Reports

Viscous Corneal Protection by Sodium Hyaluronate, Chondroitin Sulfate, and Methylcellulose

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The authors' study of the viscosities of various concentrations of sodium hyaluronate, chondroitin sulfate, and methylcellulose revealed that sodium hyaluronate and methylcellulose are pseudoplastic fluids in contrast to chondroitin sulfate, which is a Newtonian fluid. Pseudoplastic fluids are ideal for maintaining the anterior chamber, since they are more viscous at rest. Intermediate viscosity preparations of these three agents used as a thin endothelial coating gave excellent protection from intraocular lens abrasion. A highly viscous agent, eg, sodium hyaluronate 1%, in a thin layer produced extensive endothelial cell damage because it transmitted excessive shear force to the endothelium. A highly viscous agent, sodium hyaluronate 1% in a thick layer produced a physical barrier to compression with little endothelial damage. A low-viscosity agent, balanced salt solution provided insufficient protection against intraocular lens abrasion.


Extensive corneal endothelial cell loss occurs during contact with a polymethylmethacrylate intraocular lens.3 To decrease this corneal endothelial cell loss, a variety of viscous substances have been studied as protective coatings for intraocular lenses. These substances include sodium hyaluronate,6 chondroitin sulfate,7,8 methylcellulose,6,7 serum albumin,5 and polyvinylpyrrolidone.8 Harrison, Soll, Shayegan, and Clinch5 found chondroitin sulfate to be superior to sodium hyaluronate as a protective lens coating. More recently, Mac Rae et al.6 found sodium hyaluronate and chondroitin sulfate to be equally protective. Neither of these studies measured the shear dependence of the viscosities of the protective agents tested.

Two forces, compression and shear, are responsible for the mechanical damage when an intraocular lens is drawn across the endothelium. The compression force is the component of the applied force perpendicular to the corneal plane (Fig. 1), while the shear force is the component of the applied force parallel to the corneal plane (Fig. 1). The fraction of the shear force on the lens transmitted to the corneal endothelium as a drag force will increase as the viscosity of the fluid between them increases. An ideal protective agent must prevent contact with the endothelium while minimizing the drag force. An agent with too high a viscosity, ie, sodium hyaluronate 1%, transmits excessive shear force when used in a thin layer.

Our study was designed to measure the viscosities of sodium hyaluronate, chondroitin sulfate, and methylcellulose at various concentrations at clinically relevant shear rates. We then compared the protective properties of high, intermediate, and low viscosity agents. Our results demonstrated that control of viscosity is a critical factor in preventing mechanical damage to the corneal endothelium.

Materials and Methods. Viscosity measurements were obtained at 37°C with a Wells-Brookfield cone-plate model LVT microviscometer connected to a Haake model NK 22 waterbath. Various concentrations of sodium hyaluronate 1% (Healon) were prepared by mixing with balanced salt solution. Chondroitin sulfate from Cilco was obtained in concentrations of 20% and 50%, and dilutions also were made with balanced salt solution. Methylcellulose 400 cps was obtained from Sigma Chemical Company (St. Louis, MO) and prepared by dissolving in balanced salt solution.

Corneal buttons were obtained from fresh beef eyes using an 8.0-mm corneal trephine and a through-and-through technique.9 Pretreatment endothelial damage was assessed by staining with 0.1% trypan blue for 90 sec and then rinsing with balanced salt solution. Corneas with evidence of central endothelial damage were discarded. Minimal amounts of peripheral endothelial damage were present in most corneas, but this damage did not interfere with subsequent damage estimates. The corneal button was placed epithelial side down in a concave wet preparation microscope slide. The endothelium then was coated with 0.03 ml of the viscous protective material to create a thin layer. The solutions used were balanced salt solution, sodium hyaluronate 1% and 0.17%, chondroitin sulfate 20%, and methylcellulose 1%, as well as a thick layer of sodium hyaluronate 1%.
COMPRESSION
FORCE
VISCOUS AGENT
SHEAR FORCE
MINIMIZED BY
LOW VISCOITY
ENDOTHELIUM

MINIMIZED
BY HIGH
VISCOITY
SHEAR FORCE
APPLIED
FORCE

Fig. 1. The applied force on an IOL can be represented by two vectors: the compression force and the shear force. High viscosity agents transmit more of the shear force to the endothelial surface as a drag force.

Sodium hyaluronate 1% was the only commercially available material with a high enough viscosity to maintain a thick layer for testing. A Leiske intraocular lens with one haptic removed was grasped by the remaining haptic with a pair of forceps. The endothelial surface then was abraded twice with sufficient force to bend gently the haptic. The downward force that can be applied by moderately bending the haptics of a Leiske lens is 0.8 mg ± 0.1 mg. The lens was drawn across the cornea at the speed of 2 to 3 cm/sec. The corneal button was stained with trypan blue and alizarin red S. The corneal button was then examined under a microscope at ×40 and ×100, and damage estimates from 0-100% were based on the ratio of the damaged cells, ie, trypan blue staining, to the total number of cells, ie, alizarin red S staining. Several corneal buttons from each group were stained in an identical manner to those of test corneas without the abrasion phase. These control corneas did not reveal any significant damage to the corneal endothelium. The investigations utilizing animals, as described in this study, conform to the ARVO Resolution on the Use of Animals in Research.

Results. Sodium hyaluronate exhibited pseudoplastic behavior (Fig. 2A), ie, the viscosity is higher at low shear rates. In contrast, chondroitin sulfate exhibited Newtonian behavior (Fig. 2B), ie, the viscosity is relatively constant at all shear rates. Methylcellulose, like sodium hyaluronate, is pseudoplastic (Fig. 2C). Balanced salt solution had a viscosity of 0.70 cp at all shear rates from 6 sec\(^{-1}\) to 450 sec\(^{-1}\).

Theoretically, if an intraocular lens is drawn past the corneal endothelium at 2–3 cm/sec with a 0.5 mm coating of a protective agent, the shear rate is approximately 40–60 sec\(^{-1}\). Various concentrations of these substances were prepared to yield a viscosity of approximately 30 cp at a shear rate of 40–60 sec\(^{-1}\) (Fig. 3). The shear force on the corneal endothelium is then 15 dynes/cm\(^2\). The shear force for a thin layer of 1% sodium hyaluronate is 500 dynes/cm\(^2\) with the same conditions.

Little endothelial damage was seen with a thin layer of the intermediate viscosity substances (Fig. 4): sodium hyaluronate 0.17%, chondroitin sulfate 20%, and methylcellulose 1%. However, with balanced salt solution, which has a low viscosity and a thin layer...
of sodium hyaluronate 1%, which has a high viscosity, over 40% of the cells were damaged (Fig. 4). A thick layer of sodium hyaluronate 1% was protective of the corneal endothelium and showed little endothelial damage (Fig. 4).

Methylcellulose 1% had a pH of 6.95 and an osmolality of 298 mOsm, while sodium hyaluronate 0.17% had a slightly higher pH of 7.00 and an osmolality of 313 mOsm. Chondroitin sulfate 20% had a pH of 6.45 and an osmolality ranging from 900–1000 mOsm.

Discussion. Intermediate viscosity substances such as chondroitin sulfate 20% sodium hyaluronate 0.17%, and methylcellulose 1% exhibited excellent protective properties when used as coatings, ie, thin layer. A high viscosity agent such as sodium hyaluronate 1% exhibits poor protection when used as a coating because of the high shear force transmission. Low-viscosity agents such as balanced salt solution provide insufficient contact protection. A thick layer of sodium hyaluronate did not allow the lens to get close enough to the cornea to create a significant drag force with the limited amount of compression in our study. If more extensive manipulation in a surgical procedure allowed the lens to become very close to the endothelium, severe endothelial damage would be expected with sodium hyaluronate 1%.

Our results indicate that sodium hyaluronate and methylcellulose are pseudoplastic fluids unlike chondroitin sulfate, which is a Newtonian fluid. In the clinical situations where maintenance of the anterior chamber is important, a pseudoplastic material is
ideal since the viscosity is highest when the material is at rest under low shear stress. This means a pseudoplastic fluid is more viscous when it is nearly stationary and less viscous when it is flowing. In contrast chondroitin sulfate has the same viscosity at various shear rates and would not exhibit the same degree of anterior chamber support as a pseudoplastic fluid at rest. Further research is needed to identify the optimal viscosity of the agents used for corneal endothelial protection in specific clinical situations.

Key words: viscosity, endothelium, sodium hyaluronate, chondroitin sulfate, methylcellulose, intraocular lens

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References

Neuron-Specific Enolose-Containing Cells in the Rhesus Monkey Trabecular Meshwork

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Neuron-specific enolase (NSE) localizes immunohistochemically to a discontinuous band of cell clusters in the trabecular meshwork of the anterior segment of the rhesus monkey eye. To date, this enolase isomer has been found exclusively in neurons or in cells of the diffuse neuroendocrine system. On this basis, its presence provides presumptive evidence for neuroregulatory cells in the primate trabecular meshwork. Invest Ophthalmol Vis Sci 25:1332–1334, 1984

Neuron-specific enolase (NSE), an isomer of the glycolytic enzyme enolase,1,2 has been localized histochemically only in central and peripheral neurons and in neuroendocrine cells.3–5 As a result, it is believed generally that NSE is a reliable marker for such tissues.3–6 We now report the immunohistochemical localization of NSE in cells at the primate trabecular meshwork, a finding that implies the presence of neuroregulatory cells in the aqueous humor outflow pathway.

Methods. Antiserum: The primary antiserum used for immunohistochemical localization in this study was raised by injecting New Zealand white rabbits with highly purified rat brain NSE homogenized in Freund’s complete adjuvant.7 It has been characterized previously1,3; and importantly, this antiserum does not cross-react with a 105 excess of non-neuronal enolase, indicating its specificity.

Tissue preparation: Eyes were obtained immediately after death from rhesus monkeys (Macaca mulatta) killed under deep pentobarbital anesthesia as part of the polio vaccine testing program of the Bureau of Biologies of the Food and Drug Administration. The eyes were immersion fixed for 4 hr at 4°C in 0.1 M phosphate buffer, pH 7.2, with 4% paraformaldehyde and transferred overnight to 0.1 M phosphate buffer with 30% sucrose at 4°C. Cryostat tissue sections, 16–20 μm thick, were thaw mounted on gelatin-coated slides, dried at room temperature, and stored at −20°C until stained by the following indirect immunofluorescence technique.

Immunohistochemical procedure: After washing in phosphate-buffered saline (PBS), pH 7.2, the tissue sections were incubated at 37°C for 1 hr with NSE antiserum diluted 1:500 or 1:1000 and containing 0.3% Triton X-100. After incubation, the tissue sections were washed twice in PBS and then reacted for