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The rheological behaviour of animal vitreus and its comparison with vitreal substitutes

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Some important eye pathologies, such as diabetic proliferative retinopathy and retinal detachment, are strictly connected to different gel vitreus alterations. Therefore there is a strong need in ophthalmology for vitreal substitutes. Until now many synthetic and natural polymers have been tested as vitreal substitutes, but no one has proved to be an ideal vitreal substitute. An ideal vitreal substitute, apart from other characteristics, such as transparency, permanency, biocompatibility, etc. must have a rheological behaviour compatible with the surrounding tissues. The viscoelastic behaviour of different animals' gel vitreus, evaluated by means of steady shear viscosity and small-amplitude oscillatory measurements, is typical of solid-like rubbery gels with dynamic elastic modulus G' higher than the dynamic viscous modulus G'' in the typical frequency range investigated (0.05–10 Hz). On the other hand the rheological behaviour of current or candidate vitreal substitutes (silicone oil, HPMC, high molecular weight hyaluronic acid and chemically-crosslinked hyaluronic acid), analysed with the same technique, is generally different from that of natural vitreus.

1. Introduction

The vitreus cavity is the space within the human eye bounded anteriorly by the lens and its zonular fibres and more posteriorly by ciliary body, retina and optic disc. This space is occupied by the vitreus body, a gel composed almost completely of water and three main components: collagens, proteoglycans (PG) and hyaluronic acid (HA). The gel structure is formed by an ordered network of fine collagen fibrils (diameter of about 10 nm) immersed in a viscoelastic matrix composed mainly of highly hydrated HA macromolecules [1, 2]. This structure permits the vitreus body to be a transparent medium, to maintain ocular shape, to serve as a mechanical absorber of any movements and impacts for bordering tissues [3] and, finally, to help the retina to remain in position. The vitreus body, however, may lose its structural or functional properties because of various diseases. Often a retinal detachment with or without retinal breaks is associated with pathological vitreus.

Vitreal substitutes, therefore, have great relevance in ophthalmology. The permanent substitutes should have appropriate properties: biocompatibility, transparency, permanency, refractive index and viscoelasticity approximating those of natural vitreus. Moreover these materials should tamponade retinal breaks, whether present, and push the retina against the choroid. A substance has tamponade properties when it is insoluble in vitreus humour, is able to obstruct or reduce markedly the flow of preretinal fluid towards the subretinal space through breaks and have a poor tendency to diffuse into the subretinal space [4]. Several materials have been tested as vitreal substitutes: saline solutions, silicone oils, hyaluronic acid, air, gas, etc. but none of them has proved satisfactorily as ideal vitreal substitute.

This study deals with the rheological analysis of current or candidate vitreal substitutes. Moreover, the relationships between structure and viscoelasticity of vitreus bodies of animal origin were investigated, because an ideal permanent vitreal substitute must match, first of all, the mechanical properties of natural vitreus.

2. Materials

The eyeballs employed for this study were obtained from the butchery. The animals were pig (age: 4–5 months), sheep (age: 1–2 months), goat (age: 5 months), rabbit (age: 5 months) and calf (age: 10 months).

Immediately after the animals were slaughtered, the eyes were enucleated and immersed in Ringer's solution and stored at 4 °C for no longer than 2 days. The rheological tests were performed less than 30 min after the sclera, choroid and retina were carefully dissected from the vitreus.

The vitreal substitutes investigated were the following: medical grade silicone oil with cinematic viscosity of 1000 cS (Schott Duran, Germany); hydroxy propyl methyl cellulose (HPMC) 2% Cel 4000® (Bruschettini, Genova, Italy) at a concentration of 22 mg/ml in buffered physiological sodium chloride solution; Healon® (Pharmacia AB, Uppsala, Sweden), high molecular weight (about 4 000 000 g/mol) sodium hyaluronate at a concentration of 10 mg/ml in phosphate-buffered sodium chloride solution (pH = 7–7.5); Synvisc® Hylan® GF-20 (Biomatrix Medical Canada Inc., Pointe-Claire, Canada), containing Hylan fluid and Hylan gel slurry at a concentration of 8 ± 2 mg/ml in buffered physiological sodium chloride solution (pH 7.2 ± 0.3).

3. Methods of measurement

The rheological properties of vitreus and vitreal substitutes were evaluated on a Bohlin VOR Rheometer (Bohlin Reologi AB, Lund, Sweden) at a controlled temperature of 37 °C. The measuring systems were parallel plates (PP 30 cell), cone and plate (CP 5/30 cell) and Bohlin "small sample cell" with a coaxial cylinders geometry (Couette). The appropriate cell was chosen for each material depending on the volume, the consistency and the structure of the sample. The outer cylinder or the lower plate was forced to rotate or oscillate, whereas the stress transferred from the fluid to the inner cylinder or upper plate was measured by means of a linear variable displacement transducer (LVDT) system.

The non-linear flow properties of the investigated materials were evaluated through steady shear measurements to determine the viscosity η as a function of shear rate $\dot{\gamma}$, while small-amplitude oscillatory shear experiments allowed the measurement of the dynamic response of the samples and hence the determination of their linear viscoelastic properties. Moreover, this technique is very useful for the determination of structure–mechanical properties relationships [5, 6].

In this dynamic experiment the material is subjected to a sinusoidal shear strain:

$$\gamma = \gamma_0 \sin(\omega t)$$

where γ_0 is the shear strain amplitude, ω is the oscillation frequency (which can be also expressed as $2\pi f$ where f is the frequency in Hz) and t the time. The mechanical response expressed as shear stress τ of viscoelastic materials is intermediate between an ideal pure elastic solid (obeying Hooke's law) and an ideal pure viscous fluid (obeying Newton's law) and therefore is out of phase with respect to the imposed deformation as expressed by

$$\tau = G'(\omega)\gamma_0 \sin(\omega t) + G''(\omega)\gamma_0 \cos(\omega t)$$

where $G'(\omega)$ is the shear storage modulus and $G''(\omega)$ is the shear loss modulus. G' gives information about the elasticity or the energy stored in the material during deformation, whereas G'' describes the viscous character or the energy dissipated as heat.

The combined viscous and elastic behaviour is given by the absolute value of complex shear modulus G^*:

$$G^*(\omega) = \sqrt{G'^2 + G''^2}$$

or by the absolute value of complex viscosity η^* defined as

$$\eta^*(\omega) = \frac{\sqrt{G'^2 + G''^2}}{\omega}$$

which is usually confronted with the steady shear viscosity in order to evaluate the effect of large deformations and shear rates on the material structure.

The ratio between the viscous modulus and the elastic modulus is expressed by the loss tangent:

$$\tan\delta = \frac{G''}{G'}$$

where δ is the phase angle.

Strain sweep tests at a fixed oscillation frequency (consisting in monitoring the viscoelastic properties while logarithmically varying the strain amplitude γ_0) were previously performed on these materials to determine the strain amplitudes at which linear viscoelasticity is valid.

The testing procedure was as follows: first of all we performed strain sweep tests to choose the right strain amplitude, then oscillation measurements and only at the end of the sample testing session, steady shear measurements. This sequence was chosen so as not to destroy the macrostructure of these deformation-sensitive materials.

4. Results and discussion

4.1. Viscoelastic properties of gel vitreus

Vitreus body is a natural composite formed by a viscoelastic matrix of highly hydrated HA macromolecules reinforced with a network of water insoluble rigid gel-forming collagen fibrils [7–9].

The viscoelastic matrix of gel vitreus is mainly composed of hyaluronic acid, a glycosaminoglycan (GAG) consisting of residues of D-glucoronic acid and N-acetyl-D-glucosamine; it behaves in aqueous medium as an expanded random coil molecule occupying a large hydrodynamic volume overlapping with other HA macromolecules even at low concentration and molecular weight (MW), probably through physical interactions such as polymer "entanglements" responsible for its transient network structure. The viscoelasticity of HA solutions depends mainly on its concentration, molecular weight and molecular weight distribution (MWD); moreover, HA rheological properties, because of its polyelectrolite nature, depend also on ionic strength, pH, proteins and other substances present in living tissues. In vitreus, HA macromolecules account for water-retaining and provide a stabilizing effect on the collagen network.

The collagen fibrils (diameter about 10 nm) are tied together by PG bridges and run in approximately parallel bundles. The PG bridges can interact through their glycosaminoglycans chains with HA matrix via non-covalent bonds [1].

Gel vitreus composition changes with age and species, because of variations in collagen content and in HA concentration, MW and MWD. In fact there is a significant variation among different species in collagen fibrils concentration and hence in their network density because all species have about the same fibril thickness. Human gel vitreus has a low-density network of collagen fibrils, while rabbit gel vitreus (a model widely used in ophthalmic research) has a very dense collagen network. Also hyaluronate in human vitreus has a weight-average molecular weight $\bar{M}_w$ of $3\text{–}4.5 \times 10^6$, in rabbit vitreus $2\text{–}3 \times 10^6$ [10] while this value drops to about 5×10^5 in old bovine. On the other hand, in new-born calf vitreus HA has a $\bar{M}_w$ of about 3×10^6.

Apart from its biological and optical properties, vitreus plays a crucial role in the biomechanics of the eye, contributing to maintaining ocular shape, keeping the retina in position, and acting as a mechanical shock absorber for neighbouring tissues because of its peculiar viscoelastic properties.

The rheological properties of goat (caprine), sheep (ovine), pig (suine), calf (bovine) and rabbit vitreus bodies were investigated with parallel-plate geometry through frequency sweep tests, strain sweep tests and steady shear viscosity tests at $T = 37\,^\circ\mathrm{C}$. In these tests, the whole vitreus body, once isolated, was put between the two plates of the cell with the exception of bovine vitreus, which, because of its large volume, was dissected to permit the measurement.

The steady shear viscosity η and the complex viscosity η^* of all analysed vitreus bodies decrease linearly with the imposed shear rate showing a typical shear thinning (pseudoplastic) behaviour. The values of η^* are one to two orders of magnitude higher than those of η in the investigated range, indicating that vitreus structure is very sensitive to deformations and that simple viscosity is not a correct measure of energy dissipation for this system. In fact small-amplitude oscillatory tests, producing only a slight periodic deformation in the proximity of the rest state of the sample, are more representative of vitreus motions rather than steady-state viscosity measurement implying large deformations of the material.

The strain sweep tests at fixed frequency show a linear behaviour for G' and G'' versus γ_0 up to about 0.01 strain, then decaying because of destructuration of the material.

The rheological behaviour, qualitatively similar for all the analysed vitreus even if different in absolute values, is solid-like and typical of rubbery gels [11], with the dynamic elastic modulus G' always higher than dynamic viscous modulus G'' throughout the frequency range investigated (0.05–10 Hz) and with the moduli almost parallel to each other (Fig. 1a and b). Moreover, G', G'' and $\tan\delta$ do not vary significantly with frequency. It is worth noting that $\tan\delta$ varies for the different species in a restricted range (between 0.2 and 0.5 at 1 Hz).

4.2. Viscoelastic properties of current and candidate vitreal substitutes

Some eye diseases, such as diabetic proliferative retinopathy and retinal detachment, are directly related to vitreus body anomalies and degenerations. Many synthetic and natural-origin polymers have been tested as replacements for diseased vitreus but none has proved satisfactory. An ideal vitreal substitute, apart from other characteristics such as transparency, permeability, biocompatibility, tamponade properties, permanency, etc. must present a similar mechanical performance to natural vitreus and must have a rheological behaviour compatible with the surrounding tissues, protecting them from translatory and especially rotatory eye movements, body movements, vibrations, and indirect and direct traumas.

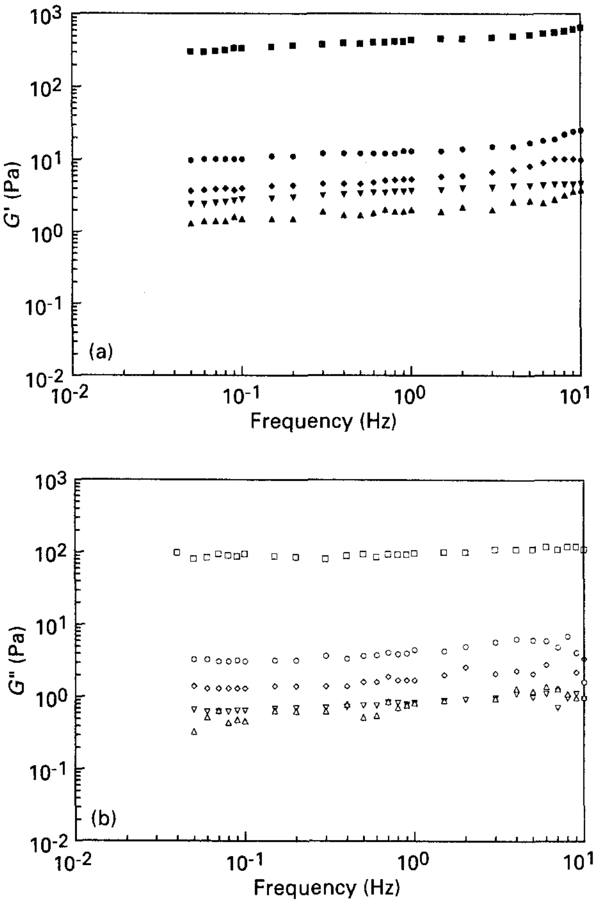

Figure 1 Frequency sweeps of vitreus body (a) at 37 °C showing dynamic elastic modulus G' (■ caprine; ● ovine; ◆ suine; ▼ rabbit; ▲ bovine) and (b) dynamic viscous modulus G'' (□ caprine; ○ ovine; ◇ suine; ▽ rabbit; △ bovine).

4.2.1. *Silicone oil*

Silicone oils (SOs) are widely used by many surgeons practising posterior segment surgery. The intraocular application of silicone oils can lead to a number of complications such as keratopathy, increased intraocular pressure and cataract formation. These complications are caused by volatiles, impurities, low molecular weight elements present in SO and their tendency to emulsify [12]. From a strictly rheological point of view, SOs behave as Newtonian liquids, having shear viscosity η constant with shear rate. The higher the viscosity the higher the capability of this fluid to dissipate mechanical energy and its resistance to flow; on the other hand SOs have no elasticity, so differing substantially from natural vitreus.

4.2.2. *Hydroxy propyl methyl cellulose*

Hydroxy propyl methyl cellulose (HPMC), a synthetic derivative of cellulose, has been extensively used in ophthalmology as a component of artificial tears, as viscoelastic aid in intraocular surgery [13] and more recently [14] has been tested as a vitreal substitute in the eyes of rabbits.

The viscoelastic response of HPMC 2% is typical of liquid-like polymeric solutions, flowing as a high viscosity liquid; in fact G'' is always greater than G' over the frequency range of interest (Fig. 2).

4.2.3. High molecular weight hyaluronic acid (Healon®)

Exogenous HA is used in those cases in which it is necessary to introduce into the vitreal cavity a substitute without tamponade properties [15–17]. The medically usable preparations of non-inflammatory sodium hyaluronan (NIF-HA as designated by Balazs) consist of a high molecular weight HA fraction which has been separated from the inflammatory HA fraction. The restricted use of NIF-HA (Healon®) in vitreoretinal surgery is mainly due to its hydrosolubility and short residence time in the vitreal cavity; for this reason it cannot act as a lasting phase separated from water and therefore act as tamponade. It is, however, known that the vitreus has tamponade properties; in fact it is possible to see clinically a retinal break without retinal detachment.

Healon® solutions behave as transient-network polymer solutions, having a predominantly viscous character ($G'' > G'$) at low frequencies and an essentially elastic behaviour ($G' > G''$) at higher frequencies (Fig. 2).

4.2.4. Chemically cross-linked hyaluronic acid (Hylan®)

Balazs *et al.* [19] have recently improved HA preparation leading to the development of the Hylan® (Biomatrix Inc., Ridgefield, N.J., USA) family of polymers, a chemically modified HA preparation containing small amounts (0.005–0.05% by weight) of aldehyde crosslinking groups covalently bonded to HA macromolecules. Hylan® is obtained through treatment of HA *in situ* in animal tissues with a mixture including a reagent (typically an aldehyde) reactive towards HA and proteins contained in these animal tissues. Hylan fluid and Hylan gel are non-inflammatory and have a significantly larger residence time than HA when placed into tissues. The Hylan gel, moreover, has a distinctive characteristic different from both Hylan fluid and HA: its lack of water solubility [18–20]. For this reason it can be seen as a candidate vitreal substitute. Synvisc® Hylan G-F 20 (Fig. 2) has a predominantly elastic response ($G' > G''$), with both moduli frequency dependent, G' approaching a plateau at high frequencies and a low cross-over frequency (less than 0.01 Hz). The elasticity depends on both the chemical crosslinks and the polymer entanglements. This viscoelastic behaviour is qualitatively more similar to natural vitreus than the other vitreal substitutes examined.

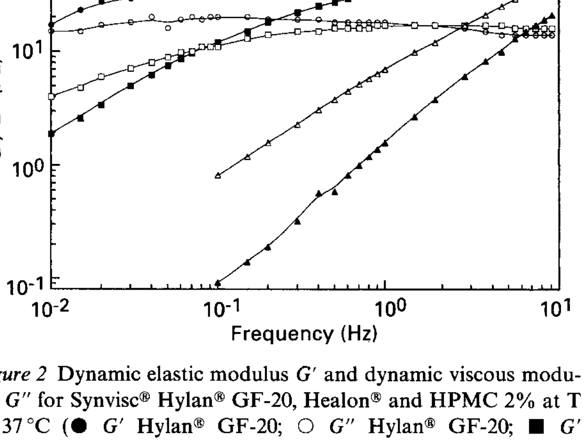

Figure 2 Dynamic elastic modulus G' and dynamic viscous modulus G'' for Synvisc® Hylan® GF-20, Healon® and HPMC 2% at T = 37 °C (● G' Hylan® GF-20; ○ G'' Hylan® GF-20; ■ G' Healon®; □ G'' Healon®; ▲ G' HPMC; △ G'' HPMC).

5. Conclusions

The peculiar mechanical behaviour of natural gel vitreus depends on its solid-like character. The current or candidate vitreal substitutes have a generally different rheological behaviour, varying from Newtonian fluid (silicone oil) to polymeric solutions of increasing viscoelasticity (Hylan® > Healon® > HPMC) and hence an increasing degree of protection for eye tissues against direct and indirect mechanical stresses. Moreover, the absolute values of viscoelastic moduli of vitreal substitutes have to be not too different from those of the replaced gel vitreus. Rheological analysis seems to be a very useful tool for understanding the structural–mechanical properties relationships of these materials and provides interesting information for the design of proper vitreal substitutes and for investigation of normal and pathological natural vitreus.

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