

Biophysical Evaluation of Vitreous Humor, Its Constituents and Substitutes

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Vitreous humor, present in the posterior cavity of eye, often becomes dysfunctional due to liquefaction, physical collapse and opacification resulting in its detachment from the retina and vision loss. Materials in clinical use are associated with many complications and search for an ideal substitute is still ongoing. In this study, detailed characterization of the vitreous, its constituents, silicone oil: the presently used substitute, and gellan gum as a possible substitute, was done on the basis of ultrastructure, viscosity, wettability and gelation. Viscosity of the natural vitreous is very high, greater than 4000 cP at a shear rate of 0.15 sec⁻¹ and shows shear thinning. Environmental Scanning Electron Micrographs (ESEM) revealed a crosslinked structure. Vitreous gel showed syneresis of 12% at 5000 x g and had a glycerol contact angle of 81.4 ± 0.98. At the original concentrations present in the vitreous, Col (0.05 mg/ml) and HA (0.15 mg/ml) individually have maximum viscosity of 0.743 cp and 19.63 cp respectively. Even at 10-fold higher concentrations, the maximum viscosity of HA and collagen individually is lower than vitreous: 164.1 cP and 11.78 cP respectively. Combination of HA and Col even at 5 fold higher than physiological concentrations has maximum viscosity of only 54.7 cP. Viscosity of 1% gellan was > 5000 cP and it underwent gelation at room and body temperature. Gellan gel did not show syneresis and had a glycerol contact angle of 75.17 ± 1.11. Its light transmission was greater than 95% in visual range and ESEM micrographs showed a crosslinked structure. Silicone oil has maximum viscosity of 2600 cP. Its light transmission is 100% in visible range and its contact angle is 91.3 ± 0.60.

The contact angle measurements indicated that Silicone oil is more hydrophobic than natural vitreous, thus it makes poor contact with retina. Moreover it is not physiological and does not form a gel. The results of this study indicate that the natural constituents of vitreous on their own are not good as substitutes and that a desirable vitreous substitute should show a maximum viscosity of = 4000 cp, should undergo gelation, syneresis = 12%, light transmission = 90% over visual range and glycerol contact angle of 80°. Though gellan appears promising, further research is required towards development of an ideal vitreous substitute.

Introduction

The vitreous body also known as vitreous humor is a fascinating result of natural evolution. Its major constituent is water but it exists in the form of gel still maintaining most of the properties of the liquid component. This gel is a transparent extracellular matrix that fills the posterior cavity of the eye (behind the lens) and is surrounded by and attached to the retina [1,2]. The vitreous gel has certain physiological functions including providing a conduit for the metabolic requirements of the lens and excluding cells and large macromolecules from the vitreous cavity in order to maintain transparency. It has also

been suggested that the vitreous provides adequate support for the retina, that it protects the eye during mechanical trauma and that it protects surrounding ocular tissue from adverse circumstances. Liquefaction of this vitreous gel takes place during ageing and in 25-30% of the cases the separation of vitreous takes place from retina, which ultimately results into posterior vitreous detachment. This process plays a central role in various blinding conditions, which includes rhegmatogenous retinal detachment, proliferative diabetic retinopathy and macular hole formation. Apart from this ageing process there are other conditions also where damage to vitreous results in blindness [1].

The vitreous can become dysfunctional due to opacification or by liquefaction and collapse. Pathological causes for this includes developmental abnormalities, various inflammatory processes, systemic diseases and degenerative processes. The damage may also occur by trauma or due to the presence of foreign bodies. All these conditions may result in extremely poor vision or even blindness. Various substitutes are used for replacing the vitreous to restore the vision and for normal functioning of the eyes. Further substitutes are also required during various surgical treatments of the eyes including cataract, surgeries [3]. Various biomaterials have been used to replace the vitreous which includes temporary as well as permanent substitutes. Some of them are in clinical use such as silicone oil, perfluorooctane, perfluorodecalin etc. They have some important characteristics such as optical clarity, permanent retention and chemical inertness but they are also associated with many complications such as poor contact with retina due to the hydrophobic nature, cataract, oil in anterior chamber, less tamponade effect in case of inferior retinal breaks, irreversible cell damage, postoperative retinal detachment etc [3].

The main aim of the present study is to do the detailed characterization of the vitreous humor; its constituents including collagen and hyaluronic acid; silicone oil: the presently used substitute; and gellan gum as a possible substitute on the basis of ultrastructure, viscosity, wettability and gelation, and to approach the clinical issue of obtaining a vitreous substitute which will be free from the complications associated with presently available substitutes.

Materials and methods

Vitreous for characterization is obtained from goat eye. The materials, selected for evaluation, include collagen, hyaluronic acid and gellan. Collagen and hyaluronic acid are selected on the basis of the fact that they are the constituents of natural vitreous body itself. So they are expected to show biocompatibility in biological environment. Gellan gum, an exocellular heteropolysaccharide, is secreted by bacteria and is an excellent gelling agent. It consists of glucose, glucuronic acid and rhamnose in the molar ratio of 2:1:1.

Viscosity measurements

The viscosity for the vitreous and for all the three materials is measured by concentric cylinder viscometer (Contraves low shear 30 viscometer, Zurich) at body temperature (37 °C). Viscosity is measured as a function of shear rate. Viscosity measurements are also done as a function of temperature at a fixed shear rate to study the transition of the liquid to gel in case of gellan.

Syneresis

Syneresis was done for vitreous and gellan. Gel was loaded in centrifuge tubes and centrifuged for 30 min at 15000 x g in Sigma cooling centrifuge at 4°C. Syneresis in % (w/w) was calculated as mass of liquid separated from gel due to centrifugation, related to the total mass of gel that was centrifuged:

$$\text{Syneresis in \%} = \left(\frac{\text{mass of serum}}{\text{mass of gel}} \right) * 100 \quad (1)$$

Light transparency

The substitute should be transparent for the transmission of light and for clear vision. Light transparency of these materials is measured by Perkin Elmer Lambda 25 UV/VIS spectrophotometer. Distilled water is used as blank and transparency is measured from 400-700 nm.

Environmental scanning electron microscopy

The morphology of the vitreous and the gellan is studied by taking the electron micrographs. Images are taken under different magnifications.

Wettability

Wettability of the vitreous and materials is calculated by measuring the contact angle. For that, the materials, to be used as substitute, are coated on the glass slides and then same liquid (here in this case, glycerol) is placed on every slide and contact angle is measured.

Results

Viscosity studies

The vitreous is in gel form so its viscosity is very high. The viscosity of the vitreous with increasing shear is given in figure 1. It is greater than 4000 cP at a shear rate of 0.15 sec⁻¹ and shows shear thinning, thus it is a non-newtonian fluid. Figure

1 also shows the viscosity of silicone oil (maximum value ~2600 cP), which is currently the most commonly used substitute.

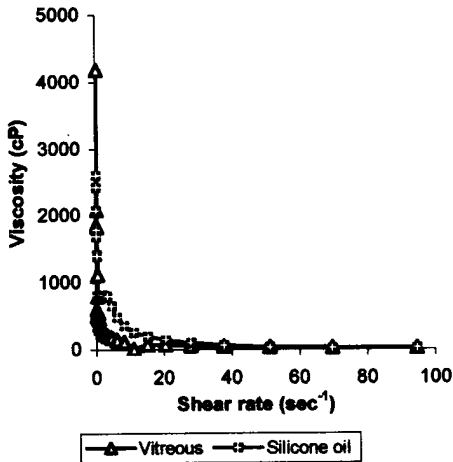


Figure 1 Viscosity of vitreous and Silicone oil as a function of shear rate

Figure 2 shows the viscosity of collagen and hyaluronic acid alone at two different concentrations: original concentration present in vitreous and 10 fold higher concentration (10x). The figure also shows the viscosity of their combination at two different concentrations: original concentration present in vitreous and 5 fold higher

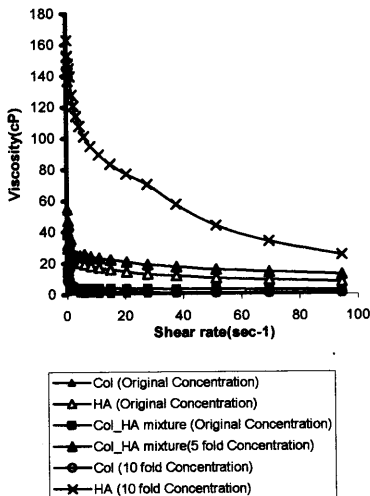


Figure 2 Viscosity of Collagen and HA (alone and combinations) as a function of shear rate

concentration. It is clear that the viscosity in all the cases is much lower than viscosity of the vitreous. Moreover, the collagen and HA did not form gel, so they are not able to provide proper tamponade to retina and thus cannot be used as vitreous substitute.

Gellan underwent gelation and after the formation of gel the viscosity is determined which was > 5000cP. Figure 3 represents the gelation of gellan with temperature. Here the viscosity is measured with decreasing temperature. As the formation of gel takes place the viscosity increases. Furthermore, when this gel was incubated at body temperature for 48 hr, then the gel maintained its structure and it did not undergo liquefaction, which represents the good mechanical strength of gel under physiological conditions.

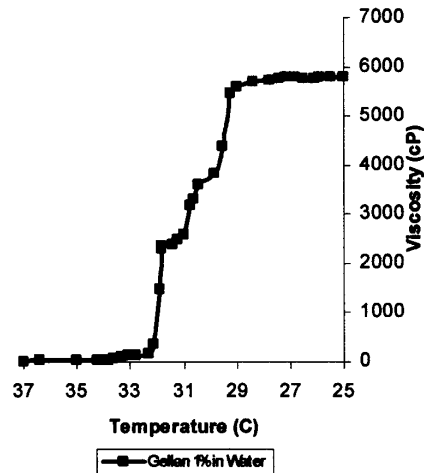


Figure 3 Viscosity of gellan (1 %) as a function of temperature showing temperature dependent gelation

Light transmission

The light transparency of the vitreous, Collagen and HA (alone and combinations), silicone oil and gellan is determined in the visible range (400-700 nm) of electromagnetic spectrum. The plot of transmittance against different wavelengths is given in figure 5. From the transmittance it is clear that the light transparency of vitreous is excellent approaching 100%, which allows maximum light to reach retina resulting into clear vision. For collagen and HA (individually and in combinations) light transmission is in acceptable range ranging from 85% -

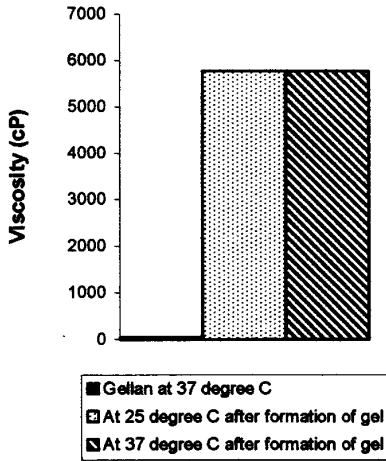


Figure 4 Viscosity of gellan (1 %) at body temperature before formation of gel, at room temperature and again at body temperature after formation of gel

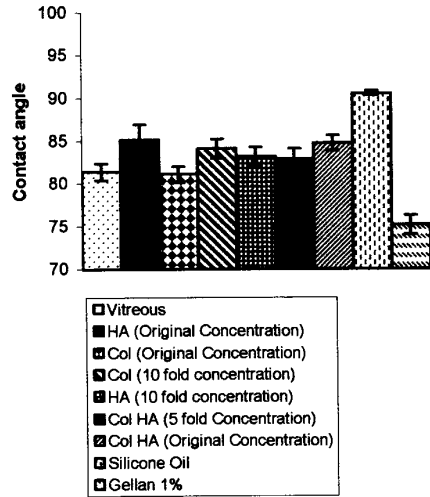


Figure 6 Contact angle of vitreous, Col and HA, silicone oil, and gellan

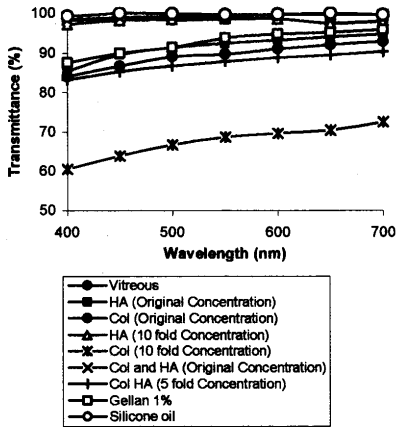


Figure 5 Transmittance of vitreous, Col and HA, silicone oil and gellan

100% except for collagen (10 fold higher concentration) where transmittance is very poor.

Contact Angle

The contact angle values are shown in Figure 6. These contact angle values indicate the adhesion properties of these specimens.

Syneresis

Syneresis was done at 15000 x g to determine the mechanical strength of the gel. For the vitreous, it

is 23.38%. No water separation resulted for gellan gel, which indicates that it has good water holding capacity.

Environmental Scanning Electron Microscopy

Microscopic structure of vitreous and gellan gel is determined by ESEM. From the ESEM images (Figure 7 and 8) it is clear that the structure of the gel is very complex.

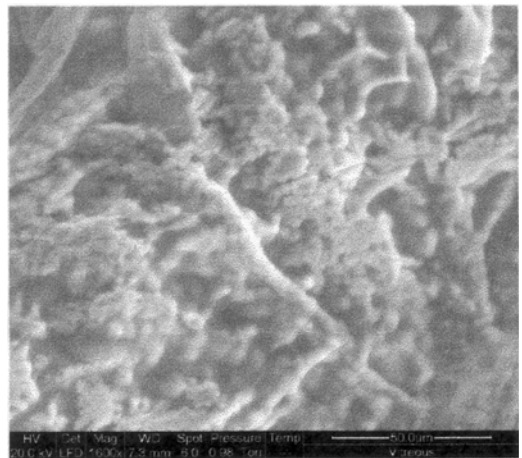


Figure 7 Environmental scanning electron micrograph of vitreous (original magnification x 1600; scale bar: 50 μm)

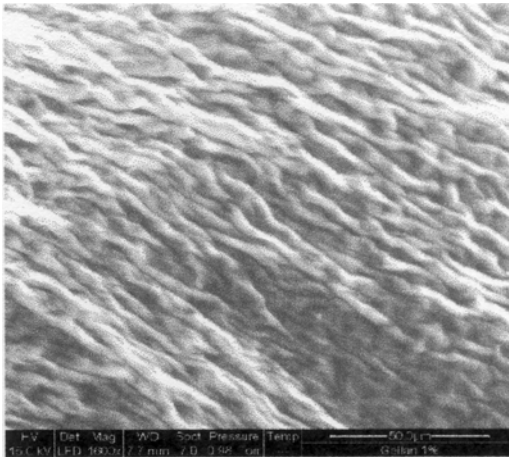


Figure 8 Environmental scanning electron micrograph of 1 % gellan (original magnification x 1600; scale bar: 50µm)

Discussions

The vitreous body is a very important intraocular element not only because of its optical role but also due to its significant role in the pathogenesis and treatment of the conditions affecting the adjacent tissue or the whole eye. Vitreoretinal conditions are the significant causes of blindness in the world [3]. There are two cases where the substitute for vitreous is required. First case is when the vitreous itself becomes dysfunctional due to opacification or by liquefaction.

There are various causes because of which vitreous becomes dysfunctional. The pathological causes include developmental abnormalities, inflammatory processes (from infection, injury or retinal disease), hemorrhage, systemic diseases (e.g. tumors, diabetes mellitus), and degenerative processes (e.g. aging changes, myopia). The traumatic destruction of the vitreous humor can also occur by mechanical, chemical and thermal trauma, or by the presence of intraocular foreign bodies. This may result in collapse of liquefied vitreous which has a great tendency to detach from the retina, and this change predisposes to damage of the retinal surface, vitreous bleeding or to retinal detachment, due to traction at any point of vitreoretinal adhesion. These conditions may result in extremely poor vision or even total blindness [1].

In principle, a total replacement of the damaged vitreous with a biocompatible, transparent material would be desirable in such cases. However, the treatment of a dysfunctional vitreous is not the most important reason for which the vitreous substitutes are needed. The surgical treatment of various complicated retinal detachments mostly requires the availability of a vitreous substitute, either temporary or permanent, ideally to be injectable into the vitreous cavity at the time of surgery [1].

In this study the characterization of vitreous, collagen, HA, gellan and silicone oil has been done. The viscosity of vitreous is very high because it is in gel form. It is a very important property to look for because it provides the tamponade to retina against the choroid and thus maintain the shape of eyeball. Silicone oil, the most frequently used vitreous substitute, has also very high viscosity but less than vitreous and is associated with many complications such as hydrophobicity, partial filling of vitreal cavity, and inhibition of effective closure of retinal break, which necessitate its removal from the vitreal cavity after sometime. Moreover, it is a liquid and does not form gel and thus fails to provide proper tamponade to retina [4]. Collagen and HA alone, as well as their combinations, have very low viscosity. The viscosity of none of the formulations of collagen and HA is high enough to make it suitable as a vitreous substitute. Gellan seems to be promising as its viscosity is >5000 cP and it undergoes gelation also. It is a biopolymer and is expected not to degrade because of its exogenous nature. Further, it retains its gel structure even after 48 hours of incubation at 37°C.

Mechanical strength of the gel is determined by syneresis experiment where the water holding capacity of gel is measured. For vitreous it was ~23% at 15,000 x g whereas for gellan there is no syneresis. It is a process in which bound water is released from the hydration layer of biopolymers and becomes free water. It has several effects. It increases the activity of water in tissues, thus it has a diluting effect. It also disturbs the osmotic balance. Irreversible syneresis results into the formation of liquid pockets in aging vitreous. This process also affects the mechanical properties of vitreous mainly viscoelasticity, sometimes resulting into detached retinas in older

persons [5]. In contrary to vitreous, gellan has good water holding capacity and thus does not release water with increase of pressure.

The contact angle values show the adhesion properties of vitreous and other samples. For a material to be used as substitute, its wettability should be same as that of vitreous so that it can make proper contact with the surrounding ocular tissues. Results show that the contact angle values of all the samples vary from that of vitreous and the wettability of gellan is significantly different. The wettability can be modified by the addition of some polymer along with gellan such as HA or collagen and thus similar wettability like that of vitreous can be achieved.

The transparency of 1 % gellan is between 90-95% which is excellent. Thus it allows maximum light to pass through it. Silicone oil is also totally transparent. From the ESEM images (Figure 7 and 8) it is clear that the structure of the vitreous gel and gellan is very complex. In vitreous, the pivotal role is played by collagen fibrils in maintaining the gel structure and it is already given in literature that

the removal of these collagen fibrils from the vitreous results into the conversion of gel into a viscous liquid [2]. ESEM images of gellan clearly represent crosslinked structure and due to this crosslinking, the gel is mechanically strong and has good water holding capacity which is also proved by syneresis experiment.

Conclusions

This study on the characterization of vitreous, its constituents, gellan and silicone oil has allowed us to reveal several points. Collagen and HA, which are the main constituents of vitreous cannot be used as vitreous substitute. Silicone oil which is the currently used substitute is associated with many complications which require its removal after sometime. Gellan has shown most promising results. However, the temperature of gelation and its adhesion properties need to be fine tuned. Further experiments are ongoing for the optimization of these properties for the development of a potential vitreous substitute.

References

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