Ophthalmic viscosurgical devices (OVDs) protect the corneal endothelial cells and facilitate the steps during intraocular surgery. Various OVDs are available for cataract and intraocular surgery. Most commercially available OVDs are based on sodium hyaluronate, and they differ in the rheologic polymer type(s), concentration, and chain length. These factors determine an OVD’s viscosity, elasticity, cohesion, and other physical and chemical properties.

Over time, additional types of OVDs have been developed, including various mixtures of sodium hyaluronate with chondroitin sulfate. The molecular weight and percentage concentration of sodium hyaluronate have been changed to create agents of varying molecular weights and viscosities. High-viscosity cohesive OVDs and low-viscosity dispersive OVDs have unique advantages and disadvantages. High-viscosity cohesive agents help maintain and preserve space as well as displace and stabilize tissue. The materials, however, tend to flow out of the eye during phacoemulsification. Low-viscosity dispersive agents tend to remain in the eye adjacent to the corneal endothelium, providing potential protection during phacoemulsification; however, these agents do not maintain space well and can be difficult to remove.

In 1999, Arshinoff reported the soft-shell technique, which combines dispersive and cohesive OVDs sequentially without mixing to derive the benefits of both types and eliminate their drawbacks. In this technique, a low-viscosity dispersive OVD agent is injected into the anterior chamber and a mound is formed on the anterior surface of the lens. A high-viscosity cohesive agent is then injected into the posterior center of the mound of...
low-viscosity dispersive OVD so the incoming viscous cohesive OVD fills the center of the eye and pushes the low-viscosity dispersive agent up and out, eventually forming a smooth, even, and pressurized layer adjacent to the corneal endothelium. As current techniques in cataract surgery induce little corneal endothelial damage in eyes with low or moderate nuclear hardness, the soft-shell technique should be more useful in cataract patients with a hard lens nucleus or primary endothelial cell disease.

There is a significant difference in the costs of the various OVDs, and this plays a crucial role when choosing the number and type of OVDs to use. The objective of the present study was to compare 2 combinations of OVDs applied using the soft-shell technique and the use of cohesive OVD alone in a relatively mild phacoemulsification rabbit model. We selected the rabbit model because it is widely accepted for use in ocular studies. The most important advantage is its repeatability and uniformity in comparison with human eye models. It has 2 major disadvantages. The first is the ability of endothelial cells to regenerate, a process which takes place approximately 1 week after surgery and may be a confounding factor that alters postoperative cell counts. The second is that the rabbit cornea is much more convex than the human cornea, making cell counts more difficult.

MATERIALS AND METHODS

New Zealand white rabbits aged 5 to 6 months and weighing 3.3 to 3.5 kg were obtained from an approved vendor (Harlan Laboratories Israel, Ltd) and maintained in accordance with the guidelines set forth by the Association for Research in Vision and Ophthalmology, the Animal Welfare Act regulations, and the Guide for the Care and Use of Laboratory Animals. This study was performed after review by the Committee for Ethical Conduct in the Care and Use of Laboratory Animals.

The rabbits were housed individually in stainless-steel cages mounted in batteries (60 cm long × 50 cm wide × 45 cm high). The room was maintained at 17°C to 23°C with a relative humidity of 30% to 70%, a 12-hour-light/12-hour-dark cycle and 22 air changes per hour. Each rabbit was given a diet of approximately 100 g/day (7078S Rabbit Diet, Harlan Teklad) and allowed free access to drinking water (filtered with a 0.1 μ filter, chlorinated, acidified), which was supplied to each cage via polyethylene bottles with stainless steel sipper tubes. The general health status and the eyes of the animals were examined on their arrival; all animals used in this study were healthy and free of ocular anomalies.

The rabbit eyes were randomly assigned to 3 equally sized groups based on the OVD used to replace the aqueous humor. In Group A, the aqueous humor was completely replaced by Biolon (sodium hyaluronate 1.0%) alone. In Group B and Group C, the soft-shell technique was used with a combination of Viscoat (sodium chondroitin sulfate 4.0%-sodium hyaluronate 3.0%) and Provisc (sodium chondroitin sulfate 1.0%) and a combination of Visiol (sodium hyaluronate 2.0%-mannitol 0.5%) and Biolon, respectively. The protective effects of the 2 combinations on endothelial cells were assessed and compared with the effect of Biolon alone.

Before surgery, a corneal endothelial cell count (ECC) was performed in all eyes using a specular microscope (Noncon Robo, Konan Medical); a confined area within which all cells were marked was indicated. The integrated software of the microscope calculates the quantity of cells. At least 3 repeated counts were performed in each eye.

Approximately 30 minutes before surgery, the pupil of each eye was dilated with phenylephrine hydrochloride (AK-Dilate 2.5%) and cyclopentolate hydrochloride USP 1% (1 drop/eye approximately every 15 minutes). Anesthesia was then induced by an intramuscular injection containing a mixture of ketamine hydrochloride 35 mg/kg (KePro) and xylazine 5 mg/kg. Supplemental anesthetic agents were administered intramuscularly as needed during surgery. One drop of oxybuprocaine hydrochloride (Localin) was instilled into each eye shortly before surgery. All surgical procedures were performed in an aseptic manner under an operating microscope (Universal S3B, Carl Zeiss Meditec). Each eye was cleaned and draped and a lid speculum placed to retract the eyelids. Subsequently, a partial-thickness limbal incision was made with a crescent blade (Sharpoint) followed by penetration into the anterior chamber with a 3.2 mm keratome knife (Katena Products, Inc.).

The insult to the corneal endothelial cells was assessed using a modification of the technique by Nemet et al. The phacoemulsification probe (Phakovisc II, Optikon 2000 SpA) was inserted into the anterior chamber via the corneal incision, taking care to avoid damage to ocular structures, particularly the cornea and lens. The probe was activated at 70% energy in the center of the anterior chamber, just adjacent to the crystalline lens. The power was alternated on and off every 10 seconds until the required 2.5 minutes of exposure net time elapsed (5.0 minutes total time).

Neomycin sulfate-polymyxin B-decamethasone 0.1% (Maxitrol) was applied to all the eyes immediately after the completion of the surgery and thereafter 4 times a day. The ECC was repeated 3 days after surgery. The rabbits were then killed humanely by an intravenous overdose of sodium pentobarbitone.
Statistical Analysis

The nonparametric Kruskal-Wallis test was used to compare preoperative and postoperative parameters between the 3 groups. The nonparametric Wilcoxon test was used to compare the changes within each group. A P value less than 0.05 was considered significant.

RESULTS

The study used 18 rabbit eyes, 6 in each group. Table 1 and Figure 1 show the preoperative and postoperative mean ECC in the study groups as well as the change in the ECC and percentage loss of ECCs. Group A had the greatest endothelial cell loss followed by Group B and then Group C. The difference in cell loss between Group C and Group A was statistically significant (P = .037). The difference in cell loss between Group B and Group A approached statistical significance (P = .055).

DISCUSSION

Our experimental rabbit model showed that the soft-shell technique with the combined application of a dispersive OVD (Visiol) and a cohesive OVD (Bilon) had a protective effect against phacoemulsification insult to corneal endothelial cells versus the effect of Bilon alone (change in ECC, 4% versus 13%; P = .037). It also showed the effectiveness of the soft-shell technique using a combination of the dispersive OVD Viscoat and the cohesive OVD Provisc versus that of Bilon alone (change in ECC, 7% versus 13%; P = .055). Although approaching significance, the difference between the latter combination and Bilon alone was not statistically significant; this can be attributed to, among other reasons, the relatively small sample of rabbit eyes.

The main reason we selected a rabbit model for this comparative OVD study was to overcome the significant inherent flaws in this type of study in human eyes; namely, the lack of repeatability and reproducibility and the uniformity of the insult to endothelial cells. Even with the same extent of cataract hardness and phacoemulsification power and time, other factors can affect the formation of free radicals. These include the location of the phaco probe needle in the anterior chamber and its proximity to the cornea, surgical technique, changes in the eye tonus during surgery, the duration of surgery, and the amount of fluid used during surgery. A major factor is the flow rate. Ophthalmic viscosurgical devices are extremely sensitive to flow rate, and their retention or elimination from the anterior chamber is determined more by the flow rate than by other factors. Although we could not measure the flow rate because of our model design, it is reasonable to assume it was similar in all eyes.

Several techniques for the use of OVDs in anterior segment surgery have been reported.12 One widely applied procedure, which combines a dispersive OVD and a cohesive OVD, is the soft-shell technique as reported by Arshinoff in 1999.4 Results in studies evaluating this technique7,13 suggest that the soft-shell technique leads to less endothelial cell loss than when it is not used.

In the present study, we used a relatively new dispersive OVD (Visiol) in 1 combination for the soft-shell technique. Visiol has a molecular weight of 1.8 million.
Da and zero-shear-rate viscosity of 60,000 mPas. One component of this OVD is mannitol, which is a scavenger of free radicals, such as those produced during phacoemulsification; these free radicals play a significant role in cell damage.14–16 This may explain why the combination including Visiol gave better results than the combination that included the dispersive OVD Viscoat. Again, this result should be interpreted keeping in mind the relatively small sample in our study. Both Visiol and Viscoat have the added benefit of being retained in the eye longer than the cohesive OVD Biolon.

Recently, Belda et al.17 indirectly assessed the protective effects of Viscoat, Visiol, and Healon (sodium hyaluronate 1.0%) on the corneal endothelium after exposure to increasing concentrations of oxidative stress induced by hydrogen peroxide (H2O2). Their results suggest that OVDs with higher concentrations of sodium hyaluronate (Visiol and Viscoat) have enhanced endothelial protective effects. They also found that Visiol was better at protecting endothelial cells against free-radical damage; they attributed this to the addition of mannitol. However, Belda et al. evaluated corneal endothelium insult by H2O2-induced oxidative stress, whereas we induced corneal endothelial cell trauma phacoemulsification. Furthermore, they used the Janus green photometry absorbance technique to determine the extent of endothelial cell damage; in this technique, baseline measurements of the ECC before the insult are not possible.

In conclusion, we found that the soft-shell technique using a combination of Visiol and Biolon was more efficacious than and had advantages over the use of Biolon alone. The combination of Viscoat and Provisc in the soft-shell technique also had a better protective effect than Biolon alone, although the difference was of borderline statistical significance.

REFERENCES