The Optimal Molecular Weight of Dispersive Type Sodium Hyaluronate for the Reduction of Corneal Endothelial Damage Induced by Sonication, Irrigation, and Aspiration

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Purpose: The purpose of this study was to investigate the optimal molecular weight of dispersive sodium hyaluronate (Na-HA) for the reduction of corneal endothelial damage induced by sonication, irrigation, and aspiration, using enucleated pig eyes.

Method: The phaco-needle of phacoemulsification and aspiration (PEA) equipment was inserted into the anterior chamber after aqueous humor replacement with a 1% Na-HA solution of various molecular weights (2420×10^3, 1460×10^3, 1100×10^3, 520×10^3 or 290×10^3). Then sonication, irrigation, and aspiration were conducted for 60 seconds. The residual rate of Na-HA in the anterior chamber and the damaged area of corneal endothelium were determined using an image analyzer.

Results: Na-HA with a molecular weight of 1100×10^3 gradually disappeared from the anterior chamber after mixing with the irrigating solution, and the damaged area in only the 1100×10^3 group was significantly smaller compared with that in the control group.

Conclusion: These results suggest that an optimal molecular weight exists for dispersive hyaluronate applied for the protection of intraocular tissues during PEA. Under the conditions of this study, Na-HA with a molecular weight of 1100×10^3 displayed the highest protective efficacy.   **Jpn J Ophthalmol 2001;45:339–347**   © 2001 Japanese Ophthalmological Society

Key Words: Cohesive, corneal endothelium, dispersive, phacoemulsification, sodium hyaluronate.

Introduction

Currently, viscoelastic materials for ophthalmic surgery, such as sodium hyaluronate (Na-HA), are broadly classified into two types, cohesive and dispersive.[1] It has been proposed that these viscoelastic materials are utilized appropriately according to their properties in specific stages of surgery. For example, when the depth of the anterior chamber is required to be maintained during insertion of the intraocular lens, the cohesive type is suitable. On the other hand, when the intraocular tissues are required

to be protected by a viscoelastic material that is retained in the anterior chamber during irrigation/aspiration in phacoemulsification, the dispersive type is recommended. In a previous study carried out using rabbit eyes and 5-aminofluorescein-labeled Na-HA with a molecular weight of 1130×10^3 (dispersive type) and 2010×10^3 (cohesive type),[2] we demonstrated that dispersive Na-HA exhibited a protective effect against corneal endothelial damage induced by sonication, irrigation, and aspiration. The protective effect of dispersive viscoelastic materials on the intraocular tissues during phacoemulsification and aspiration (PEA) has been recognized in other clinical and nonclinical studies.[3–5]

Na-HA solution can exist as cohesive or dispersive viscoelastic material, depending on its molecular

weight. This is because the extent of entanglement among Na-HA molecules varies depending on the molecular weight. It is known that an isotonic solution which contains 1% Na-HA with a molecular weight of 1620×10^3 or more exhibits cohesive properties and that one with a molecular weight of less than 1620×10^3 exhibits dispersive properties.[6]

As described above, a dispersive viscoelastic material retained in the anterior chamber during PEA is suited to protecting the intraocular tissues from various damaging factors. However, studies have not been carried out to investigate systematically the optimal molecular weight of Na-HA when used for dispersive purposes. Therefore, in this study, we studied the relationship between the molecular weight of Na-HA and its residual rate in the anterior chamber, and between the molecular weight of Na-HA and its effectiveness in protecting the corneal endothelium, using enucleated pig eyes and 1% solutions of Na-HA of various molecular weights, supplemented with sodium fluorescein.

Materials and Methods

Sodium Hyaluronate

Sodium hyaluronate, with the molecular weight of 2420×10^3, 1460×10^3, 1100×10^3, 520×10^3, or 290×10^3 was used in this study. These Na-HAs were prepared by Seikagaku Corporation (Tokyo) and dissolved in phosphate-buffered physiological saline (PBS) at a final concentration of 1%. The actual molecular weight was calculated using the equation derived by Laurent et al,[7] based on the limiting viscosity. The Na-HA with a molecular weight of 2420×10^3 was classified as being cohesive, and Na-HAs with other molecular weights were classified as dispersive.[1,6] Sodium fluorescein was added to these Na-HA solutions to give a final concentration of 500 μg/mL, and to make it possible to determine the amount of Na-HA remaining in the anterior chamber.

Enucleated Pig Eyes

Pig eyes, enucleated on the day of examination, were obtained from Tokyo Shibaura Zoki (Tokyo).

Sonication, Irrigation, and Aspiration

An appropriate volume of physiological saline was injected through the optic disc of an enucleated pig eye, and the intraocular pressure was adjusted to approximately 7 mm Hg. An incision 3 mm wide was made at the limbus using a keratome, and the anterior aqueous humor was eliminated as much as pos-

sible. Then, 250 μL of Na-HA solution was carefully injected through the incision, and immediately after this, sonication, irrigation, and aspiration were performed in the anterior chamber using the phaco-needle attached to the PEA equipment (Phacompo®; Allergan/Optical Micro Systems, North Andover, MA, USA).

During sonication, the anterior chamber was irrigated with an irrigating solution (BSS® plus, Alcon Lab, Fort Worth, TX, USA) at a flow rate of 24 mL/minute under vacuum pressure of 70 mm Hg. In order to determine the protective effect more clearly, sonication was carried out at 100% full power of the PEA equipment. The angle of the phaco-needle was 45°, and the bevel of the needle was placed facing downwards against the lens. The phaco-needle was positioned in the center of the pupil, at the side nearest to the lens. The position of the aspiration port was not uniform in each case. The duration of sonication, irrigation, and aspiration was 60 seconds. The emulsification of lens (phacoemulsification) was not conducted during the sonication, irrigation, and aspiration. The conditions for sonication, irrigation and aspiration are indicated in Table 1.
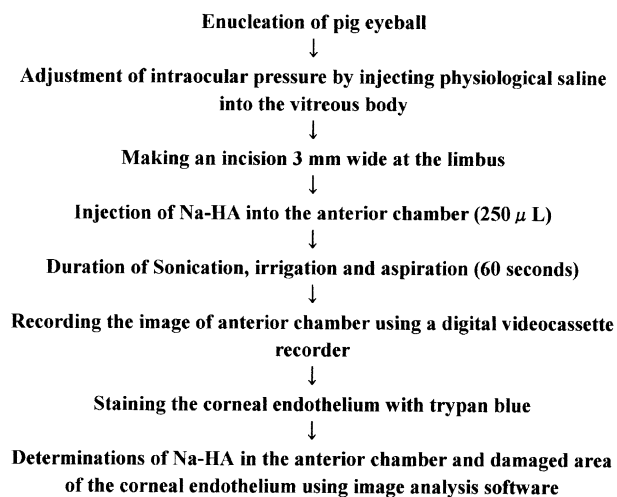
Determination of Na-HA in the Anterior Chamber

Images of the anterior chamber were recorded during the period following injection of the Na-HA solution up until completion of sonication, irrigation, and aspiration, using a digital videocassette recorder (DVCAM®, DSR-20; Sony, Tokyo). The images were transferred to a computer from the recorded videotape in order to calculate the amount of Na-HA in the anterior chamber using image analysis software (Image-Pro® plus; Media Cybernetics, Silver Spring, MD, USA). The region of yellow-green fluorescence derived from sodium fluorescein was identified using an established threshold level, and then the area of this region was calculated as the residual amount of Na-HA using the analysis software. The images recorded were those immediately before

Table 1. Conditions for Sonication, Irrigation, and Aspiration

Equipment for PEA: Phacompo®
Irrigating Solution: BSS® plus
Ultrasonic Output of PEA Equipment: 100% of power used
Aspiration Rate: 24 mL/minute
Vacuum Pressure: 70 mm Hg
Size of Incision: 3 mm wide (under watertight condition)

Enucleation of pig eyeball
↓
Adjustment of intraocular pressure by injecting physiological saline
into the vitreous body
↓
Making an incision 3 mm wide at the limbus
↓
Injection of Na-HA into the anterior chamber (250 μ L)
↓
Duration of Sonication, irrigation and aspiration (60 seconds)
↓
Recording the image of anterior chamber using a digital videocassette
recorder
↓
Staining the corneal endothelium with trypan blue
↓
Determinations of Na-HA in the anterior chamber and damaged area
of the corneal endothelium using image analysis software

Figure 1. Outline of experimental procedures.

sonication, irrigation, and aspiration, and 2, 4, 10, 30, and 60 seconds after the start of the procedures. The residual amount of Na-HA at each time interval after the start of sonication, irrigation, and aspiration was expressed as a residual rate (percentage) for the value immediately before the start of these procedures.

Determination of Damaged Corneal Endothelium

The area of damaged corneal endothelium was measured after sonication, irrigation, and aspiration for 60 seconds. The corneal endothelium was stained with a 0.25% trypan-blue PBS solution, and fixed with 10% neutral formalin.[2,8] Damaged regions of the corneal endothelium were stained by the trypan-blue. Vital staining with trypan-blue was also conducted for the normal corneal endothelium derived from nontreated pig eye. Images of the corneal endothelium were transferred to a computer; the area of damaged corneal endothelium was measured using image analysis software (Image-Pro® plus). The damaged areas were expressed as a percentage of the whole corneal endothelium; that is, the percentage of damaged area was calculated. The experimental procedure is outlined in Figure 1.
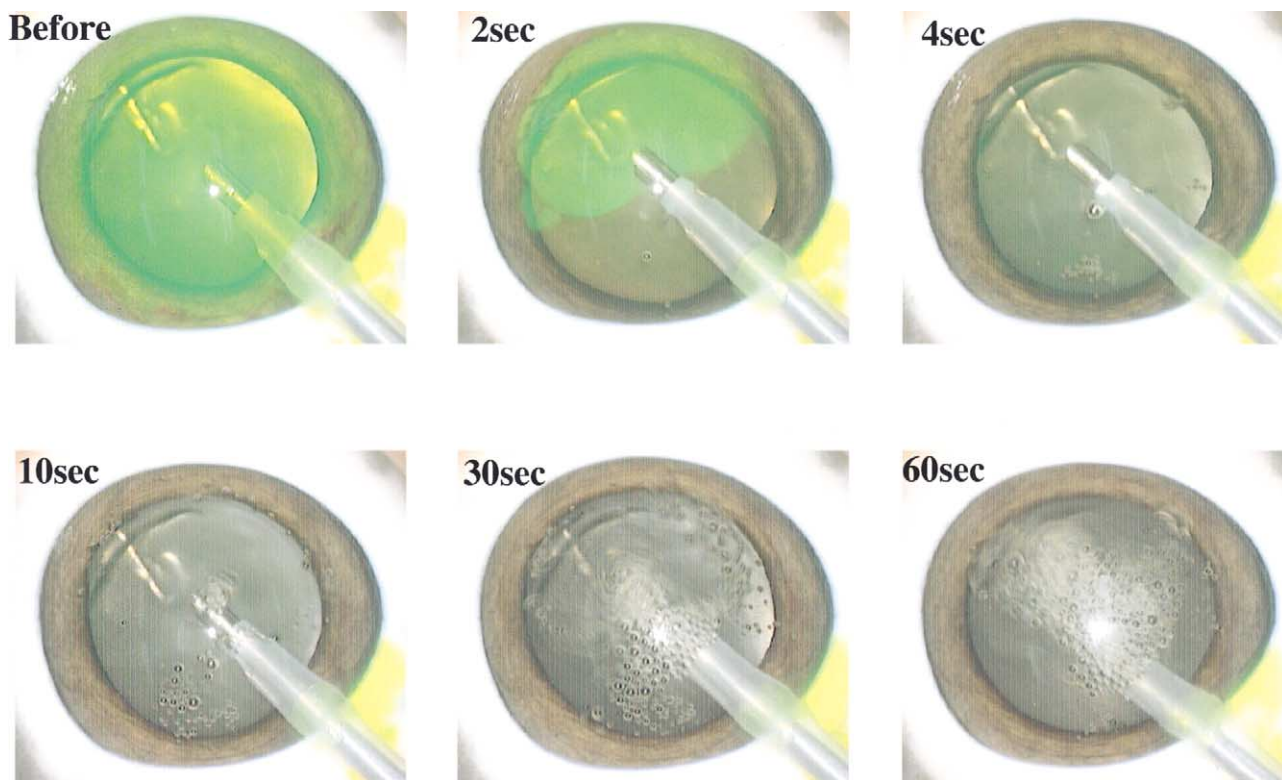
Figure 2. Images of anterior chamber in sodium hyaluronate (Na-HA) (2420×10^3) group. Yellow-green fluorescence in anterior chamber derives from Na-HA (2420×10^3) containing Na-fluorescein. No fluorescence is observed in anterior chamber at and after 4 seconds. Conditions of procedures are described in Table 1.

Experimental Groups

There were six groups of enucleated eyes in this study, control, Na-HA (2420×10^3), Na-HA (1460×10^3), Na-HA (1100×10^3), Na-HA (520×10^3) and Na-HA (290×10^3). Each group consisted of 8 eyes. In the control group, sonication, irrigation, and aspiration were conducted without prior injection of Na-HA solution.

Statistical Analysis

For the residual rate of Na-HA in the anterior chamber and the percentage of damaged area, the mean and the standard deviation of each group were calculated. Differences in mean value of the residual rate of Na-HA were analyzed among Na-HA groups using the data collected at 2, 4, 10, 30, and 60 seconds. Differences in the mean value of the percentage of damaged area were analyzed between each Na-HA group and the control group. Tukey multiple comparison test was applied for an analysis of inter-group difference in the mean value.

Results

The Residual Rate of
Na-HA in the Anterior Chamber

Images of the anterior chamber immediately before sonication, irrigation, and aspiration, and after 2, 4, 10, 30, and 60 seconds are shown in Figures 2–6. In the Na-HA (2420×10^3) and Na-HA (290×10^3) groups, Na-HA almost completely disappeared from the anterior chamber within 4 seconds after the start of procedures (Figures 2 and 6). In the Na-HA (520×10^3) group, Na-HA was also eliminated within 30 seconds (Figure 5). By comparison, in the Na-HA (1100×10^3) group, Na-HA gradually disappeared, mixing with the irrigating solution, but was still detectable even after 60 seconds. In this group, Na-HA was almost uniformly distributed and adhered to the corneal endothelium at all intervals of measurement (Figure 4). Although residual Na-HA
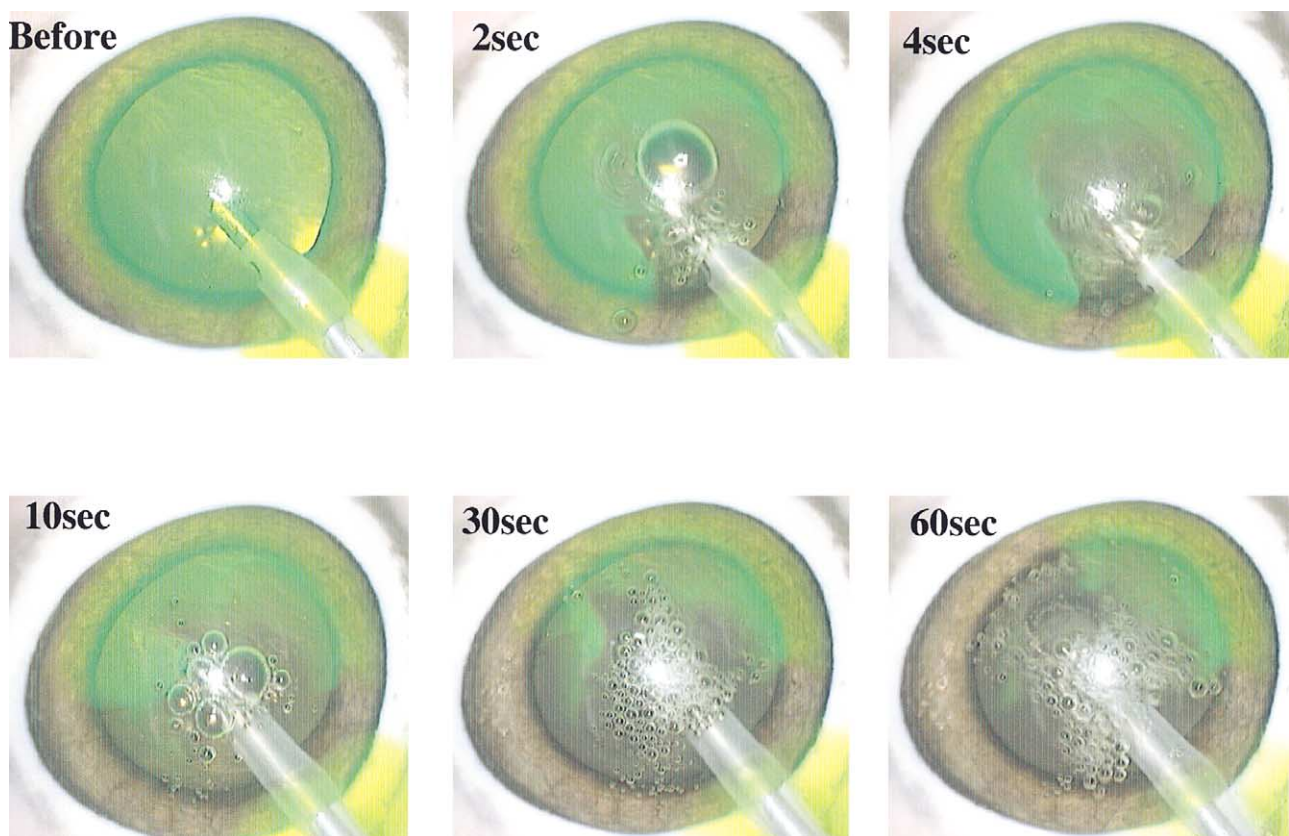
Figure 3. Images of anterior chamber in sodium hyaluronate (Na-HA) (1460×10^3) group. Yellow-green fluorescence in anterior chamber derives from Na-HA (1460×10^3) containing Na-fluorescein. Although fluorescence is detected even after 60 seconds, the fluorescence close to the phaco-needle disappeared within 4 seconds, and distribution of fluorescence is found to be uneven at and after 4 seconds.

was also detected in the anterior chamber at 60 seconds in the Na-HA (1460×10^3) group, Na-HA close to the phaco-needle was eliminated within 4 seconds; also, the distribution of residual Na-HA (1460×10^3) was found to be uneven at and after 4 seconds (Figure 3).

The residual rate of Na-HA at each time point is shown in Figure 7. The residual rates in the Na-HA (1100×10^3) group at 4, 10, 30, and 60 seconds were significantly higher than in other Na-HA groups. Furthermore, even at 2 seconds, the residual rate in the Na-HA (1100×10^3) group was significantly higher than in other Na-HA groups, with the exception of the Na-HA (1460×10^3) group.

The residual rate of Na-HA in the anterior chamber after sonication, irrigation, and aspiration for 60 seconds was 0% in the Na-HA (2420×10^3), Na-HA (520×10^3), and Na-HA (290×10^3) groups; $33.3 \pm 14.9\%$ in the Na-HA (1460×10^3) group; and $62.4 \pm 8.1\%$ in the Na-HA (1100×10^3) group.

The Area of Damaged Corneal Epithelium

Photographs of the corneal endothelium following staining with trypan-blue are shown in Figure 8. The damaged region stained was clearly smaller in the Na-HA (1100×10^3) group than in other groups.

The percentage of the damaged area in each group, calculated by the image analysis software, is shown in Figure 9. Only in the Na-HA (1100×10^3) group, the percentage of the damaged area was significantly smaller than in the control group.

Discussion

In this study, an appropriate amount of sodium fluorescein was mixed with Na-HA solutions to make it possible to detect Na-HA in the anterior chamber. Although ideally Na-HA should be labeled with a covalent bond, a simple combination with coloring matter was sufficient to allow detection of behavior over a short period; 60 seconds. This was confirmed by the fact that the behavior of Na-HA (2420×10^3) or Na-HA (1100×10^3) in the anterior chamber was similar to that of Na-HA used in a previous study that was prepared by covalent-bonding with 5-aminofluorescein.[2]

Na-HA (2420×10^3) was the only cohesive viscoelastic material used in this study. This material in-
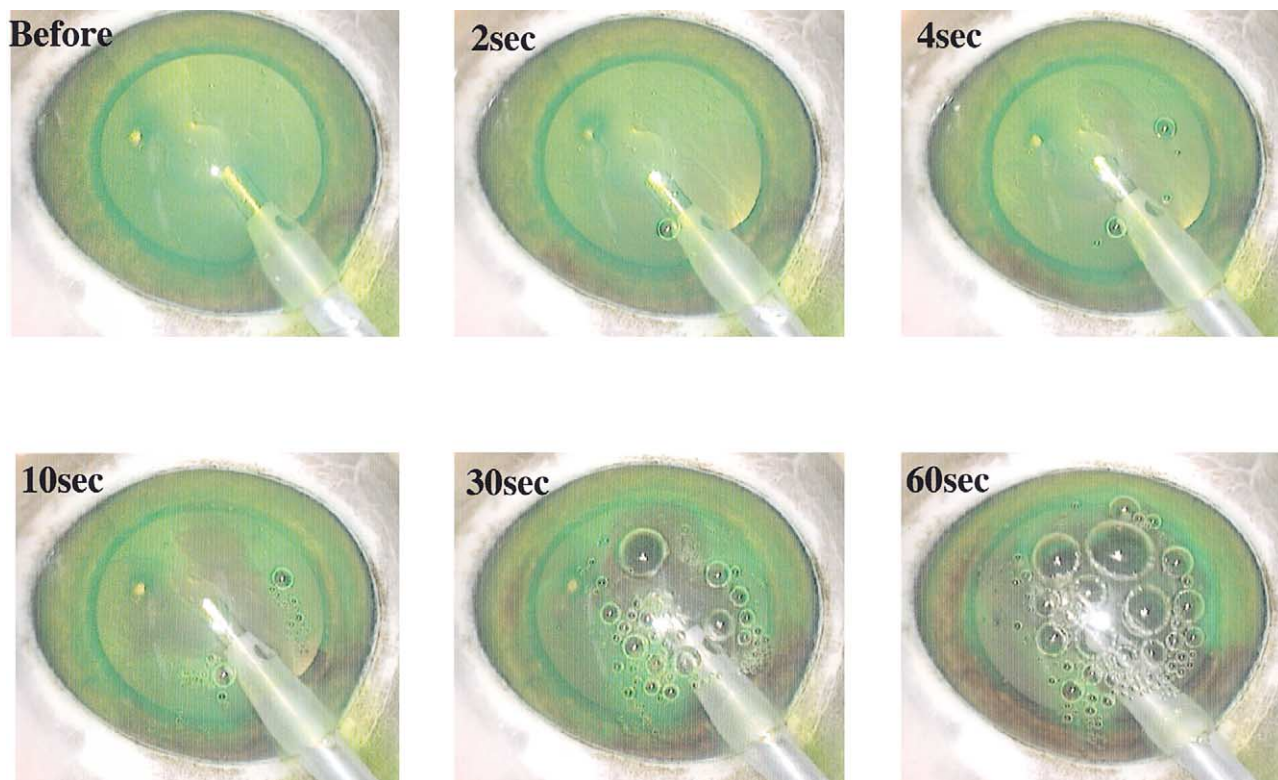
Figure 4. Images of anterior chamber in sodium hyaluronate (Na-HA) (1100×10^3) group. Yellow-green fluorescence in anterior chamber derives from Na-HA (1100×10^3) containing Na-fluorescein. Almost uniformly distributed fluorescence is observed in anterior chamber at all intervals after start of procedures.
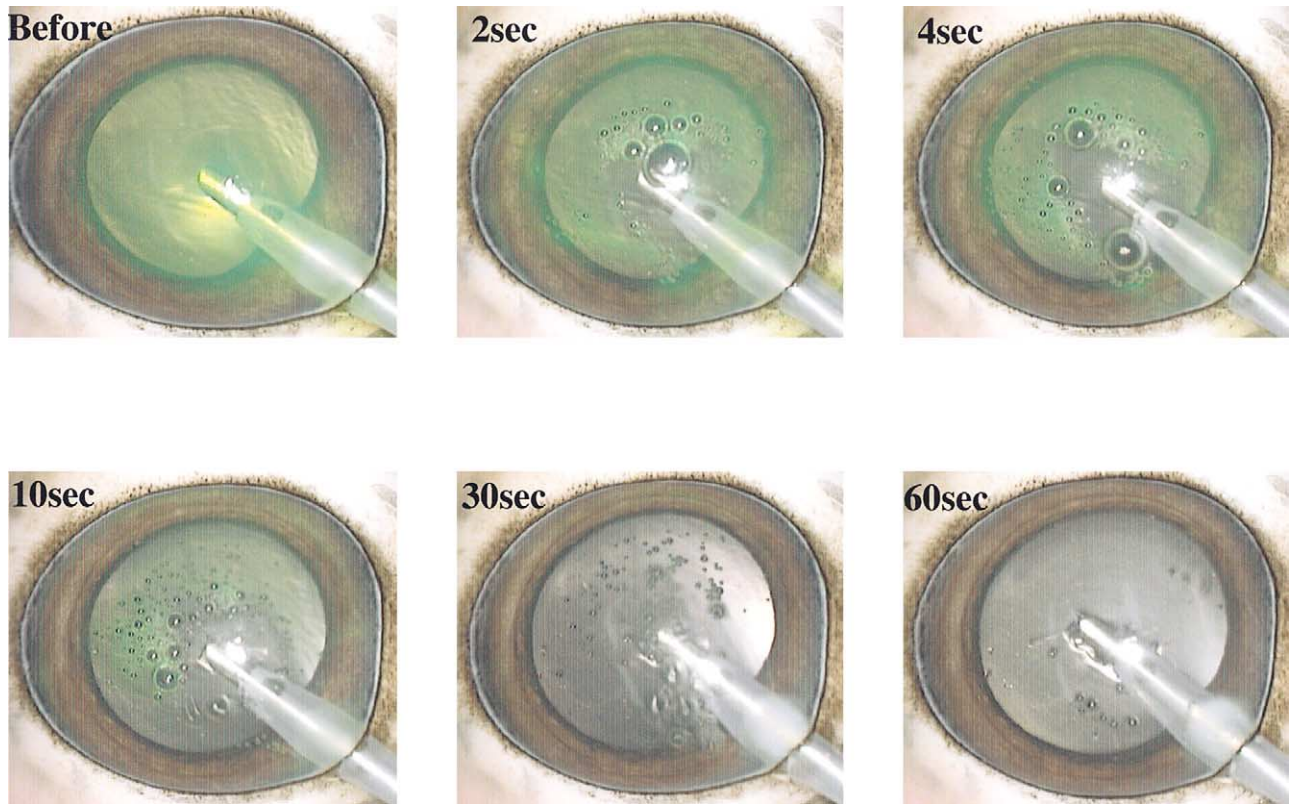
Figure 5. Images of anterior chamber in sodium hyaluronate (Na-HA) (520×10^3) group. Yellow-green fluorescence in anterior chamber derives from Na-HA (520×10^3) containing Na-fluorescein. No fluorescence is observed in anterior chamber at and after 30 seconds.
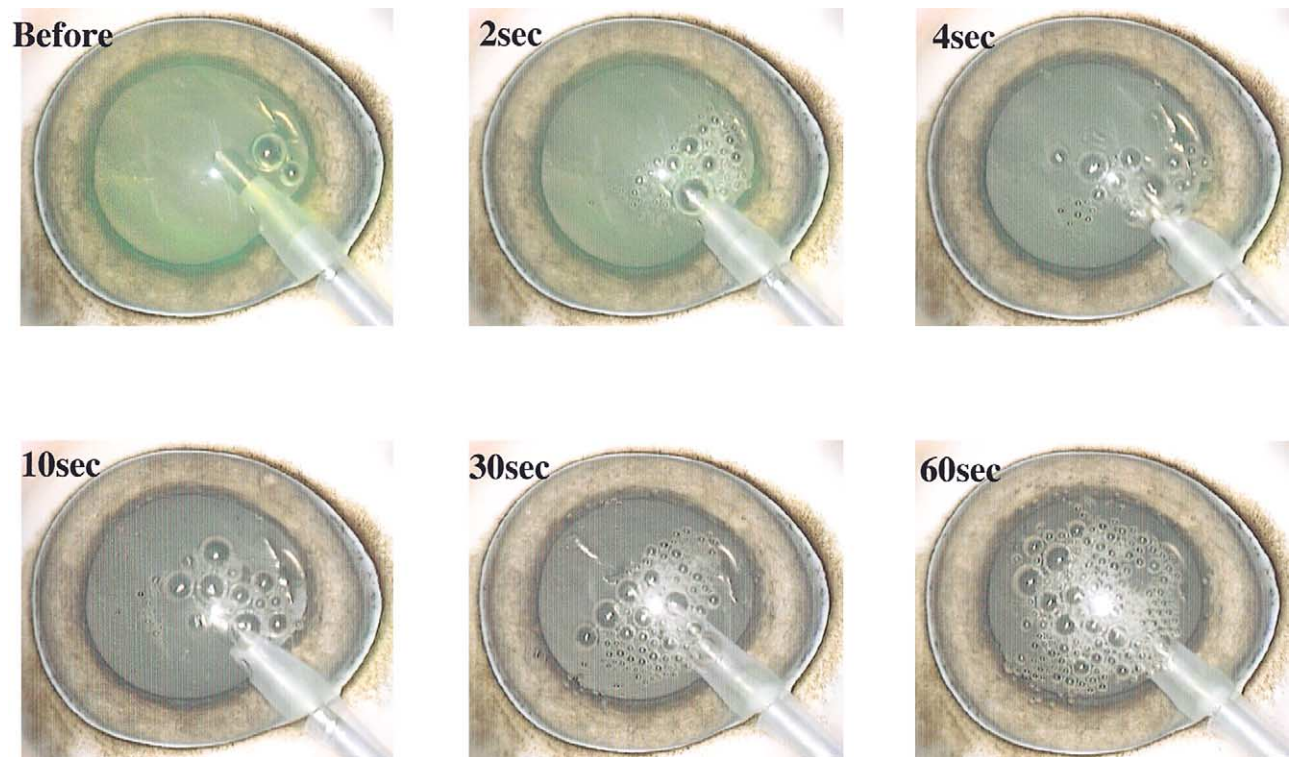
Figure 6. Images of anterior chamber in the sodium hyaluronate (Na-HA) (290×10^3) group. Yellow-green fluorescence in anterior chamber derives from Na-HA (290×10^3) containing Na-fluorescein. No fluorescence is observed in anterior chamber at and after 4 seconds.
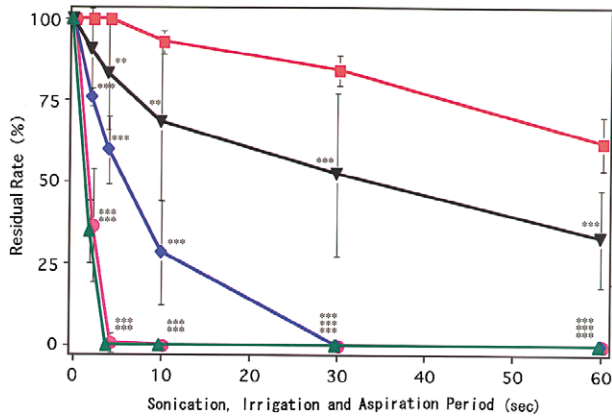
Figure 7. Residual rate of sodium hyaluronate (Na-HA) in anterior chamber after 60-second procedures. Residual amount of Na-HA at each time interval after start of sonication, irrigation, and aspiration is expressed as residual rate (percentage) for value immediately before start of these procedures. Values are represented by mean ± standard deviation for 8 eyes. ▲ (green): 2420×10^3 group, ▼(black): 1460×10^3 group, ■ (red): 1100×10^3 group, ◆ (blue): 520×10^3 group, ● (red): 290×10^3 group. **$P < .01$, ***$P < .001$ (Tukey multiple comparison test, Na-HA (1100×10^3) vs. other Na-HA groups). Residual rates in Na-HA (1100×10^3) group at 4, 10, 30 and 60 seconds were significantly higher than in other Na-HA groups. Furthermore, even at 2 seconds, residual rate was significantly higher in Na-HA (1100×10^3) group than in other Na-HA groups excepting Na-HA (1460×10^3) group.

stantly evacuated from the anterior chamber immediately after the start of sonication, irrigation, and aspiration (Figures 2 and 7); this was comparable to what has been reported previously.[2–5] Because the molecular network of Na-HA (2420×10^3) is completely entangled at a concentration of 1%,[6] Na-HA (2420×10^3) behaved as a mass of solid gel against the irrigation and aspiration. The condition in the anterior chamber was almost the same as that in the control group after elimination of Na-HA. Therefore, the percentage of damaged area in the Na-HA (2420×10^3) group was the same as that found in the control group (Figures 8 and 9).

Na-HA (1460×10^3), Na-HA (1100×10^3), Na-HA (520×10^3), and Na-HA (290×10^3) are classified as dispersive viscoelastic materials. The Na-HA (520×10^3) was eliminated from the anterior chamber within 30 seconds after the start of procedures, and Na-HA (290×10^3), within 4 seconds (Figures 5–7). The percentage of damaged area in these groups was similar to that in the control group (Figures 8 and 9). The molecular entanglement of the 1% Na-HA (520×10^3) solution was weak, and that

of the 1% Na-HA (290×10^3) solution was almost negligible.[6] Because there are no great differences in rheological properties between these solutions and the irrigating solution, these materials were eliminated from the anterior chamber at an early stage after the start of irrigation and aspiration. This explains why they exhibited no protective efficacy on the endothelium.

Na-HA (1100×10^3) was observed to remain in the anterior chamber, adhering almost uniformly to the corneal endothelium during sonication, irrigation, and aspiration (Figure 4). The residual rate at 60 seconds was approximately 60% (Figure 7).The percentage of damaged corneal endothelial area was significantly lower in this group than in the control group (Figure 9). Although the molecular entanglement of 1% Na-HA (1100×10^3) is incomplete, an appropriate interaction exists among the molecules of Na-HA.[6] Thus, 1% Na-HA (1100×10^3) shows properties between those of solid and liquid, and therefore, the Na-HA was gradually eliminated from the anterior chamber as it mixed with the irrigating solution. It is assumed that the relatively uniform layer of Na-HA (1100×10^3) adhering to the corneal endothelium may have acted as a barrier against any damage caused by sonication, irrigation, and aspiration.

In comparison, Na-HA (1460×10^3) close to the phaco-needle was eliminated within 4 seconds, and the distribution of residual Na-HA (1460×10^3) was found to be uneven at and after 4 seconds (Figure 3). Because there were no differences in the area of corneal endothelial damage between this group and the control group (Figure 9) in spite of its residual rate being approximately 33% at 60 seconds (Figure 7), in order to protect the corneal endothelium from damage induced by sonication, irrigation, and aspiration, it seems that Na-HA has to remain in the anterior chamber uniformly adhering to the corneal endothelium.

The results obtained in this study suggest that an optimal molecular weight exists for dispersive Na-HA employed to protect intraocular tissues during PEA. A dispersive Na-HA in which there is almost no molecular entanglement would not be expected to exhibit protective capability during PEA. It is important to employ a dispersive Na-HA displaying satisfactory "residual capability" during PEA. Under the conditions of this study, a 1% solution of Na-HA with a molecular weight of approximately 1100×10^3 exhibited the highest residual and protective capability. In addition, based on our observation of images obtained during sonication, irrigation, and aspiration, in order to protect the corneal endothe-
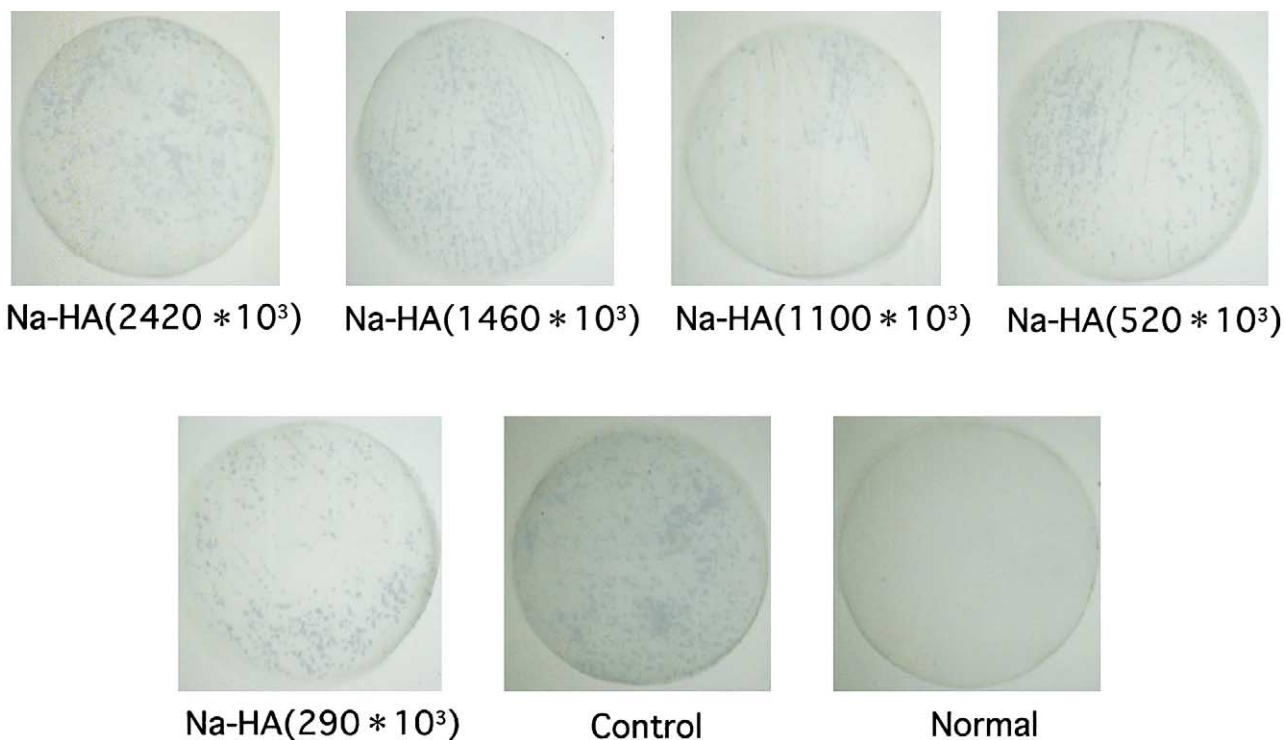
Figure 8. Photographs of corneal endothelium specimens after sonication, irrigation, and aspiration for 60 seconds (magnification at time of photography: ×1). Damaged regions of corneal endothelium are stained by trypan-blue. Damaged region stained is clearly smaller in sodium hyaluronate (Na-HA) (1100×10^3) group than in other groups.
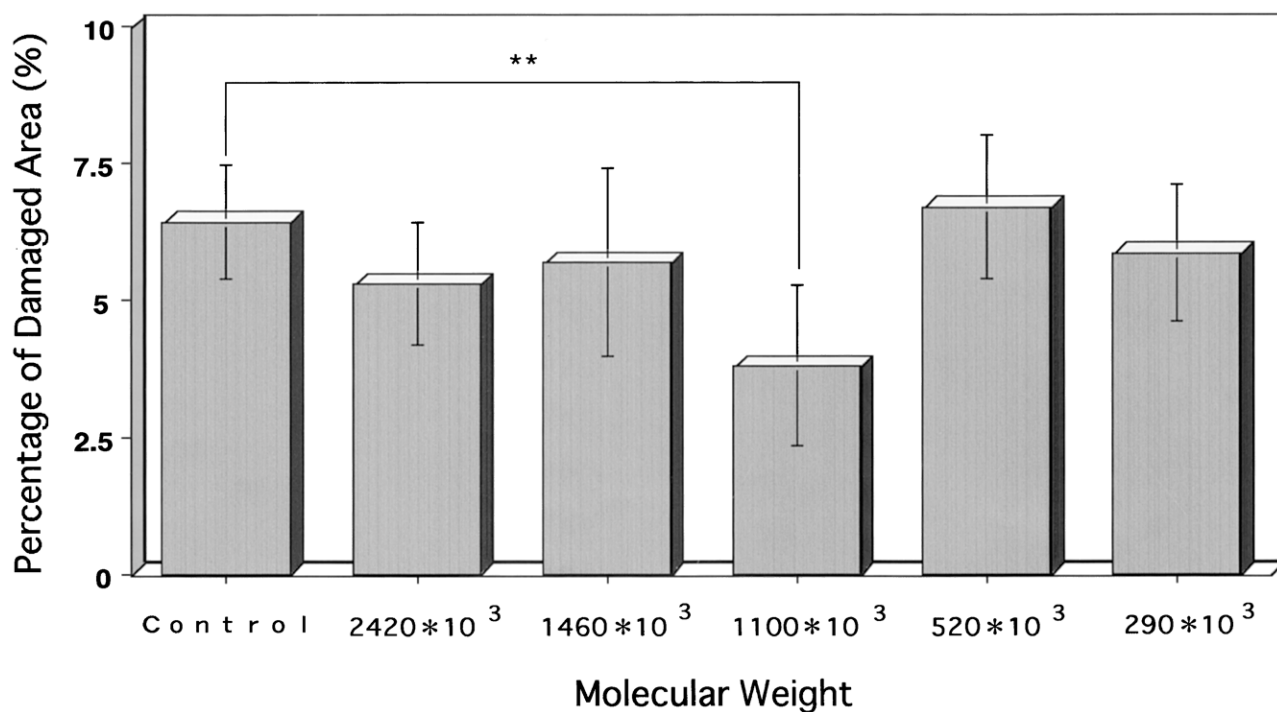
Figure 9. Area of damaged corneal endothelium after sonication, irrigation, and aspiration for 60 seconds. Damaged areas are expressed as percentage of whole corneal endothelium; that is, percentage of damaged area is calculated. Values are represented by mean ± standard deviation for 8 eyes. **$P < .01$ (Tukey multiple comparison test, each sodium hyaluronate (Na-HA) group vs. control group). Only in Na-HA (1100×10^3) group, percentage of damaged area was significantly smaller than that in control group.

lium effectively from damage, it seems important for Na-HA to remain in the anterior chamber, uniformly adhering to the corneal endothelium. Regarding the application of dispersive viscoelastic materials to protect the intraocular tissues, "residual capability in the anterior chamber with uniform adhesion to the tissues" will be a key factor.

This study was conducted under certain limitations: that is, isolated pig eyes were used; lens emulsification and aspiration were not conducted; the phaco-needle of the PEA equipment was fixed at the center of the anterior chamber; the position of aspiration port was not uniform; and the level of ultrasonic output was set at 100% higher than that used in clinical practice. However, it is considered suitable to apply appropriate dispersive viscoelastic materials in order to protect the anterior intraocular tissues from damaging factors produced during PEA such as ultrasound, radiation stream, heat shock, free radicals, cavitation, or pieces of emulsified lens nucleus.[9–11] When a 1% Na-HA solution is used as a dispersive viscoelastic material, Na-HA with a molecular weight of approximately 1100×10^3 is recommended, as described above.

Our objective in conducting this study is not to persuade the ophthalmic surgeon to use a specific viscoelastic material during PEA, but to report on the most effective use of the viscoelastic materials available on the market depending on the properties of each material. We hope the results of this study will provide the ophthalmic surgeon involved in cataract/intraocular lens implantation surgery with the information required to minimize damage to the intraocular tissues, particularly in the corneal endothelium, during PEA.

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