge) with two primary functions of cutting and suction are used in vitrectomy. The main issue is the change in the value of viscosity in chopped vitreous compared to the value of intact vitreous[36].

Recent advances in developing high-end multifunctional vitrectomy machines and ultrahigh-speed cutters, powerful illuminating light sources and chandelier endoillumination systems, and wide-angle viewing systems (WAVs), enhanced developing several new techniques and

enabled using much smaller gauge systems for treating challenging cases safer and more efficient[26]. Despite all the advancement in vitrectomy instrumentation, the fluid flow of vitrectomy is still not undesrtood well, though such knowledge is essential to achieve the optimized performance [36].

1.6 Motivation

Previous studies have shown a correlation between the macromolecular organization of vitreous and its related pathologies such as rhegmatogenous, retinal tear, tractional retinal detachment, glaucoma and etc. This is true while there is still alot of unsolved mystery about vitreous humor. Better understanding of the rheology of vitreous would enhance further development of surgery procedures and therapeutic treatments[30]. Most of vitro-retinal surgical procedures include a vitrectomy, as was descibed in details in Section 1.5. More understanding on the viscoelastic properties of vitreous would help modify cutters to obtain better flow rate performances[17].
In addition to that, better knowledge on this matter is essential not only with respect to the normal physiology of vitreous and its structure, but also for better understanding of the aging phenomena of vitreous and vitreous related pathologies.

The biggest challenge is to characterize viscoelastic properties of vitreous humor because of its fragile structure. In order to measure its bulk rheological properties, the eye must be dissected and the vitreous needs to be removed[33]. Therefore, a suitable technique that is able to measure its properties with minimal damage is necessary. In-situ techniques are good solutions for this application. However, current techniques have their own issues: Some of them measure the local properties such as in microrheology [20],[21] or have lack of desirable sensitivity such as light scattering techniques [46]. As for microrheology in small samples, there is less accuracy (magnetic beads are used as microspheres) [46]. Also, in conventional macroscopic rheometers, large amounts of sample are needed and it is difficult to test delicate biological samples such as vitreous gel. Considering all the above difficulties and the need for a better technique, a device was designed to address these issues. The invented rheology

device is a cylindrical probe with a diameter ranging from less than 1 mm(micro-probe) to large diameter (macro-probe). This device has been used to obtain creep comliance of the viscoelastic materials such as vitreous gel [33].

The purpose of this study is to further characterize the previously invented probe. In order to do that, creep compliance tests were desigend and conducted on pair of eyes in three different species. These tests were also perfomed to investigate any possible rheological similarities in a pair of eyes in different species.

CHAPTER 2

Materials and Methods

2.1 Materials

In this section a summary of material handling and preparation for the specimens (pig, cow and rabbit vitreous) is provided.

2.1.1 Vitreous Gel Study

Freshly harvested porcine, cow and rabbit eyes were purchased from Sierra Medical Supplies (Whittier, CA, USA). Eyes were acquired on the day of experimentation and the tests were performed within 12 hours after postmortem to ensure the consistency in the results.

Porcine eye is often used as an vivo animal model in opthalmological research because of its morphological similarities to human eye. Porcine eye has been used for neuroretinal studies, glaucoma research, cornal transplant and aberrometry studies[27]. Porcine eye has also been an animal model for characterization and optimization of related surgical devices because of the substantial similarities of its mechanical properties to that of human vitreous[11],[39].

In this study, rabbit and cow eyes were also prepared to furthermore characterize the function of the rheology rod (cylindrical probe). First, the eyes were cleaned up and extra tissues around them were removed. Second, cubic foams were prepared as fixtures in order to hold the eyes in position while the test is running. These foams were made in 8.5 mm width, 8.5 mm in length and 60 mm in depth. Incision was made on the sclera at 2 mm of the cornea for pig eyes. For rabbit eyes, this incision was made at 1 mm of the cornea,

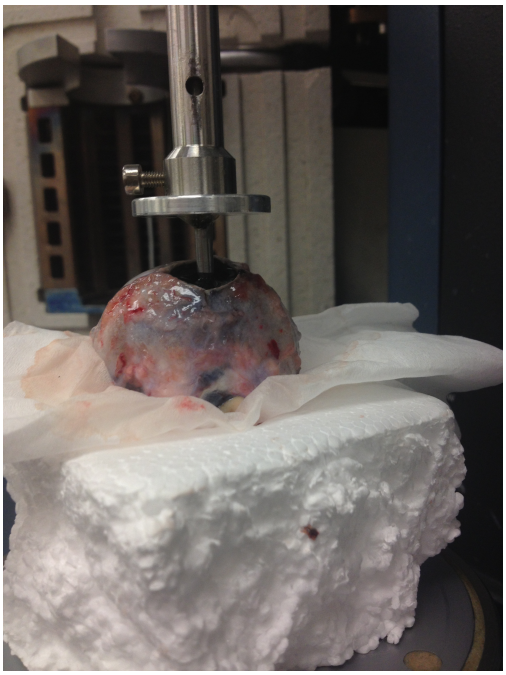


Figure 2.1: The probe inserted in a cow eye from a window opened at 4 mm from cornea

considering to be far enough from the anterior segment of the eye not to interfere with the lens and cornea. Lastly, for cow eyes, the incision was made at 4mm of the cornea. A cross shape window, streching 5 mm in each direction was made on the sclera that the tip of the probe (7mm) can be inserted in the posterior without touching the sclera.

2.2 Rheology Experimental Setup

Rheology is the study of matter that exhibits both fluid-like and solid-like behavior which is called viscoelasticity. We can conclude alot of useful information about the nature of a polymer like the vitreous gel from measuring its viscoelastic properties. This information includes but its not limited to the interaction in both the short-range and long-range interrelation of macromolecules[12].

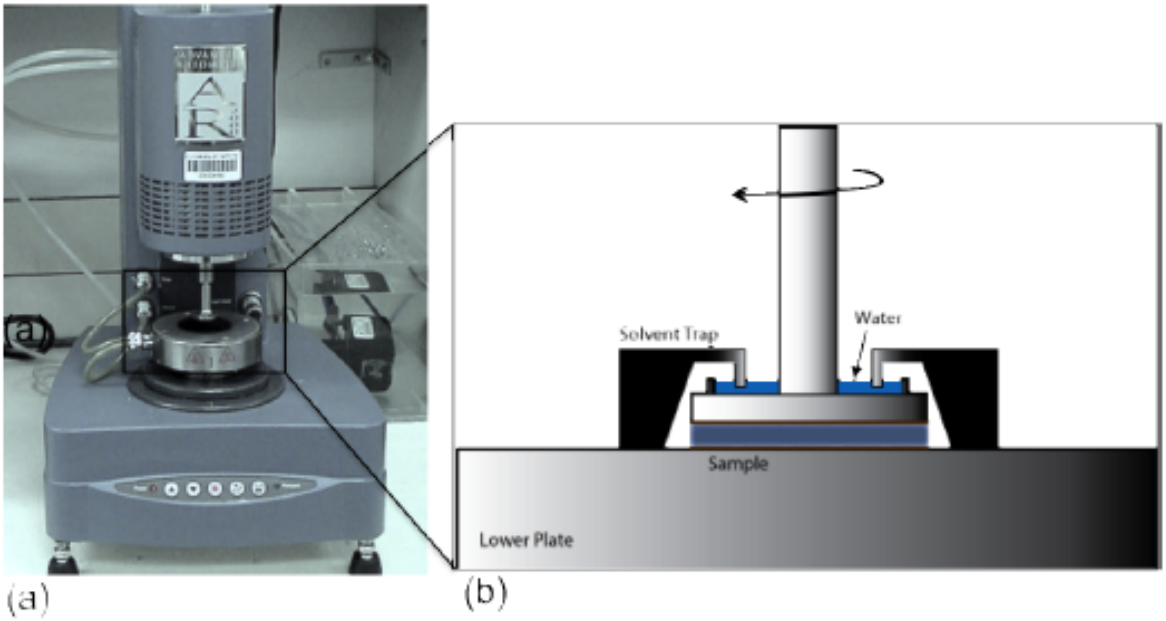


Figure 2.2: AR-2000 TA Instruments rheometer with solvent trap and parallel plates[32]

2.2.1 Rheology Measurement

For viscoelastic properties of the vitreous gel, a stressed-controlled shear rheometer (AR-2000, TA Instruments) was used in this study. As it is shown in Fig2.2(a), the system has an upper rotating plate with several diameters or cone geometry and a lower

fixture that can detect normal force (0.1 N) through an internal force transducer. The TA AR2000 rheometer is a highly sensitive instrument equipped with a feedback controller that simultaneously monitors and controls torque, normal force, and angular velocity. In order to minimize the slippage of delicate samples and provide the effective zero-slip condition[45], the upper and bottom plates can be covered with silicon carbide sandpaper. To minimize the effect of water evaporation and liquid loss of the vitreous during the experimet, a solvent trap sealed with vacuum oil can be used to enclose the testing chamber as shown in Fig 2.2(b).

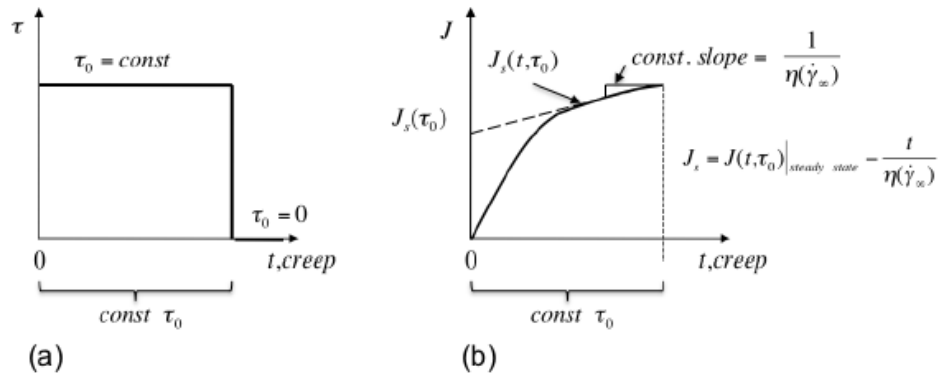


Figure 2.3: (a) Constant stress applied in a finite time to obtain creep compliance; (b) creep compliance, at long times the viscosity is obtained by fittig a linear line[33]

2.2.2 Creep Experiments

When under stress, a new arrangement of the configurations of polymers is obtained. There is a rapid response to the local aspects of the new distribution but the reponse to long-range aspects is slow. There is a wide and continuos range of time scales covering the response of the polymer network to the applied stress. Such experiment can be utilized by applying a constant stress (σ) in a finite time t and recording the deformation (γ). Such experiment is called *creep experiment* [33]. The creep compliance *J(t)* can be derived as,

$$J(t) = \frac{\gamma(t)}{\sigma}[12] \tag{2.1}$$

For perfect elastic solid, creep compliance is $J = \frac{1}{G}$, where G is the Maxwell modulus. For a viscoelastic material, the situation is different. First, there is no direct relationship and large compliance usually resembles small elastic behavior. Also, at long times the creep compliance reaches steady state flow, where the slope of compliance is an inverse of steady flow viscosity η_s shown in Fig2.3 [12]

One can model the behavior of the linear viscoelastic material by the behavior of a mechanical model in which there is a sufficient number of elastic elements, spring, and viscous elements, dashpots. A general Voigt-Kelvin model as illustrated in Fig. 2.4 is an arrangement of springs and dashpots subjected to creep experiments. Therefore, the applied stress

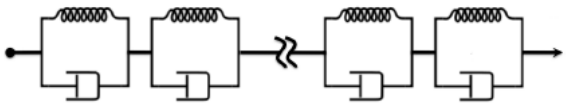


Figure 2.4: Voigt-Kelvin spectrum model

is analogous to σ and the displacement of the terminals is analogous to γ. For each spring there is a contribution of shear modulus, G_i and each dashpot represents a viscosity η_i[12]. The model is expressed as,

$$\text{Voigt Kelvin model: } J(t) = \Sigma J_i(1 - e^{-t/\tau_i}) + \frac{t}{\eta_m}[12] \tag{2.2}$$

Creep compliance experiments were conducted on three species; pig, cow and rabbit for constant shear stress of $\tau = 11.37$ Pa. In these series of tests, the rheology probe, was used to enhance the in-situ characterization of vitreous.The probe is a cylinder with a diameter ranging from less than 1mm to larger. The probe used in this study has a diameter of 1.7 mm. The outer surface is coated with diamond and the opposite end of the probe is attached to the rotational actuator of a shear rheometer. The diamond coated tip inserts in the sample while the actuator rotates the probe with a constant torque. The rotational displacement caused by the sample is recorded by the attached sensor to the actuator Fig2.5[32]. In general, the probe can be used to analyze the viscoelastic response to different kinds of time-dependent patterns of stress and strain. This is feasible by using appropriate equations [33].
In this project, the probe was used to further investigate the viscosity pattern of vitreous by performing the creep compliance tests on three different species. For each species, a pair of eyes were prepared according to methodology explained above.

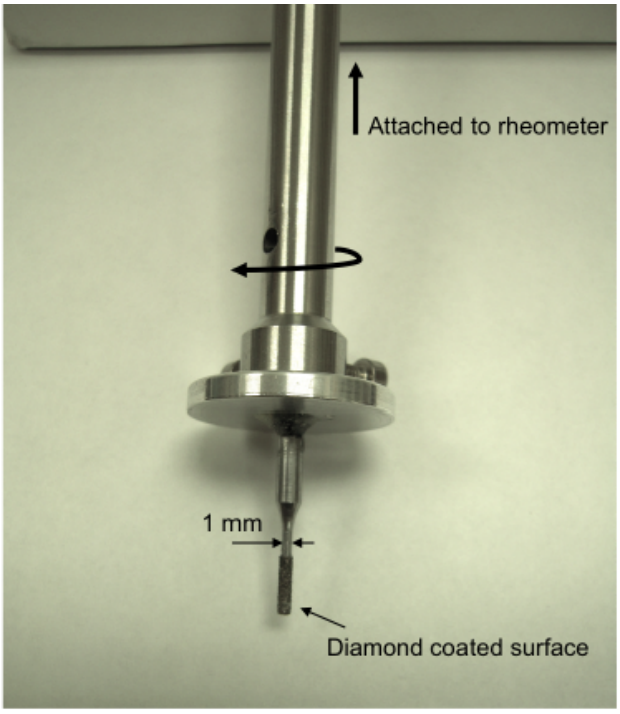


Figure 2.5: The Stainless-steel rheology rod with diamond coated surface. The rod attaches to AR2000 rheometer for actuation and sensing[33]

CHAPTER 3

Results and Discussion

3.1 Creep Compliance Results

The in-situ creep compliance of the vitreous gel was obtained for 10 pairs of three species.This was done using the rheology probe with increase in duration of the experiment to gain different time scales. The creep compliance values were plotted as a function of time in log-log format in order to avoid the lage range of magitudes in data. The results are shown below:

Pig Eye Results Results for pig eyes are shown in Fig3.1. For better comparison between a pair of eyes, the creep compliance graphs of both eyes in a pair are plotted together.

Rabbit Eye Results Results for rabbit eyes are shown in Fig3.2 . For better comparison between a pair of eyes, the creep compliance graphs of both eyes in a pair are plotted together.

Cow Eye Results Results are shown in Fig3.3. For better comparison between a pair of eyes, the creep compliance graphs of both eye in a pair are plotted together.

In agreement with previous studies done on creep compliance of vitreous, all the logarithmic graph of creep compliances presented above, follow the same the pattern of certain zones on time scale[33]. The time scales are described as shown in Fig3.4.

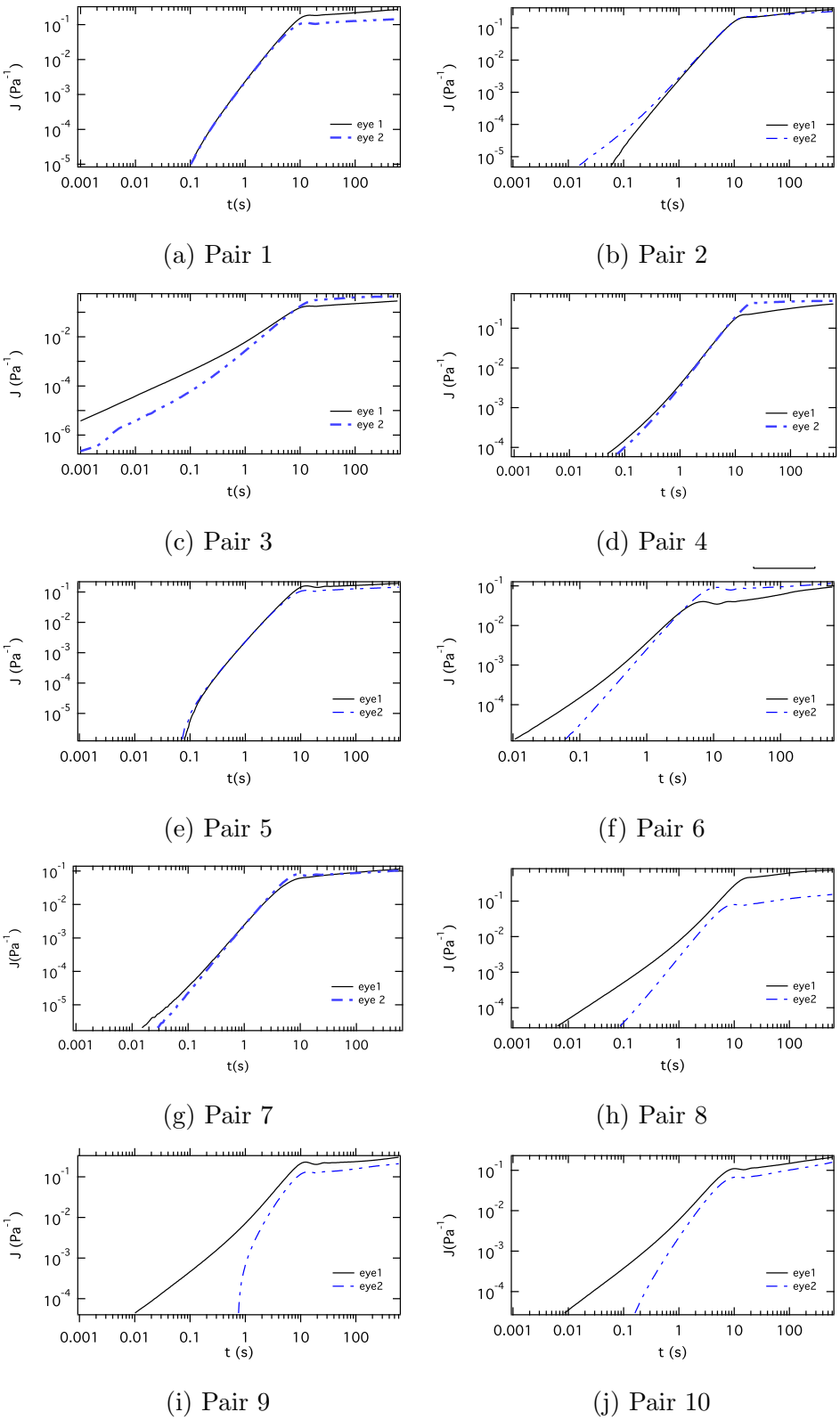
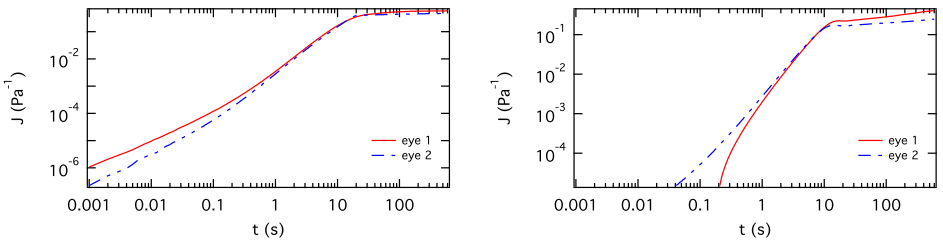
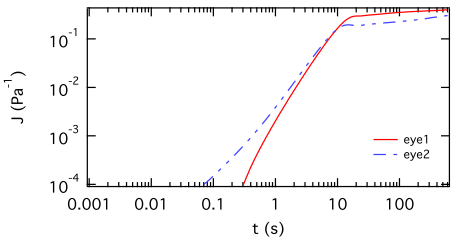


Figure 3.1: **Compliance J (Pa^{-1}) as a function of time t (s):** For ten pairs of pig eyes. In each graph the creep compliance for both eyes of a pair is shown

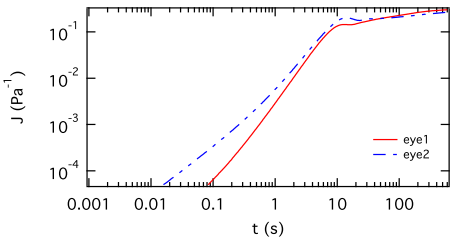


(a) Pair 11

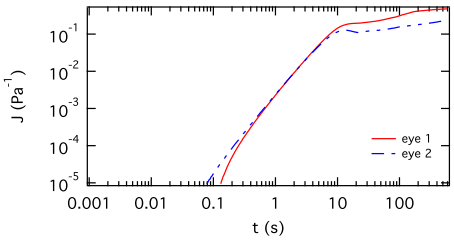
(b) Pair 12



(c) Pair 13



(d) Pair 14



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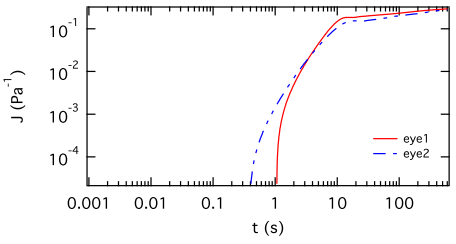

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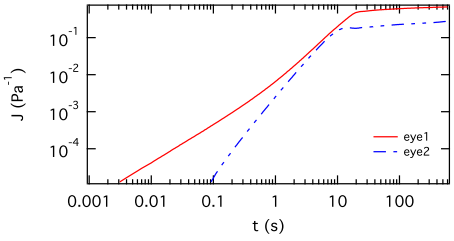

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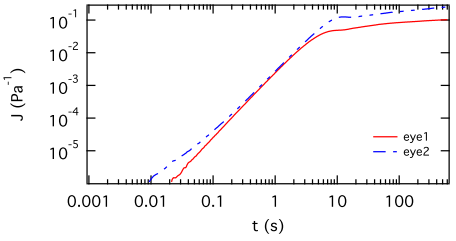

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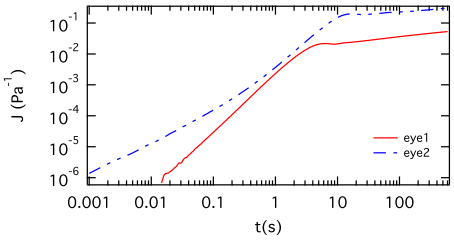
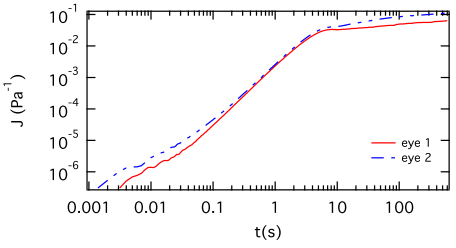
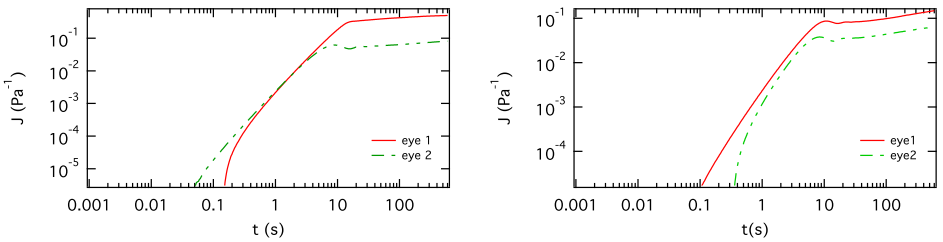


(i) Pair 19



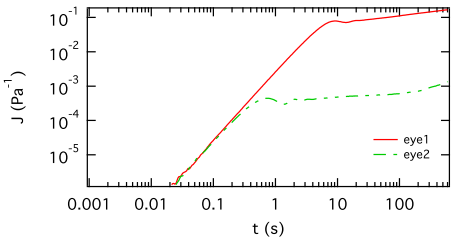
(j) Pair 20

Figure 3.2: **Creep Compliance J (Pa^{-1}) as a function of time t (s):** For ten pairs of rabbit eyes. In each graph the creep compliance for both eyes of a pair is shown

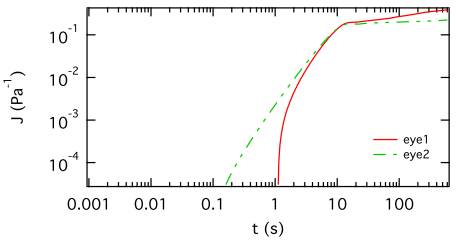


(a) Pair 21

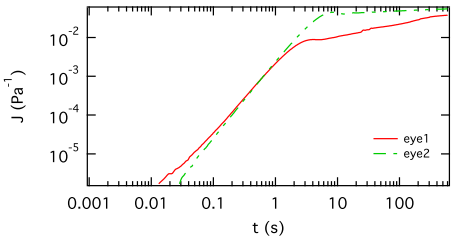
(b) Pair 22



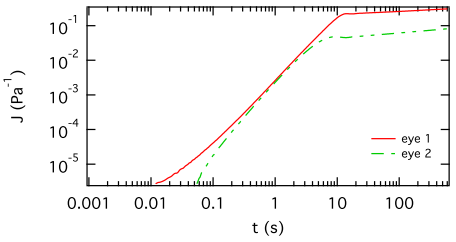
(c) Pair 23



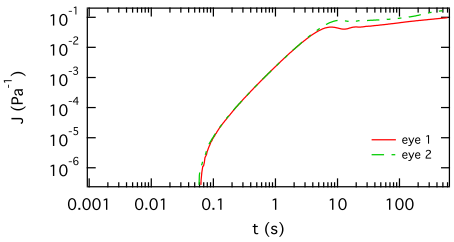
(d) Pair 24



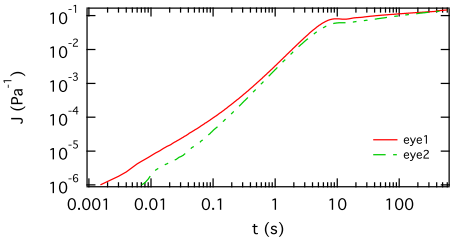
(e) Pair 25



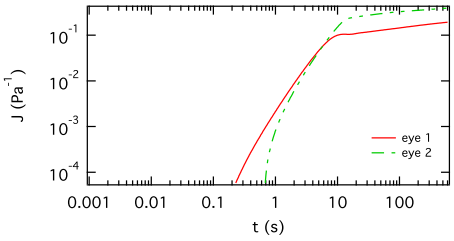
(f) Pair 26



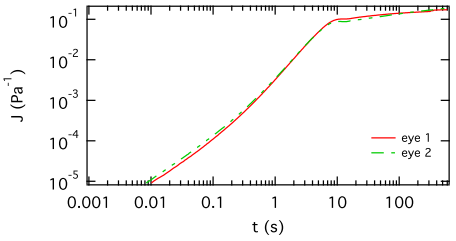
(g) Pair 27



(h) Pair 28



(i) Pair 29



(j) Pair 30

Figure 3.3: **Creep Compliance J (Pa^{-1}) as a function of time t (s):** For ten pairs of cow eyes. In each graph the creep compliance for both eyes of a pair is shown

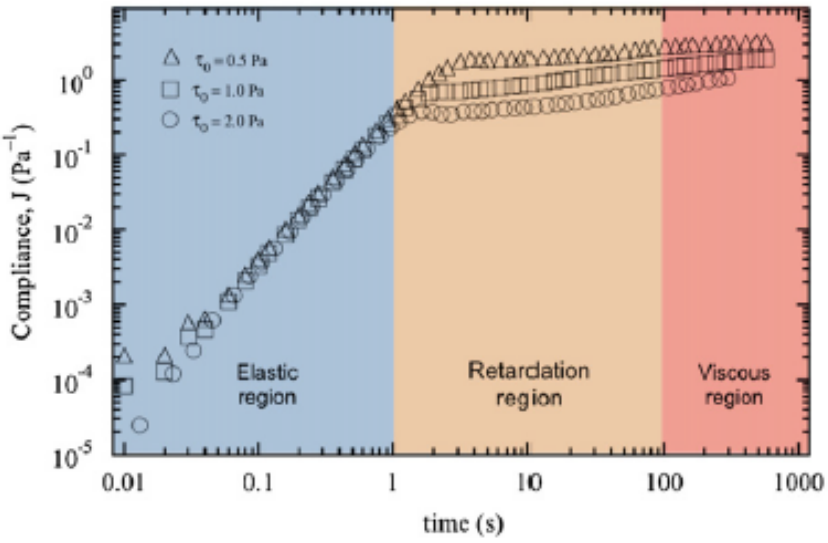


Figure 3.4: Compliance versus time for different shear stresses showing three distinguished regions of Elasticity, Retardation and Viscous Region[33]

The first region, Elastic Region, is the region where vitreous is showing solid-like behaviour, i.e. responding elastically to shear stress in short time. The second region following is Retardation Region. During this time scale the retardation from viscous behavior occurs. Lastly, the steady state stage is obtained where vitreous behaves like liquid[33]. In addition to that, according to the same study, we can model the creep compliance with Voigt-Kelvin as shown as equation 2.2. This model further indicates that vitreous has two time scales in response to a contstant stree in a creep experiment. Therefore, using this method, the time scales described above were quantified; the short time scale is in association with the collagen structure. This is in satisfaction with the initial reported response of cross-linked collagen structure which is elastic within a short time scale[34],[18]. Also, It has been hypothized that the second time scale obtained from Voigt-Kelvin is accociated with hyaluronic microfibrils and network[33].

3.2 Comparison Among Three Species

The steady state compliance values were calculated for each of the graphs by fitting a linear curve to the third region of compliance, J_0. It must be noted that this result is a correct steady state compliance value only if the steady state has reached and attained for a long time. Furthermore, the average value for the first time scale, t_0 and average value for viscosity were calculated and plotted to be compared statiscally for all three species.

The values of t_0 are shown in Fig 3.5. The highest value belongs to cow eyes. Folllowing value is for rabbit and the lowest value is for pig eyes. This suggests that the elastic region lasts the most for cow eyes . In reference to Table 1.1 cow eyes have the highest concentration of collagen among three species(cow, rabbit and pig) which is in agreement with the highest value of t_0 as in Fig 3.5. Also, the average value of t_0 for rabbit eyes are slightly higher than for pig eyes in agreement with their collagen concentration according to Table 1.1. It must be noted that because of the enormous average value of t_0 for cow eyes, the error bar for this value has not been plotted.

Fig3.6 illustrates a comparison of creep compliance values at the steady state among pig, rabbit and cow eyes.This plot is also representing the same pattern discussed before. For cow eyes, with higher percentage of collagen, the average compliance value which is the inverse of elasticity, is the smallest. As for pig and rabbit eyes this value is close to each other but higher than of the cow. The calculated average viscosity is shown in Fig3.7, It must be noted that the average value of viscosity for cow eyes was enormous and it is not conclusive in this study. However, the viscosity value for rabbit and pig eyes were close to each other confirming the previous discussion1.1.

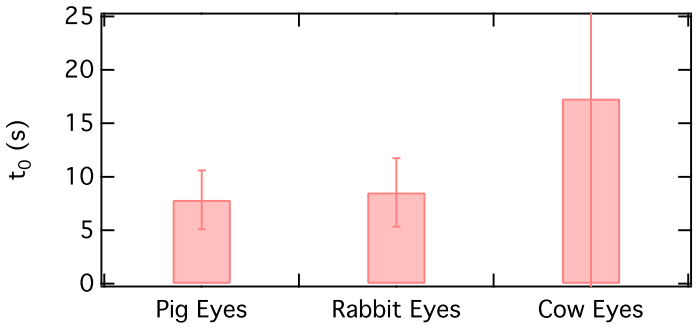


Figure 3.5: Comparison of t_0 at the steady state gained from creep compliance plots among three species

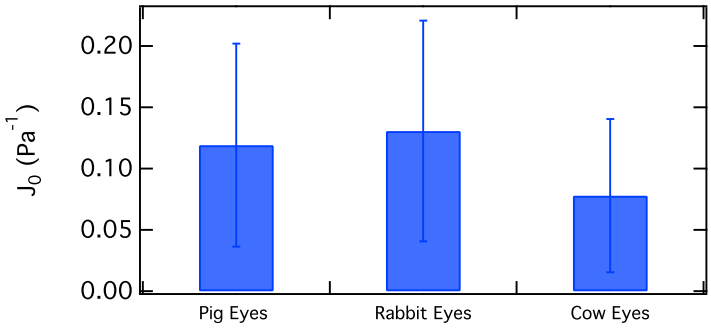


Figure 3.6: Comparison of steady state creep compliance values among three species

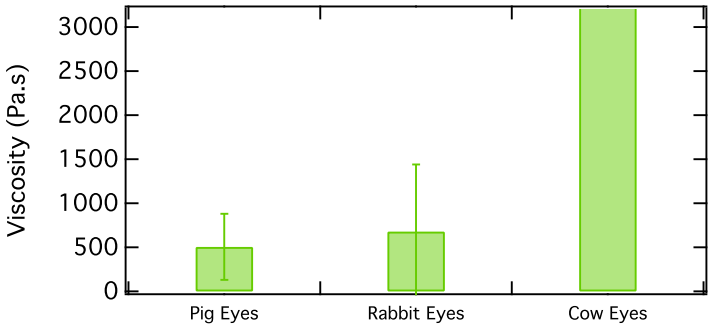


Figure 3.7: Comparison of viscosity at the steady state gained from creep compliance plots among three species

CHAPTER 4

Conclusion

In this thesis, a testing plan was designed to further characterize the rheology probe. A series of in-situ creep compliance experiments were conducted using the probe. The obtained data was analyzed and it was with good agreement with existing viscoelastic models. The extracted data was used to investigate the relationship between the viscoelastic properties of vitreous gel and its macromolecular structure .

Most of retinal surgical procedures are to involved with vitrectomy, a technique in which the vitreous gel is removed from the cavity. However, there are many aspects of this procedure that are not well understood. The missing piece that has been neglected in the design and evaluation of vitrectomy devices, is understanding of viscoelastic behavior of vitreous before and after the procedure. The viscoelastic properties of vitreous is directly affected plays an important role in therapeutic treatments and intravitreal drug injection. Due to the complexity of vitreous gel, it is essential to furthermore widen our knowledge of its macromolecular structure. The important aspect of the rheology probe is its small size that enables bulk characterization with minimal damage to the structure of the material. Therefore, it is possible to quantify viscoelastic changes in fragile materials such as vitreous gel. In order to characterize the probe, creep compliance experiments were performed on three different species. The results showed similar pattern of creep deformation response for all the species that were used in this study. By using the associated two-element retardation model, the distinct responses of the bipolymeric system of vitreous gel was observed as predicted. The initial stage with short time scale that is associated with solid-like behaviour, was observed for all the results. This region was associated with the structure of collagen while the second respond for longer duration time was associated with hyaluronan, confirming

that both collagen structure and hyaluronan network contribute to the overall rheology of the vitreous gel in all three species. By thoroughly inspecting the creep compliance data, another significant observation was noted: The value of plateau compliance (J_0)differs among three species and contributes to characterizing of the probe. Also, the plateau compliance as well as the general trends of the compliance plots, including the first short time scale were very similar within the pair eyes of individual animals. However, these values differ from one species to the other one. Data from cow eyes showed the least similarity in the compliances within a pair comparing to rabbit and pig eyes. As a result, we found a clear similarity between the viscoelastic behavior of the vitreous gel in two eyes of a pair in all three species. This finding can facilitate future investigation of vitreous gel. For instance, in enzymatic activities performed on vitreous of a pair of eyes, one eye can act as the control sample while the other one belongs to the treatment group.

For future work, we suggest more study to be done on using the probe and its limitations such as minimum and maximun applicable stress used for different samples. Currently, there are some challenges regarding the limitations in applied stress of the probe. Eliminating these limitations would enhance the investigation on small effects of enzymes on vitreous or liquefaction and related complications. Thus, eventually the result would help preventing vitreous related complications in drug delivery, pharmacologic vitreolysis studies and vitrectomy instrumentation.

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