최정열 석사 학위논문

초음파유화술시 각막내피세포 보호를 위한 온도감응성 고분자 물질(폴록사머 407)의 적용 : 돼지 및 토끼안에서의 실험적 연구

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논 문 초 록

목적 : 초음파수정체 유화술시 각막내피세포 보호를 위하여 온도감응성 고분자 물질 (폴록사머407)을 돼지안과 토끼안에서 기존의 히알루론산 기반의 점탄물질을 대용으로 적용가능한지 평가하고자 하였다.

방법 : 형광물질을 염색한 여러 농도의 폴록사머 하이드로겔 [(20, 22, 24, 26% (w/w%)], 응집성 점탄물질 (1% 히알루론산 나트륨) 그리고 분산성 점탄물질 (3% 히알루론산 나트륨 -4% 콘드로이틴 황산)을 32도에 보관된 돼지눈의 전방내에 주입하였다. 돼지눈 45안을 동등하게 각 15안씩 3군으로 나눠 돼지눈에서의 연속적 초음파 유화술을 시행하며 전방내 유지시간을 측정하였다. 체내실험으로 토끼 12마리 12안을 26% 폴록사머군과 분산성 점탄물질 두 군으로 나눠 간헐적 초음파 유화술을 시행하였고, 술 전 및 술 후 3일차 각막내피세포밀도를 측정하였다.

결과 : 전방내에서 21도 관류용액으로도 겔-졸 전이가 일어나지 않는 적절한 농도의 폴록사머 하이드로겔은 26%였다. 돼지눈에서의 전방내 평균 유지시간은 다음과 같았다. 응집성 점탄물질 : 5.53±1.77, 분산성 점탄물질 125.00±29.34, 26% 폴록사머 221.53±42.48초 ($p<0.001$). 토끼안에서의 체내실험은 26% 폴록사머군에서의 내피세포밀도 감소는 5.27%, 분산성 점탄물질군은 18.27%였다 ($p=0.029$).

결론 : 온도감응성 고분자물질인 폴록사머 하이드로겔은 초음파 유화술 시 각막내피세포 보호를 위한 기존의 히알루로산 기반의 점탄물질을 대체할 수 있는 물질로 이용될 수 있다.

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주요어 : 온도감응성 고분자물질, 폴록사머, 각막내피세포, 초음파유화술, 점탄물질,

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목 차

INTRODUCTION

Although phacoemulsification is currently the most common technique used in cataract surgery, it is still associated with the risk of permanent damage to the corneal endothelium in the hard nucleus. Corneal decompensation from excessive endothelial cell loss after cataract surgery is a common complication encountered by ophthalmic surgeons[1,2]. Therefore, substantial efforts have been made, through advancements in techniques and development of ophthalmic viscosurgical devices (OVDs), to minimize corneal endothelial cell damage associated with cataract surgery.

During phacoemulsification, OVDs protect the corneal endothelium by preventing direct contact between the corneal endothelium and the nucleus, surgical instruments, and ultrasound-generated heat energy. Additionally, hyaluronic acid protects the corneal endothelium by binding to specific endothelial cells and coating the inner surface of corneal endothelial cells[3,4]. Over time, various hyaluronic acid-based OVD components have been developed by including various mixtures of sodium hyaluronate with chondroitin sulfate and varying concentrations of sodium hyaluronate[5]. A recent head-to-head comparison of different OVDs and mixed treatment options revealed that the use of viscoadaptive OVDs, the soft shell technique[6], resulted in superior outcomes in terms of corneal endothelial protection[7]. However, the differences between the OVDs in terms of absolute loss of endothelial cell density were <100 cells/mm^2. When performing cataract surgery in cases with an extremely hard nucleus, the use of a large amount of ultrasonic energy is unavoidable. Moreover, in such cases, viscoadaptive OVDs and use of the soft shell technique[6] cannot completely protect against corneal endothelial damage because OVDs

are aspirated with phacoemulsification. Currently, repeated injection of hyaluronic acid-based OVD is required during phacoemulsification in cases with hard cataract to maintain corneal endothelial protection. The introduction of femtosecond laser cataract surgery has enabled surgeons to reduce the required amount of ultrasound energy. However, the clinical significance of the extent of endothelial cell loss compared to that with conventional phacoemulsification remains debatable[8]. We believe that this issue cannot be adequately addressed by current hyaluronic acid-based OVDs. Therefore, new interventions that can persist longer and mechanically protect better from free radicals and nucleus particles than the current hyaluronic acid-based OVDs during phacoemulsification are required. Retention time of OVDs in the anterior chamber is closely associated with the anti-free radical effect on the corneal endothelium. The effect of OVDs on free radicals depends on the retention of the materials within the anterior chamber[9].

In a previous study, we obtained promising results by inserting a senofilcon A mechanical protector under the corneal endothelium to protect the corneal endothelium during phacoemusification[10]. However, we experienced difficulty in maintaining the stability of the senofilcon A mechanical protector under high vacuum and high flow rate. Therefore, we hypothesized that the application of a semisolid thermoreversible (poloxamer) hydrogel as a protective shell under the corneal endothelium would offer superior performance compared with that of a mechanical protector. We have termed this method of poloxamer hydrogel application as the poloxamer shell technique, with the poloxamer shell acting as a mechanical barrier and protecting the corneal endothelium from free radicals, heat, and ultrasound energy generated by the phacoemulsification probe. Poloxamer block

copolymers comprise ethylene oxide (EO) and propylene oxide (PO) blocks arranged in a triblock structure: EOx-POy-EOx. All poloxamers have similar chemical structures, but with different molecular weights and different values of x and y in each block. Poloxamer 407, one of the most commonly used poloxamers, is a nontoxic copolymer with an average molecular weight of 11,500. It is a white powder that contains 70% hydrophilic EO units and 30% hydrophobic PO units[11]. An aqueous poloxamer solution has thermoreversible properties. This thermogelling phenomenon is reversible, transitioning from sol to gel phase according to the temperature, and can be modified by adjusting the concentration of the poloxamer solution[12]. A solution of the poloxamer hydrogel is a clear liquid at room temperature, but when warmed to body temperature, it undergoes gel-to-sol transition to yield a solid gel form[11,13]. Because of these thermal gelation and nontoxic properties, the poloxamer hydrogel provides a convenient and efficient means for forming a physical barrier during surgery. Therefore, commercial products containing poloxamer hydrogels are used as anti-adhesive and contracture preventive agents in abdominal[14] and plastic surgeries[15]. Ophthalmic usage was tested to increase the residence time on the corneal surface to minimize washout by tears[16,17]. In a previous study, an injectable intraocular lens refilling formulation with 25% poloxamer hydrogel produced no inflammatory response or toxicity in rabbit eyes[18].

This experimental study aimed to evaluate the applicability of the poloxamer hydrogel as a substitute for OVDs during phacoemulsification and to determine the optimal concentrations of the poloxamer hydrogel at room temperature. To accomplish this, we performed both in vitro and in vivo experiments. In the in vitro

testing of porcine eyes, we measured the retention time to evaluate the adherence of the hydrogel and evaluated the behavior of poloxamer hydrogels in the anterior chamber during phacoemulsification. In the in vivo testing, we used a rabbit model to evaluate the protective effects of thermosensitive hydrogels during phacoemulsification.

MATERIALS AND METHODS

Preparation of the poloxamer gel

Poloxamer 407 (P407, Sigma-Aldrich Co.) hydrogel was prepared using the cold method.19 Briefly, the poloxamer powder was added to water at 4–5°C with continuous magnetic stirring until a homogenous solution was formed. A series of dilutions ranging from 18% to 26% P407, with 2% intervals, was prepared in a balanced salt solution (BSS). A sterile formulation of thermosensitive hydrogels of different concentrations (percentage determined as weight of P407/weight of diluent × 100) was created. To aid in the visualization of the gel in the anterior chamber, we stained the P407 with 10% fluorescein sodium.

Temperature measurement

To simulate the actual surgical settings, we used a smartphone-based forward-looking infrared (FLIR) camera to measure the ocular temperature in the operating room during cataract surgery. Thermal imaging cameras are commercially available as smartphone attachment devices that provide thermal imaging analysis in a noninvasive manner. Thermographs of the eyeball and BSS were obtained using the FLIR ONE Personal Vision System for iOS (FLIR Systems, Inc.) after the eye was opened with a speculum under the

surgical drape. Thermal readings were obtained after the automatic calibration of the device.

Part I : An in vitro porcine study

Forty-five porcine eyes, which were obtained from an abattoir within 6 h after the animals were sacrificed, were divided equally between the following groups: the cohesive OVD (sodium hyaluronate 1% [Provisc]) group, dispersive OVD (sodium hyaluronate 3%-chondroitin sulfate 4% [Viscoat]) group, and poloxamer hydrogel group. Before beginning the experiments, the porcine eyes were incubated in a 32°C water bath filled with BSS to simulate the temperature in the anterior chamber during actual cataract surgery. The eyes were mounted, and the surgical procedures were performed under an operating microscope, similar to the procedure performed during human cataract surgery. A 1.2-mm side-port incision was made with a slit knife, and 0.45 mL of the OVD or poloxamer was fully injected into the anterior chamber. The main clear corneal incision was made with a 3.00-mm beveled knife. We subsequently evaluated the ability of the different concentrations of poloxamer hydrogels to undergo gelation and to form poloxamer shells in the anterior chamber at approximately 30 - 32°C. Phacoemulsification was performed using a Sovereign Compact Phacoemulsification System (Abbott Medical Optics, Inc.). The phacoemulsification tip was inserted in the center of the anterior chamber of all eyes, and the bevel was positioned upward to the corneal endothelium. The phacoemulsification power was set to 35%, and the rates of aspiration and vacuum were maintained at 30 cc/min and 300 mmHg, respectively. While continuing phacoemulsification at the central cornea without crystalline lens removal, we measured the retention time of the

fluorescein-stained OVD and poloxamer hydrogel in the anterior chamber with an operating microscope and side view camera (Figure 1).

Measurement of retention time

The retention times of the OVDs and poloxamer hydrogel were recorded with a surgical microscopic view, and a side view camera was used to record the flow in the anterior chamber (Figure 1). In instances where it was difficult to determine the presence of dispersive OVD in the anterior chamber via the vertical microscopic view, we measured retention time using the side view camera. The retention time was defined as the interval between the initiation of OVD aspiration through the phacoemulsification tip and the time of completion of aspiration through the phacoemulsification tip, as determined via both the vertical microscopic view and the side view camera in the anterior chamber[20]. Additionally, the completion of retention in the anterior chamber was also considered when the OVD or poloxamer hydrogel was removed within the central 8.0-mm cornea and remained at the angle or far periphery without further aspiration despite the continued phacoemulsification at center.

Part II : In vivo rabbit study

Animals

Rabbits were obtained from a vendor (KOATECH CO., Ltd) that was internationally certified by the Association for Assessment and Accreditation of Laboratory Animal Care. The rabbits were handled according to the guidelines of the Association for Research in Vision and Ophthalmology Statement for Use of Animals in Ophthalmic and Vision Research. The study protocol was approved by the

Institutional Animal Care and Use Committee (IACUC) of the Seoul Metropolitan Government-Seoul National University Boramae Medical Center (IACUC # 2017-0019) and followed the guidelines of animal ethics. The room was maintained at 20.5 - 22°C. The rabbits were treated according to the preoperative and postoperative management processes used in our previous study.10 For this study, we used 12 eyes of 12 New Zealand white rabbits weighing 2.7 - 3.0 kg and aged 18 - 20weeks.

Comparison of endothelial cell changes

The preoperative and third postoperative central corneal endothelial cell counts (ECCs) were measured using a noncontact autofocus specular microscope (EM-4000, Tomey Corp.). The 12 rabbit eyes were divided equally into two groups: the dispersive OVD (sodium hyaluronate 3%-chondroitin sulfate 4% [Viscoat]) group and the 26% poloxamer group (n = 6 per group). After making the 1.2-mm side port incision, we completely filled the anterior chamber with dispersive OVD or 26% poloxamer hydrogel without fluorescein staining. The phacoemulsification power was set to 80%, and the rates of aspiration and vacuum were controlled at 10 mL/min and 10 mmHg, respectively, to minimize the washing out of the OVD or poloxamer hydrogel and to maximize the ultrasound energy exposure during phacoemulsification. The phacoemulsification tip was inserted through the 2.75-mm main incision. The position of the phacoemulsification tip was maintained in the center of the anterior chamber, with the bevel-up toward the corneal endothelium, and 10-s intermittent phacoemulsification was activated for 5 min (total elapsed ultrasound exposure of 2.5 min). After phacoemulsification, 0.15 mL of cold BSS (15°C) was irrigated through the main incision to remove the OVD or poloxamer hydrogel remaining in the anterior chamber.

The surgical procedure for each animal was performed by the same senior cataract surgeon (Y.K.H.), and the examination was performed in a blinded and randomized manner by the same ophthalmologist (J.Y.C.). The animals were checked four times a day for signs of infection or inflammation with a portable slit lamp, and neomycin sulfate - polymyxin B - dexamethasone 0.1% was instilled at every check.

Statistical analyses

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 19.0 (SPSS, Inc., Chicago, IL, USA). Normality was checked using the KolmDIAG2020014064ogorov - Smirnov test, and retention times for the different OVDs and poloxamer hydrogels were compared using the Kruskal - Wallis test. Differences were considered statistically significant according to the Bonferroni-corrected significance level, with a P-value <0.017 indicating a significant difference. The nonparametric Mann - Whitney U test was used to compare the changes in parameters between the groups within each study. The level of statistical significance was defined as a P-value <0.05.

RESULTS

Ocular temperature under the surgical field

Figure 2 shows the temperature of the corneal surface, as measured by an infrared thermal imaging camera during surgery. The operating theater was maintained at a temperature of 21°C, and the BSS used in the operating theater was maintained at 21.1°C (Figure 2A). The temperature of the cornea after the surgical drape and opening with

the speculum was 31.8°C (Figure 2B). At the end of cataract surgery, after the stromal hydration of the main incision site, the ocular temperature was 27.1°C (Figure 2C).

In vitro study

Determination of the optimal poloxamer hydrogel concentration

The poloxamer hydrogel samples, with concentrations of 18% - 22%, had low elasticity and formed viscous solutions at a temperature of 21°C (Figure 3A). The 24% poloxamer hydrogel exists in a viscoelastic state between the solution and gel. The 26% poloxamer hydrogel was in gel form at the operating room temperature of 21°C (Figure 3B). The gelation of the poloxamer hydrogel did not occur immediately in the anterior chamber below a concentration of 24%. The concentration of <26% poloxamer hydrogels was immediately dissolved in contact with a BSS (Figure 4A - D). A semisolid form of poloxamer hydrogel that achieves complete gelation at 21°C is required to generate the poloxamer shell in the anterior chamber, and accordingly, the 26% concentration of poloxamer was optimal. The 24% and 26% poloxamer hydrogels could not be extruded using 25-gauge, bent, blunt-tip, thin-walled cannulas. A bent 24-gauge pinpoint needle was ideal for the injection of the poloxamer hydrogel into the anterior chamber through the 1.2-mm side port incision.

Retention time during phacoemulsification

The mean retention times were 5.53 ± 1.77s in the cohesive OVD group, 125.00 ± 29.34 s in the dispersive OVD group, and 221.53 ± 42.48 s in the 26% poloxamer hydrogel group (Figure 5) (Video 1, available at http://jcrsjournal.org), with significant differences among the three groups ($P<0.001$, analysis of variance). The Kruskal - Wallis

multiple comparison test revealed that the 26% poloxamer hydrogel group had longer retention time during phacoemulsification compared with the retention times for the cohesive OVD ($P<0.001$) and dispersive OVD groups ($P<0.001$). The poloxamer hydrogel left in the anterior chamber was easily removed by manual irrigation through an incision, with a BSS solution at 15°C (Video 1, available at http://jcrsjournal.org).

In vivo study

The differences between preoperative and 3-day postoperative ECCs are shown in Table 1. The dispersive OVD group showed a significantly greater decrease in ECC than the poloxamer shell group ($P = .029$, Mann-Whitney). Postoperative infection or toxic anterior segment syndrome was not detected, and anterior chamber inflammation was controlled by postoperative eye drops in both groups.

DISCUSSION

In this study, we applied a poloxamer hydrogel to form a dome-shaped shell under the corneal endothelium to test the hypothesis that the poloxamer shell would act as a mechanical protective barrier during phacoemulsification. To the best of our knowledge, this is the first study in which the poloxamer hydrogel was used as a possible material to protect the corneal endothelium. Prior to commencing this study, we considered ocular temperature during cataract surgery to be an important factor because the temperature of the surgical environment determines not only the phase of the thermosensitive poloxamer hydrogel but also its injectability. In this study, the ocular temperature measured by the

infrared thermal camera during cataract surgery was lower than the normal human body temperature. The lowest temperature calculated using a numeric model to study heat exchange inside the eye was approximately 34°C; the model accounted for the supine position and dynamics of aqueous humor[21]. Eom et al[22] measured ocular temperature during cataract surgery using a thermal imaging camera and reported that the ocular temperature, at 30.1°C, was lower than the normal human body temperature. The lower ocular temperature indicates that the application of thermosensitive hydrogel in intraocular surgery is more difficult than in other surgical fields that use body temperature for gelation. Different from the use of poloxamer in non-ophthalmic surgical fields, the feasibility of the poloxamer hydrogel for ophthalmic applications has been debatable[23]. One of the main obstacles for the ophthalmic use of poloxamer hydrogel is that tears on the ocular surface can dilute the poloxamer hydrogel. In the present study, we selected the liquid form of the poloxamer hydrogel for injection into the anterior chamber, and the injected liquid hydrogel was expected to form a gel at body temperature. However, dilution by the aqueous humor in the anterior chamber represented a major obstacle for the adequate delivery of the liquid poloxamer hydrogel. In our preliminary test using porcine eyes, rapid and prompt gelation in the anterior chamber could not be achieved after the injection of liquid or viscous poloxamer hydrogel at a concentration of 24% and temperature of 21°C. Considering this dilution effect and the low ocular temperature, phase transition of the poloxamer hydrogel in the anterior chamber requires a relatively longer time than it does when it is used in other surgical applications at body temperature[23]. Additionally, during the phase transition in the anterior chamber, dilution with aqueous humor results in an increased

sol-to-gel transition temperature. An increase in poloxamer concentration is associated with a decrease in the phase transition temperature, but an increase in the viscosity of the poloxamer hydrogel[12,24,25]. The OVDs must have low viscosity so that they can be injected into the eye through fine-bore cannulas[26]. Although most of the poloxamer hydrogels exhibit shear-thinning behavior (pseudoplasticity), when the poloxamer hydrogel reaches complete gelation, a large amount of force has to be applied to extrude the solutions out of the small-caliber syringes[27]. In this study, we were unable to inject the 26% poloxamer hydrogel into the anterior chamber through the 25-gauge bent needle. A rheologic study of poloxamer 407 revealed that it was less pseudoplastic than hyaluronic acid-based OVDs[26,28]. Higher concentration of poloxamer hydrogel exhibits less pseudoplastic and decreased viscoelastic behavior[26-28]. Pseudoplasticity is important characteristics of OVD because various shear thinning steps are present during cataract surgery. However, the use of 24-gauge pin point needles with a 1-mL syringe enabled easy injection of the 26% poloxamer hydrogel into the anterior chamber.

OVDs can perform a protective function by coating the ocular structure, specifically the corneal endothelium[29]. Previous studies have measured retention time and adhesiveness as indirect methods of evaluating corneal endothelial protection and behavior of OVD[30,31]. The large area coated by OVDs and their prolonged retention in the anterior chamber further protect the corneal endothelium during cataract surgery by minimizing the interaction between the ocular tissue and surgical instruments. In vitro study have revealed that dispersive OVDs are retained longer than cohesive OVDs[31]. In the present study, we used actual surgical phacoemulsification parameters;

however, phacoemulsification was performed under the central cornea without movement or lens removal to observe the behavior of the poloxamer hydrogel.

The equal exposure to phacoemulsification used in the present study could minimize the effect caused by variations in irrigation and ultrasound exposure compared with that in the previous study[30]. Among the 15 eyes in the poloxamer group, the poloxamer shell was completely emulsified within 3 min in only one specimen, and the other poloxamer shells persisted for at least 3 min of phacoemulsification compared with the eyes in the dispersive OVD group, which exhibited complete aspiration within 2 min. We expected the poloxamer shell to stay in place as a semisolid gel without fracturing or aspiration. However, contrary to our expectations, the poloxamer hydrogel was significantly slowly fragmented and emulsified around the phacoemulsification tip (Figure 6A and 6B). Our observations regarding the behavior of poloxamer hydrogels during phacoemulsification are important because combining the dispersive OVD and poloxamer shell technique would allow the emulsified area of the poloxamer shell to be subsequently covered by a dispersive OVD. Moreover, similar to the soft shell technique[6], this combination technique could prolong the endothelial protective coverage by providing a second barrier. Additionally, poloxamer shells could function as OVD pockets that maintain the dispersive OVD in the potential space between the endothelium and the poloxamer shell. By performing retention testing, we investigated the possibility of a poloxamer hydrogel as a protective shell for the corneal endothelium against phacoemulsification.

Despite the small sample size, the results of our in vivo rabbit eye experiment showed that the poloxamer shell technique had a

protective effect against phacoemulsification insult to the corneal endothelium. The poloxamer hydrogel remained in the anterior chamber for 5 min of intermittent phacoemulsification (Figure 6C) (Video 2, available at http://jcrsjournal.org). Additionally, we found that the 26% poloxamer hydrogel conferred significantly better endothelial protection than the dispersive OVD (sodium hyaluronate 3%-chondroitin sulfate 4% [Viscoat]) did. The change in ECC in the poloxamer group was similar to that observed in our previous study[10] with a senofilcon A mechanical protector, which was associated with a postoperative ECC decrease of 4%.

Compared to senofilcon A mechanical protectors, the poloxamer hydrogel offers better intraocular stability, safety, and ease of manipulation for injection and removal. In our previous study[10], the use of senofilcon A mechanical protectors in the anterior chamber induced toxic anterior segment syndrome, where no toxicity was observed with the poloxamer hydrogel in the present study. Extended indwelling of the poloxamer hydrogel resulted in dissolution upon contact with aqueous humor, but ocular inflammation was not detected for 3 months in a previous study[18]. Additionally, the poloxamer hydrogel was easily injected using 24-gauge needles and removed rapidly by irrigating with 1.5-mL cold (15°C) BSS through the main incision.

In the present study, the poloxamer shell technique resulted in promising outcomes in terms of protection of the corneal endothelium relative to the outcomes of cohesive OVDs; however, the study has some limitations. The small sample size limits the drawing of definitive conclusions from the findings. Furthermore, because this preliminary study was designed to investigate the feasibility of poloxamer hydrogel as an OVD substitute for the protection of the

corneal endothelium, we did not compare the poloxamer shell technique with other types of OVDs (other than dispersive OVDs) in rabbit eyes. As we removed the remaining poloxamer hydrogels with cold BSS irrigation at the end of surgery, we did not evaluate its safety issue, specifically its effects on intraocular pressure or the toxic effects of indwelling in the anterior chamber. Moreover, this study was evaluated with limited clinical specular microscopy indicating corneal endothelial cell damage. Thus, further indices by measuring central corneal thickness and histopathologic examination are required. Further large-scale studies comparing poloxamer hydrogels with different types of OVD are required in the future.

In conclusion, the results of the present study indicate that the poloxamer hydrogel is a feasible substitute for OVDs in terms of corneal endothelial protection during phacoemulsification. The use of the novel poloxamer shell technique described herein provides a new approach and a surgical device worthy of further study and modifications.

WHAT WAS KNOWN

● The ophthalmic viscosurgical device (OVD) and soft shell technique cannot completely protect against the damage to the corneal endothelium in the extremely hard nucleus.

● The poloxamer hydrogel possesses the thermoreversible property of phase transitioning from sol to gel at body temperature and its gelling temperature is related to the concentration of the poloxamer hydrogel.

WHAT THIS PAPER ADDS

● Poloxamer hydrogel showed excellent retention and adherence in

the anterior chamber during phacoemulsification compared with cohesive and dispersive OVDs.

● The poloxamer shell technique using thermosensitive hydrogel protects against corneal endothelial cell damage during phacoemulsification in rabbit eyes.

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Figure Legends

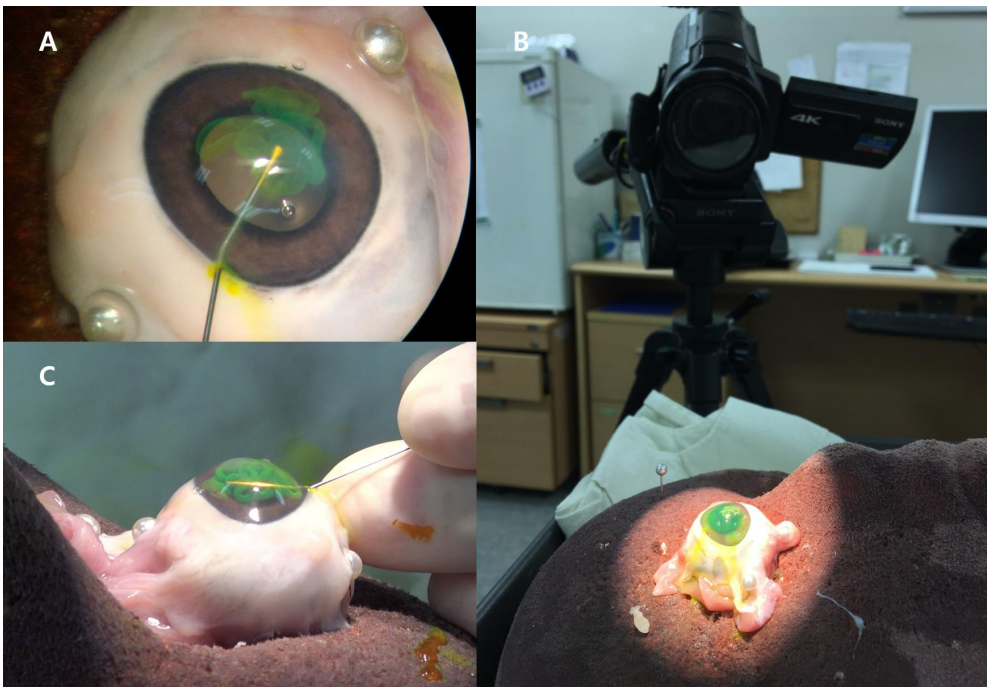

Figure 1. Retention time measurement using a surgical microscopic view and a side view camera.

(A) Surgical microscopic view of the injection of a fluorescein-stained cohesive ophthalmic viscosurgical device (OVD) into a porcine eye.

(B) A side view camera was used to observe the behavior of OVDs and poloxamer hydrogels in the anterior chamber.

(C) Side camera view of injection of a fluorescein-stained cohesive OVD into a porcine eye.

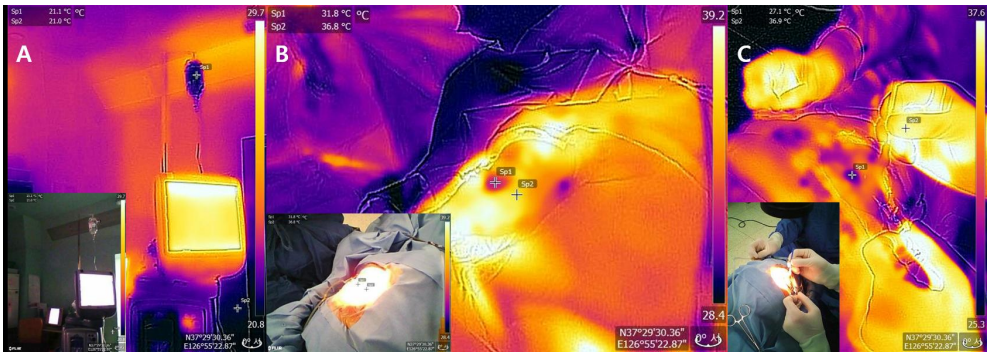

Figure 2. Temperature measured using a forward-looking infrared camera.

(A) Temperature of the balanced salt solution at the beginning of the surgery at a room temperature of 21°C. Sp1 indicates the 21.1°C temperature of the irrigation solution, and Sp2 indicates the 21°C room temperature.

(B) Ocular thermograph at the beginning of cataract surgery. Sp1 indicates the 31.8°C temperature of the eyeball at a room temperature of 21°C. Sp2 indicates a body temperature of 36.8°C.

(C) Ocular thermograph at the end of the cataract surgery, obtained after stromal hydration. Sp1 indicates the 27.1°C temperature of the eyeball. Sp2 indicates the 36.9°C temperature.

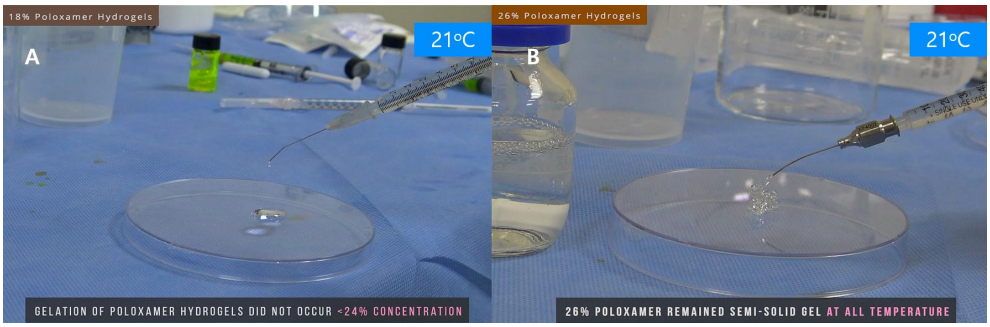

Figure 3. Different concentrations of poloxamer hydrogels at 21°C temperature.

(A)18% poloxamer hydrogels were viscous solutions at 21°C operating room temperature and had low elasticity.

(B) The 26% poloxamer hydrogels were semisolid gels at 21°C operating room temperature, and a bent 24-gauge pinpoint needle was ideal for the injection of the poloxamer hydrogel.

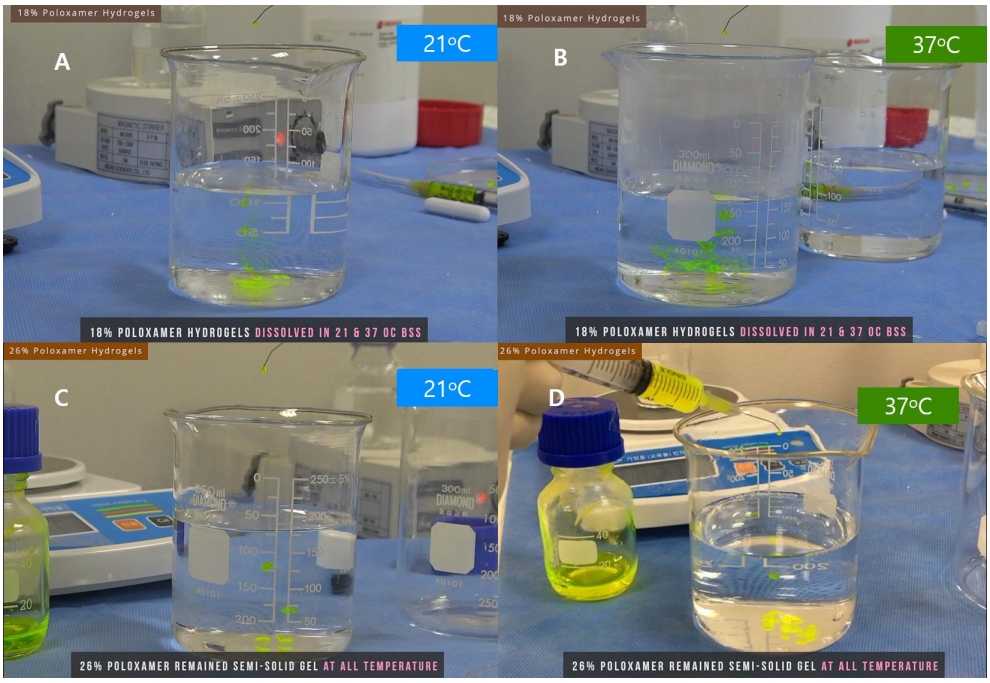

Figure 4. Behavior of 18% and 26% poloxamer hydrogels in contact with different temperatures of balanced salt solution.

(A, B) 18% poloxamer hydrogels were dissolved at all temperatures on contact with 21°C and 37°C balanced salt solution.

(C, D) 26% poloxamer hydrogel remains semisolid gel at all temperatures without dissolution on contact with 21°C and 37°C balanced salt solution.

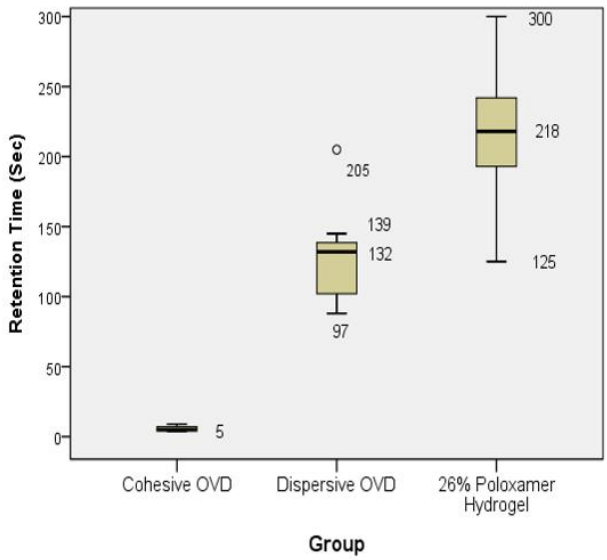

Figure 5. Retention time until the ophthalmic viscosurgical devices (OVDs) or the 26% poloxamer hydrogel was removed by phacoemulsification of the porcine eyes. The mean retention time ± standard deviation were 5.53 ± 1.77 s in the cohesive OVD group, 125.00 ± 29.36 s in the dispersive OVD group, and 221.53 ± 42.48 s in the 26% poloxamer hydrogel group. The Bonferroni multiple comparison results indicated significant differences in retention times between the 26% poloxamer and cohesive OVD (P<0.001), the cohesive and dispersive OVD (P<0.001), and the dispersive OVD and 26% poloxamer groups (P<0.001).

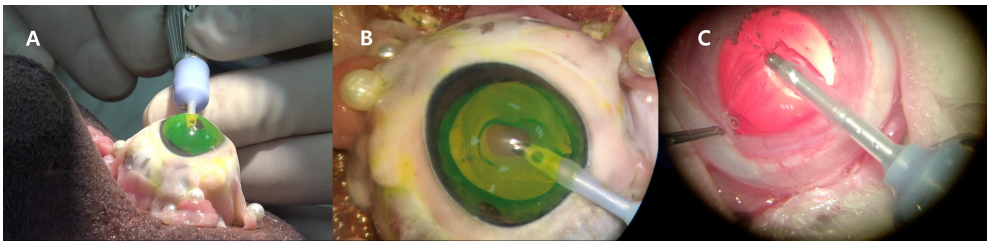

Figure 6. Poloxamer shell in the anterior chamber after phacoemulsification in porcine and rabbit eyes.
(A) Side camera view of 26% poloxamer hydrogels at 3 min of continuous phacoemulsification in a porcine eye shows that the poloxamer shell is maintained without aspiration. Only above the phacoemulsification tip is emulsified.
(B) Surgical microscopic view of 26% poloxamer hydrogels at 3 min of continuous phacoemulsification in a porcine eye shows that the poloxamer shell is maintained without aspiration. Only above the phacoemulsification tip is emulsified.
(C) In a rabbit eye, 26% poloxamer hydrogel remained in the anterior chamber throughout the 5-min intermittent phacoemulsification.

Table 1. Summary of preoperative and postoperative endothelial cell counts.

Group	Eyes	ECC (cells/mm^3)		Endothelial cell loss		
		Pre	Post	Decrease (cells/mm^3)	% change	*P* value
Dispersive OVD Group (n=6)	Rabbit 1	2707	2068	639	23.61	
	Rabbit 2	2466	2266	200	8.11	
	Rabbit 3	2890	2512	378	13.08	
	Rabbit 4	3278	2873	405	12.36	
	Rabbit 5	3048	2272	776	25.5	
	Rabbit 6	3086	2252	834	27.03	
	Mean ± SD	2912.5±291.2	2373.8±282.3	538.7±249.8	18.27	0.029
26% Poloxamer hydrogel Group (n=6)	Rabbit 7	2893	2876	17	0.59	
	Rabbit 8	2529	2428	101	3.99	
	Rabbit 9	2608	2480	128	4.91	
	Rabbit 10	2755	2601	154	5.59	
	Rabbit 11	2738	2731	7	0.26	
	Rabbit 12	2962	2480	128	16.3	
	Mean ± SD	2747.5±164	2599.3±174.2	148.2±173.9	5.27	

ECC = endothelial cell count; OVD = ophthalmic viscosurgical devices; SD = standard deviation.

Supplemental videos

Video 1 (available at http://jcrsjournal.org)

In vitro behavior of ophthalmic viscosurgical devices (OVDs) and 26% poloxamer hydrogels is described as follows: The measurement of retention time in the anterior chamber during phacoemulsification. Behavior of fluorescein-stained cohesive and dispersive OVDs, and 26% poloxamer hydrogel in porcine eyes. Removal of remaining poloxamer hydrogel using cold BSS through the main incision.

Video 2 (available at http://jcrsjournal.org)

In vivo behavior of 26% poloxamer hydrogels is shown in this video. Throughout the 5-min intermittent phacoemulsification, the anterior chamber stability was maintained under the poloxamer shell, and the poloxamer shell remained in place during the entire phacoemulsification period.

Application of thermoreversible hydrogel, poloxamer 407 for protection of the corneal endothelium during phacoemulsification: An experimental study in porcine and rabbit eyes

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Purpose : To evaluate the utility of thermoreversible (poloxamer) hydrogels as a substitute for ophthalmic viscosurgical devices (OVDs) during phacoemulsification, and to compare their endothelial protective effect with that of hyaluronic acid-based OVDs during phacoemulsification in porcine and rabbit eyes.
Methods : Fluorescein-stained poloxamer hydrogels (20,22,24, and 26% [weight/weight%]), and cohesive (sodium hyaluronate 1% [Provisc]) and dispersive (sodium hyaluronate 3.0%-chondroitin sulfate 4.0% [Viscoat] OVDs were injected into the anterior chamber of porcine eyes incubated at 32°C. In the in vitro study, the retention time was measured in 3 groups of 45 porcine eyes during continuous phacoemulsification. In the in vivo study, the endothelial cell count (ECC) was measured before and 3 days after intermittent phacoemulsification in 12 rabbit eyes randomized to a poloxamer hydrogel or a dispersive OVD group.
Results : The optimum concentration of thermosensitive hydrogel was 26%, at which no gel-to-sol phase transition occurred in the anterior chamber, with a 21°C irrigation solution. In the in vitro study, the mean retention times were 5.53 seconds ± 1.77, 125.00 ± 29.34 seconds, and 221.53 ± 42.48 seconds in the cohesive OVD, dispersive OVD, and 26% poloxamer hydrogel groups, respectively ($P < .001$). In the in vivo study, the mean decrease in ECC was significantly lower in the 26% poloxamer hydrogel group than in the dispersive OVD group ($P = .029$).
Conclusion : Thermoreversible hydrogels might be suitable substitutes for hyaluronic acid-based OVDs for corneal endothelial protection during phacoemulsification.

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keywords : Thermoreversible hydrogels, Poloxamer, corneal endothelium, phacoemulsification, ophthalmic viscosurgical device
***Student Number* : 2018-24065**

최정열 석사 학위논문

초음파유화술시 각막내피세포 보호를 위한 온도감응성 고분자 물질(폴록사머 407)의 적용 : 돼지 및 토끼안에서의 실험적 연구

2020 년 8월

서울대학교 대학원

의학과 안과학

최 정 열

논 문 초 록

목적 : 초음파수정체 유화술시 각막내피세포 보호를 위하여 온도감응성 고분자 물질 (폴록사머407)을 돼지안과 토끼안에서 기존의 히알루론산 기반의 점탄물질을 대용으로 적용가능한지 평가하고자 하였다.

방법 : 형광물질을 염색한 여러 농도의 폴록사머 하이드로겔 [(20, 22, 24, 26% (w/w%)], 응집성 점탄물질 (1% 히알루론산 나트륨) 그리고 분산성 점탄물질 (3% 히알루론산 나트륨 -4% 콘드로이틴 황산)을 32도에 보관된 돼지눈의 전방내에 주입하였다. 돼지눈 45안을 동등하게 각 15안씩 3군으로 나눠 돼지눈에서의 연속적 초음파 유화술을 시행하며 전방내 유지시간을 측정하였다. 체내실험으로 토끼 12마리 12안을 26% 폴록사머군과 분산성 점탄물질 두 군으로 나눠 간헐적 초음파 유화술을 시행하였고, 술 전 및 술 후 3일차 각막내피세포밀도를 측정하였다.

결과 : 전방내에서 21도 관류용액으로도 겔-졸 전이가 일어나지 않는 적절한 농도의 폴록사머 하이드로겔은 26%였다. 돼지눈에서의 전방내 평균 유지시간은 다음과 같았다. 응집성 점탄물질 : 5.53±1.77, 분산성 점탄물질 125.00±29.34, 26% 폴록사머 221.53±42.48초 ($p<0.001$). 토끼안에서의 체내실험은 26% 폴록사머군에서의 내피세포밀도 감소는 5.27%, 분산성 점탄물질군은 18.27%였다 ($p=0.029$).

결론 : 온도감응성 고분자물질인 폴록사머 하이드로겔은 초음파 유화술 시 각막내피세포 보호를 위한 기존의 히알루로산 기반의 점탄물질을 대체할 수 있는 물질로 이용될 수 있다.

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주요어 : 온도감응성 고분자물질, 폴록사머, 각막내피세포, 초음파유화술, 점탄물질,

학 번 : 2018-24065

목 차

INTRODUCTION

Although phacoemulsification is currently the most common technique used in cataract surgery, it is still associated with the risk of permanent damage to the corneal endothelium in the hard nucleus. Corneal decompensation from excessive endothelial cell loss after cataract surgery is a common complication encountered by ophthalmic surgeons[1,2]. Therefore, substantial efforts have been made, through advancements in techniques and development of ophthalmic viscosurgical devices (OVDs), to minimize corneal endothelial cell damage associated with cataract surgery.

During phacoemulsification, OVDs protect the corneal endothelium by preventing direct contact between the corneal endothelium and the nucleus, surgical instruments, and ultrasound-generated heat energy. Additionally, hyaluronic acid protects the corneal endothelium by binding to specific endothelial cells and coating the inner surface of corneal endothelial cells[3,4]. Over time, various hyaluronic acid-based OVD components have been developed by including various mixtures of sodium hyaluronate with chondroitin sulfate and varying concentrations of sodium hyaluronate[5]. A recent head-to-head comparison of different OVDs and mixed treatment options revealed that the use of viscoadaptive OVDs, the soft shell technique[6], resulted in superior outcomes in terms of corneal endothelial protection[7]. However, the differences between the OVDs in terms of absolute loss of endothelial cell density were <100 cells/mm^2. When performing cataract surgery in cases with an extremely hard nucleus, the use of a large amount of ultrasonic energy is unavoidable. Moreover, in such cases, viscoadaptive OVDs and use of the soft shell technique[6] cannot completely protect against corneal endothelial damage because OVDs

are aspirated with phacoemulsification. Currently, repeated injection of hyaluronic acid-based OVD is required during phacoemulsification in cases with hard cataract to maintain corneal endothelial protection. The introduction of femtosecond laser cataract surgery has enabled surgeons to reduce the required amount of ultrasound energy. However, the clinical significance of the extent of endothelial cell loss compared to that with conventional phacoemulsification remains debatable[8]. We believe that this issue cannot be adequately addressed by current hyaluronic acid-based OVDs. Therefore, new interventions that can persist longer and mechanically protect better from free radicals and nucleus particles than the current hyaluronic acid-based OVDs during phacoemulsification are required. Retention time of OVDs in the anterior chamber is closely associated with the anti-free radical effect on the corneal endothelium. The effect of OVDs on free radicals depends on the retention of the materials within the anterior chamber[9].

In a previous study, we obtained promising results by inserting a senofilcon A mechanical protector under the corneal endothelium to protect the corneal endothelium during phacoemusification[10]. However, we experienced difficulty in maintaining the stability of the senofilcon A mechanical protector under high vacuum and high flow rate. Therefore, we hypothesized that the application of a semisolid thermoreversible (poloxamer) hydrogel as a protective shell under the corneal endothelium would offer superior performance compared with that of a mechanical protector. We have termed this method of poloxamer hydrogel application as the poloxamer shell technique, with the poloxamer shell acting as a mechanical barrier and protecting the corneal endothelium from free radicals, heat, and ultrasound energy generated by the phacoemulsification probe. Poloxamer block

copolymers comprise ethylene oxide (EO) and propylene oxide (PO) blocks arranged in a triblock structure: EOx-POy-EOx. All poloxamers have similar chemical structures, but with different molecular weights and different values of x and y in each block. Poloxamer 407, one of the most commonly used poloxamers, is a nontoxic copolymer with an average molecular weight of 11,500. It is a white powder that contains 70% hydrophilic EO units and 30% hydrophobic PO units[11]. An aqueous poloxamer solution has thermoreversible properties. This thermogelling phenomenon is reversible, transitioning from sol to gel phase according to the temperature, and can be modified by adjusting the concentration of the poloxamer solution[12]. A solution of the poloxamer hydrogel is a clear liquid at room temperature, but when warmed to body temperature, it undergoes gel-to-sol transition to yield a solid gel form[11,13]. Because of these thermal gelation and nontoxic properties, the poloxamer hydrogel provides a convenient and efficient means for forming a physical barrier during surgery. Therefore, commercial products containing poloxamer hydrogels are used as anti-adhesive and contracture preventive agents in abdominal[14] and plastic surgeries[15]. Ophthalmic usage was tested to increase the residence time on the corneal surface to minimize washout by tears[16,17]. In a previous study, an injectable intraocular lens refilling formulation with 25% poloxamer hydrogel produced no inflammatory response or toxicity in rabbit eyes[18].

This experimental study aimed to evaluate the applicability of the poloxamer hydrogel as a substitute for OVDs during phacoemulsification and to determine the optimal concentrations of the poloxamer hydrogel at room temperature. To accomplish this, we performed both in vitro and in vivo experiments. In the in vitro

testing of porcine eyes, we measured the retention time to evaluate the adherence of the hydrogel and evaluated the behavior of poloxamer hydrogels in the anterior chamber during phacoemulsification. In the in vivo testing, we used a rabbit model to evaluate the protective effects of thermosensitive hydrogels during phacoemulsification.

MATERIALS AND METHODS

Preparation of the poloxamer gel

Poloxamer 407 (P407, Sigma-Aldrich Co.) hydrogel was prepared using the cold method.19 Briefly, the poloxamer powder was added to water at 4–5°C with continuous magnetic stirring until a homogenous solution was formed. A series of dilutions ranging from 18% to 26% P407, with 2% intervals, was prepared in a balanced salt solution (BSS). A sterile formulation of thermosensitive hydrogels of different concentrations (percentage determined as weight of P407/weight of diluent × 100) was created. To aid in the visualization of the gel in the anterior chamber, we stained the P407 with 10% fluorescein sodium.

Temperature measurement

To simulate the actual surgical settings, we used a smartphone-based forward-looking infrared (FLIR) camera to measure the ocular temperature in the operating room during cataract surgery. Thermal imaging cameras are commercially available as smartphone attachment devices that provide thermal imaging analysis in a noninvasive manner. Thermographs of the eyeball and BSS were obtained using the FLIR ONE Personal Vision System for iOS (FLIR Systems, Inc.) after the eye was opened with a speculum under the

surgical drape. Thermal readings were obtained after the automatic calibration of the device.

Part I : An in vitro porcine study

Forty-five porcine eyes, which were obtained from an abattoir within 6 h after the animals were sacrificed, were divided equally between the following groups: the cohesive OVD (sodium hyaluronate 1% [Provisc]) group, dispersive OVD (sodium hyaluronate 3%-chondroitin sulfate 4% [Viscoat]) group, and poloxamer hydrogel group. Before beginning the experiments, the porcine eyes were incubated in a 32°C water bath filled with BSS to simulate the temperature in the anterior chamber during actual cataract surgery. The eyes were mounted, and the surgical procedures were performed under an operating microscope, similar to the procedure performed during human cataract surgery. A 1.2-mm side-port incision was made with a slit knife, and 0.45 mL of the OVD or poloxamer was fully injected into the anterior chamber. The main clear corneal incision was made with a 3.00-mm beveled knife. We subsequently evaluated the ability of the different concentrations of poloxamer hydrogels to undergo gelation and to form poloxamer shells in the anterior chamber at approximately 30 - 32°C. Phacoemulsification was performed using a Sovereign Compact Phacoemulsification System (Abbott Medical Optics, Inc.). The phacoemulsification tip was inserted in the center of the anterior chamber of all eyes, and the bevel was positioned upward to the corneal endothelium. The phacoemulsification power was set to 35%, and the rates of aspiration and vacuum were maintained at 30 cc/min and 300 mmHg, respectively. While continuing phacoemulsification at the central cornea without crystalline lens removal, we measured the retention time of the

fluorescein-stained OVD and poloxamer hydrogel in the anterior chamber with an operating microscope and side view camera (Figure 1).

Measurement of retention time

The retention times of the OVDs and poloxamer hydrogel were recorded with a surgical microscopic view, and a side view camera was used to record the flow in the anterior chamber (Figure 1). In instances where it was difficult to determine the presence of dispersive OVD in the anterior chamber via the vertical microscopic view, we measured retention time using the side view camera. The retention time was defined as the interval between the initiation of OVD aspiration through the phacoemulsification tip and the time of completion of aspiration through the phacoemulsification tip, as determined via both the vertical microscopic view and the side view camera in the anterior chamber[20]. Additionally, the completion of retention in the anterior chamber was also considered when the OVD or poloxamer hydrogel was removed within the central 8.0-mm cornea and remained at the angle or far periphery without further aspiration despite the continued phacoemulsification at center.

Part II : In vivo rabbit study

Animals

Rabbits were obtained from a vendor (KOATECH CO., Ltd) that was internationally certified by the Association for Assessment and Accreditation of Laboratory Animal Care. The rabbits were handled according to the guidelines of the Association for Research in Vision and Ophthalmology Statement for Use of Animals in Ophthalmic and Vision Research. The study protocol was approved by the

Institutional Animal Care and Use Committee (IACUC) of the Seoul Metropolitan Government-Seoul National University Boramae Medical Center (IACUC # 2017-0019) and followed the guidelines of animal ethics. The room was maintained at 20.5 - 22°C. The rabbits were treated according to the preoperative and postoperative management processes used in our previous study.10 For this study, we used 12 eyes of 12 New Zealand white rabbits weighing 2.7 - 3.0 kg and aged 18 - 20weeks.

Comparison of endothelial cell changes

The preoperative and third postoperative central corneal endothelial cell counts (ECCs) were measured using a noncontact autofocus specular microscope (EM-4000, Tomey Corp.). The 12 rabbit eyes were divided equally into two groups: the dispersive OVD (sodium hyaluronate 3%-chondroitin sulfate 4% [Viscoat]) group and the 26% poloxamer group (n = 6 per group). After making the 1.2-mm side port incision, we completely filled the anterior chamber with dispersive OVD or 26% poloxamer hydrogel without fluorescein staining. The phacoemulsification power was set to 80%, and the rates of aspiration and vacuum were controlled at 10 mL/min and 10 mmHg, respectively, to minimize the washing out of the OVD or poloxamer hydrogel and to maximize the ultrasound energy exposure during phacoemulsification. The phacoemulsification tip was inserted through the 2.75-mm main incision. The position of the phacoemulsification tip was maintained in the center of the anterior chamber, with the bevel-up toward the corneal endothelium, and 10-s intermittent phacoemulsification was activated for 5 min (total elapsed ultrasound exposure of 2.5 min). After phacoemulsification, 0.15 mL of cold BSS (15°C) was irrigated through the main incision to remove the OVD or poloxamer hydrogel remaining in the anterior chamber.

The surgical procedure for each animal was performed by the same senior cataract surgeon (Y.K.H.), and the examination was performed in a blinded and randomized manner by the same ophthalmologist (J.Y.C.). The animals were checked four times a day for signs of infection or inflammation with a portable slit lamp, and neomycin sulfate - polymyxin B - dexamethasone 0.1% was instilled at every check.

Statistical analyses

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 19.0 (SPSS, Inc., Chicago, IL, USA). Normality was checked using the KolmDIAG2020014064ogorov - Smirnov test, and retention times for the different OVDs and poloxamer hydrogels were compared using the Kruskal - Wallis test. Differences were considered statistically significant according to the Bonferroni-corrected significance level, with a P-value <0.017 indicating a significant difference. The nonparametric Mann - Whitney U test was used to compare the changes in parameters between the groups within each study. The level of statistical significance was defined as a P-value <0.05.

RESULTS

Ocular temperature under the surgical field

Figure 2 shows the temperature of the corneal surface, as measured by an infrared thermal imaging camera during surgery. The operating theater was maintained at a temperature of 21°C, and the BSS used in the operating theater was maintained at 21.1°C (Figure 2A). The temperature of the cornea after the surgical drape and opening with

the speculum was 31.8°C (Figure 2B). At the end of cataract surgery, after the stromal hydration of the main incision site, the ocular temperature was 27.1°C (Figure 2C).

In vitro study

Determination of the optimal poloxamer hydrogel concentration

The poloxamer hydrogel samples, with concentrations of 18% - 22%, had low elasticity and formed viscous solutions at a temperature of 21°C (Figure 3A). The 24% poloxamer hydrogel exists in a viscoelastic state between the solution and gel. The 26% poloxamer hydrogel was in gel form at the operating room temperature of 21°C (Figure 3B). The gelation of the poloxamer hydrogel did not occur immediately in the anterior chamber below a concentration of 24%. The concentration of <26% poloxamer hydrogels was immediately dissolved in contact with a BSS (Figure 4A - D). A semisolid form of poloxamer hydrogel that achieves complete gelation at 21°C is required to generate the poloxamer shell in the anterior chamber, and accordingly, the 26% concentration of poloxamer was optimal. The 24% and 26% poloxamer hydrogels could not be extruded using 25-gauge, bent, blunt-tip, thin-walled cannulas. A bent 24-gauge pinpoint needle was ideal for the injection of the poloxamer hydrogel into the anterior chamber through the 1.2-mm side port incision.

Retention time during phacoemulsification

The mean retention times were 5.53 ± 1.77s in the cohesive OVD group, 125.00 ± 29.34 s in the dispersive OVD group, and 221.53 ± 42.48 s in the 26% poloxamer hydrogel group (Figure 5) (Video 1, available at http://jcrsjournal.org), with significant differences among the three groups ($P<0.001$, analysis of variance). The Kruskal - Wallis

multiple comparison test revealed that the 26% poloxamer hydrogel group had longer retention time during phacoemulsification compared with the retention times for the cohesive OVD ($P<0.001$) and dispersive OVD groups ($P<0.001$). The poloxamer hydrogel left in the anterior chamber was easily removed by manual irrigation through an incision, with a BSS solution at 15°C (Video 1, available at http://jcrsjournal.org).

In vivo study

The differences between preoperative and 3-day postoperative ECCs are shown in Table 1. The dispersive OVD group showed a significantly greater decrease in ECC than the poloxamer shell group ($P = .029$, Mann-Whitney). Postoperative infection or toxic anterior segment syndrome was not detected, and anterior chamber inflammation was controlled by postoperative eye drops in both groups.

DISCUSSION

In this study, we applied a poloxamer hydrogel to form a dome-shaped shell under the corneal endothelium to test the hypothesis that the poloxamer shell would act as a mechanical protective barrier during phacoemulsification. To the best of our knowledge, this is the first study in which the poloxamer hydrogel was used as a possible material to protect the corneal endothelium. Prior to commencing this study, we considered ocular temperature during cataract surgery to be an important factor because the temperature of the surgical environment determines not only the phase of the thermosensitive poloxamer hydrogel but also its injectability. In this study, the ocular temperature measured by the

infrared thermal camera during cataract surgery was lower than the normal human body temperature. The lowest temperature calculated using a numeric model to study heat exchange inside the eye was approximately 34°C; the model accounted for the supine position and dynamics of aqueous humor[21]. Eom et al[22] measured ocular temperature during cataract surgery using a thermal imaging camera and reported that the ocular temperature, at 30.1°C, was lower than the normal human body temperature. The lower ocular temperature indicates that the application of thermosensitive hydrogel in intraocular surgery is more difficult than in other surgical fields that use body temperature for gelation. Different from the use of poloxamer in non-ophthalmic surgical fields, the feasibility of the poloxamer hydrogel for ophthalmic applications has been debatable[23]. One of the main obstacles for the ophthalmic use of poloxamer hydrogel is that tears on the ocular surface can dilute the poloxamer hydrogel. In the present study, we selected the liquid form of the poloxamer hydrogel for injection into the anterior chamber, and the injected liquid hydrogel was expected to form a gel at body temperature. However, dilution by the aqueous humor in the anterior chamber represented a major obstacle for the adequate delivery of the liquid poloxamer hydrogel. In our preliminary test using porcine eyes, rapid and prompt gelation in the anterior chamber could not be achieved after the injection of liquid or viscous poloxamer hydrogel at a concentration of 24% and temperature of 21°C. Considering this dilution effect and the low ocular temperature, phase transition of the poloxamer hydrogel in the anterior chamber requires a relatively longer time than it does when it is used in other surgical applications at body temperature[23]. Additionally, during the phase transition in the anterior chamber, dilution with aqueous humor results in an increased

sol-to-gel transition temperature. An increase in poloxamer concentration is associated with a decrease in the phase transition temperature, but an increase in the viscosity of the poloxamer hydrogel[12,24,25]. The OVDs must have low viscosity so that they can be injected into the eye through fine-bore cannulas[26]. Although most of the poloxamer hydrogels exhibit shear-thinning behavior (pseudoplasticity), when the poloxamer hydrogel reaches complete gelation, a large amount of force has to be applied to extrude the solutions out of the small-caliber syringes[27]. In this study, we were unable to inject the 26% poloxamer hydrogel into the anterior chamber through the 25-gauge bent needle. A rheologic study of poloxamer 407 revealed that it was less pseudoplastic than hyaluronic acid-based OVDs[26,28]. Higher concentration of poloxamer hydrogel exhibits less pseudoplastic and decreased viscoelastic behavior[26-28]. Pseudoplasticity is important characteristics of OVD because various shear thinning steps are present during cataract surgery. However, the use of 24-gauge pin point needles with a 1-mL syringe enabled easy injection of the 26% poloxamer hydrogel into the anterior chamber.

OVDs can perform a protective function by coating the ocular structure, specifically the corneal endothelium[29]. Previous studies have measured retention time and adhesiveness as indirect methods of evaluating corneal endothelial protection and behavior of OVD[30,31]. The large area coated by OVDs and their prolonged retention in the anterior chamber further protect the corneal endothelium during cataract surgery by minimizing the interaction between the ocular tissue and surgical instruments. In vitro study have revealed that dispersive OVDs are retained longer than cohesive OVDs[31]. In the present study, we used actual surgical phacoemulsification parameters;

however, phacoemulsification was performed under the central cornea without movement or lens removal to observe the behavior of the poloxamer hydrogel.

The equal exposure to phacoemulsification used in the present study could minimize the effect caused by variations in irrigation and ultrasound exposure compared with that in the previous study[30]. Among the 15 eyes in the poloxamer group, the poloxamer shell was completely emulsified within 3 min in only one specimen, and the other poloxamer shells persisted for at least 3 min of phacoemulsification compared with the eyes in the dispersive OVD group, which exhibited complete aspiration within 2 min. We expected the poloxamer shell to stay in place as a semisolid gel without fracturing or aspiration. However, contrary to our expectations, the poloxamer hydrogel was significantly slowly fragmented and emulsified around the phacoemulsification tip (Figure 6A and 6B). Our observations regarding the behavior of poloxamer hydrogels during phacoemulsification are important because combining the dispersive OVD and poloxamer shell technique would allow the emulsified area of the poloxamer shell to be subsequently covered by a dispersive OVD. Moreover, similar to the soft shell technique[6], this combination technique could prolong the endothelial protective coverage by providing a second barrier. Additionally, poloxamer shells could function as OVD pockets that maintain the dispersive OVD in the potential space between the endothelium and the poloxamer shell. By performing retention testing, we investigated the possibility of a poloxamer hydrogel as a protective shell for the corneal endothelium against phacoemulsification.

Despite the small sample size, the results of our in vivo rabbit eye experiment showed that the poloxamer shell technique had a

protective effect against phacoemulsification insult to the corneal endothelium. The poloxamer hydrogel remained in the anterior chamber for 5 min of intermittent phacoemulsification (Figure 6C) (Video 2, available at http://jcrsjournal.org). Additionally, we found that the 26% poloxamer hydrogel conferred significantly better endothelial protection than the dispersive OVD (sodium hyaluronate 3%-chondroitin sulfate 4% [Viscoat]) did. The change in ECC in the poloxamer group was similar to that observed in our previous study[10] with a senofilcon A mechanical protector, which was associated with a postoperative ECC decrease of 4%.

Compared to senofilcon A mechanical protectors, the poloxamer hydrogel offers better intraocular stability, safety, and ease of manipulation for injection and removal. In our previous study[10], the use of senofilcon A mechanical protectors in the anterior chamber induced toxic anterior segment syndrome, where no toxicity was observed with the poloxamer hydrogel in the present study. Extended indwelling of the poloxamer hydrogel resulted in dissolution upon contact with aqueous humor, but ocular inflammation was not detected for 3 months in a previous study[18]. Additionally, the poloxamer hydrogel was easily injected using 24-gauge needles and removed rapidly by irrigating with 1.5-mL cold (15°C) BSS through the main incision.

In the present study, the poloxamer shell technique resulted in promising outcomes in terms of protection of the corneal endothelium relative to the outcomes of cohesive OVDs; however, the study has some limitations. The small sample size limits the drawing of definitive conclusions from the findings. Furthermore, because this preliminary study was designed to investigate the feasibility of poloxamer hydrogel as an OVD substitute for the protection of the

corneal endothelium, we did not compare the poloxamer shell technique with other types of OVDs (other than dispersive OVDs) in rabbit eyes. As we removed the remaining poloxamer hydrogels with cold BSS irrigation at the end of surgery, we did not evaluate its safety issue, specifically its effects on intraocular pressure or the toxic effects of indwelling in the anterior chamber. Moreover, this study was evaluated with limited clinical specular microscopy indicating corneal endothelial cell damage. Thus, further indices by measuring central corneal thickness and histopathologic examination are required. Further large-scale studies comparing poloxamer hydrogels with different types of OVD are required in the future.

In conclusion, the results of the present study indicate that the poloxamer hydrogel is a feasible substitute for OVDs in terms of corneal endothelial protection during phacoemulsification. The use of the novel poloxamer shell technique described herein provides a new approach and a surgical device worthy of further study and modifications.

WHAT WAS KNOWN

● The ophthalmic viscosurgical device (OVD) and soft shell technique cannot completely protect against the damage to the corneal endothelium in the extremely hard nucleus.

● The poloxamer hydrogel possesses the thermoreversible property of phase transitioning from sol to gel at body temperature and its gelling temperature is related to the concentration of the poloxamer hydrogel.

WHAT THIS PAPER ADDS

● Poloxamer hydrogel showed excellent retention and adherence in

the anterior chamber during phacoemulsification compared with cohesive and dispersive OVDs.

● The poloxamer shell technique using thermosensitive hydrogel protects against corneal endothelial cell damage during phacoemulsification in rabbit eyes.

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Figure Legends

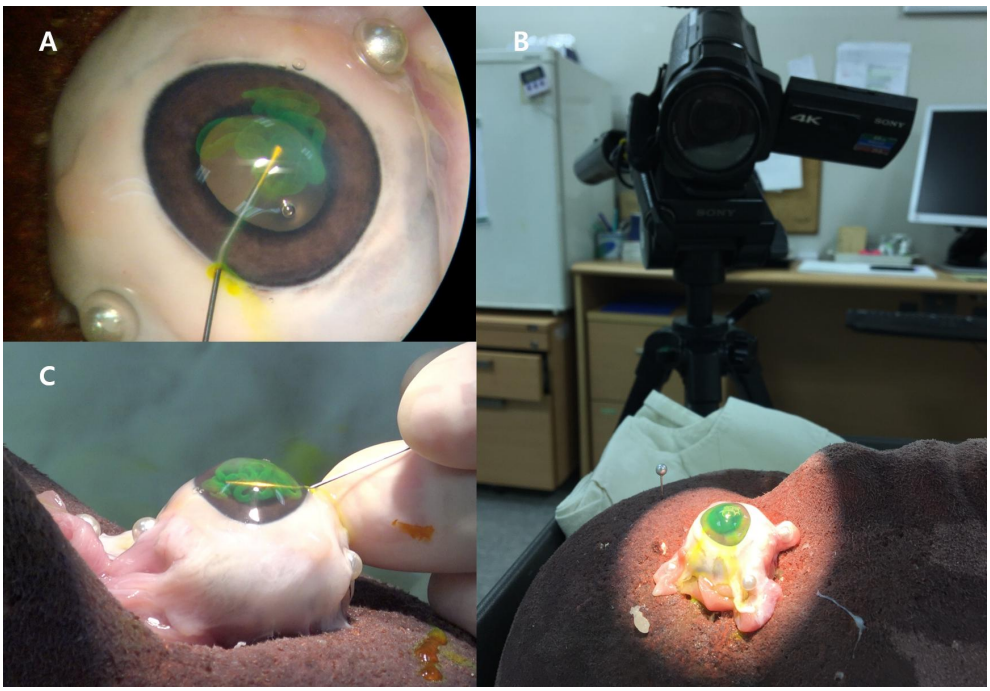

Figure 1. Retention time measurement using a surgical microscopic view and a side view camera.

(A) Surgical microscopic view of the injection of a fluorescein-stained cohesive ophthalmic viscosurgical device (OVD) into a porcine eye.

(B) A side view camera was used to observe the behavior of OVDs and poloxamer hydrogels in the anterior chamber.

(C) Side camera view of injection of a fluorescein-stained cohesive OVD into a porcine eye.

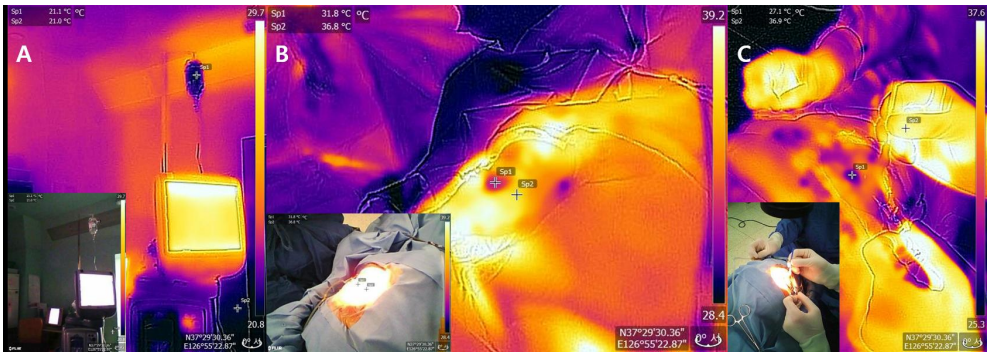

Figure 2. Temperature measured using a forward-looking infrared camera.

(A) Temperature of the balanced salt solution at the beginning of the surgery at a room temperature of 21°C. Sp1 indicates the 21.1°C temperature of the irrigation solution, and Sp2 indicates the 21°C room temperature.

(B) Ocular thermograph at the beginning of cataract surgery. Sp1 indicates the 31.8°C temperature of the eyeball at a room temperature of 21°C. Sp2 indicates a body temperature of 36.8°C.

(C) Ocular thermograph at the end of the cataract surgery, obtained after stromal hydration. Sp1 indicates the 27.1°C temperature of the eyeball. Sp2 indicates the 36.9°C temperature.

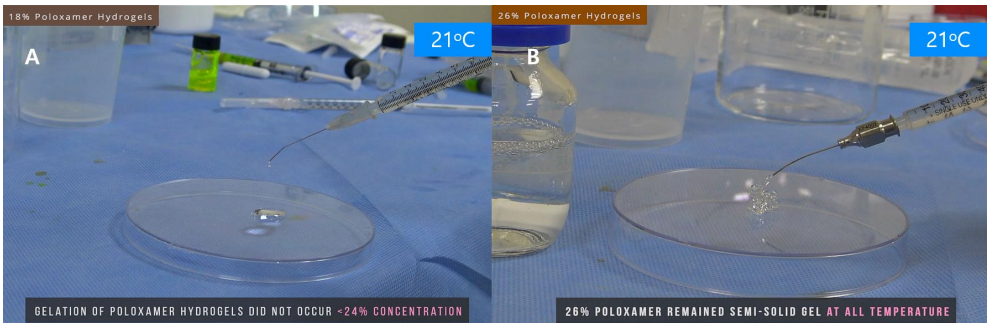

Figure 3. Different concentrations of poloxamer hydrogels at 21°C temperature.

(A)18% poloxamer hydrogels were viscous solutions at 21°C operating room temperature and had low elasticity.

(B) The 26% poloxamer hydrogels were semisolid gels at 21°C operating room temperature, and a bent 24-gauge pinpoint needle was ideal for the injection of the poloxamer hydrogel.

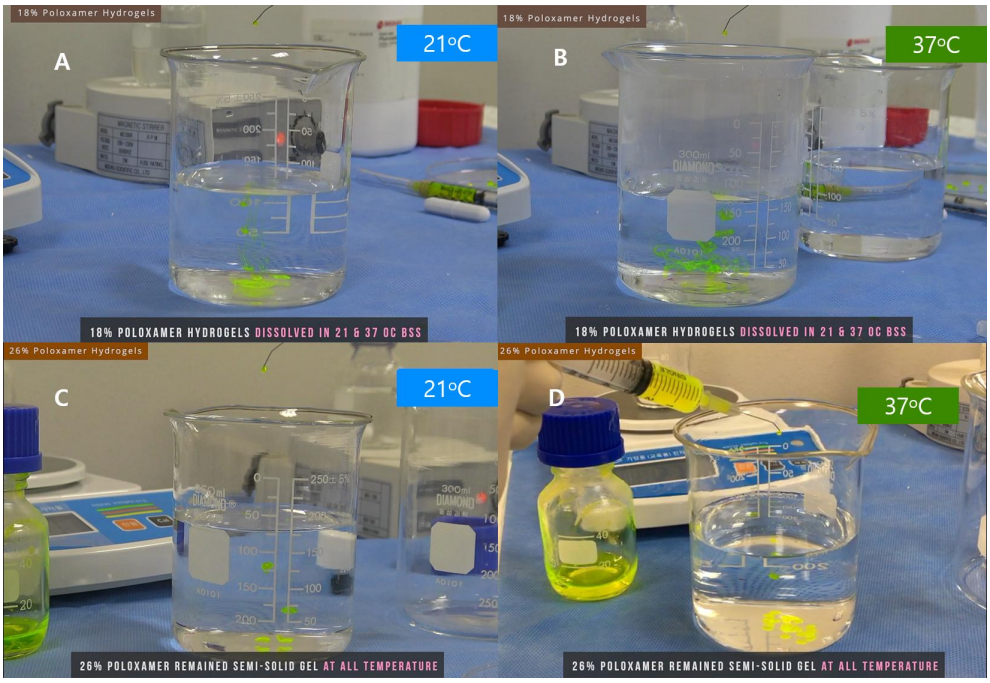

Figure 4. Behavior of 18% and 26% poloxamer hydrogels in contact with different temperatures of balanced salt solution.

(A, B) 18% poloxamer hydrogels were dissolved at all temperatures on contact with 21°C and 37°C balanced salt solution.

(C, D) 26% poloxamer hydrogel remains semisolid gel at all temperatures without dissolution on contact with 21°C and 37°C balanced salt solution.

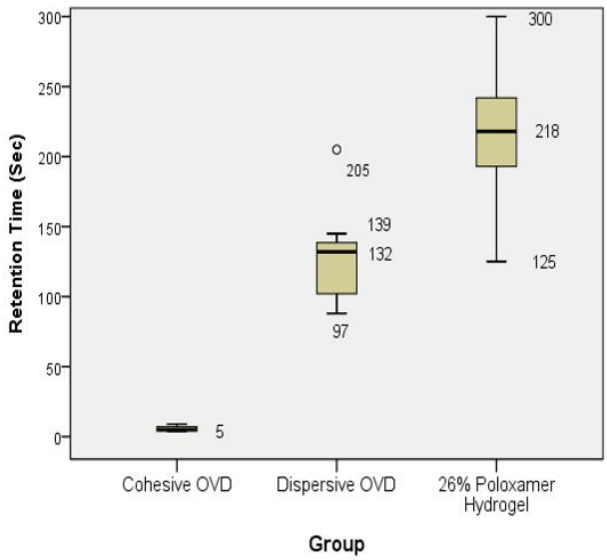

Figure 5. Retention time until the ophthalmic viscosurgical devices (OVDs) or the 26% poloxamer hydrogel was removed by phacoemulsification of the porcine eyes. The mean retention time ± standard deviation were 5.53 ± 1.77 s in the cohesive OVD group, 125.00 ± 29.36 s in the dispersive OVD group, and 221.53 ± 42.48 s in the 26% poloxamer hydrogel group. The Bonferroni multiple comparison results indicated significant differences in retention times between the 26% poloxamer and cohesive OVD (P<0.001), the cohesive and dispersive OVD (P<0.001), and the dispersive OVD and 26% poloxamer groups (P<0.001).

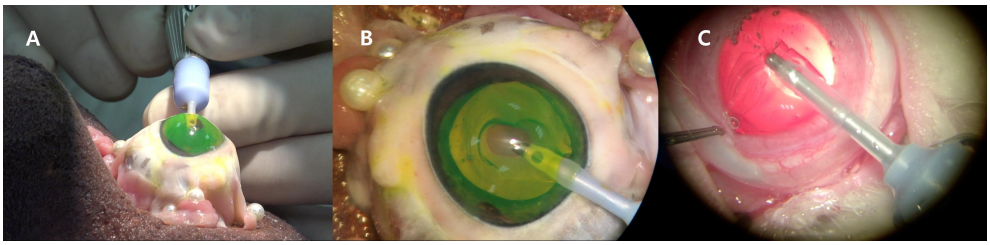

Figure 6. Poloxamer shell in the anterior chamber after phacoemulsification in porcine and rabbit eyes.
(A) Side camera view of 26% poloxamer hydrogels at 3 min of continuous phacoemulsification in a porcine eye shows that the poloxamer shell is maintained without aspiration. Only above the phacoemulsification tip is emulsified.
(B) Surgical microscopic view of 26% poloxamer hydrogels at 3 min of continuous phacoemulsification in a porcine eye shows that the poloxamer shell is maintained without aspiration. Only above the phacoemulsification tip is emulsified.
(C) In a rabbit eye, 26% poloxamer hydrogel remained in the anterior chamber throughout the 5-min intermittent phacoemulsification.

Table 1. Summary of preoperative and postoperative endothelial cell counts.

Group	Eyes	ECC (cells/mm^3)		Endothelial cell loss		
		Pre	Post	Decrease (cells/mm^3)	% change	*P* value
Dispersive OVD Group (n=6)	Rabbit 1	2707	2068	639	23.61	
	Rabbit 2	2466	2266	200	8.11	
	Rabbit 3	2890	2512	378	13.08	
	Rabbit 4	3278	2873	405	12.36	
	Rabbit 5	3048	2272	776	25.5	
	Rabbit 6	3086	2252	834	27.03	
	Mean ± SD	2912.5±291.2	2373.8±282.3	538.7±249.8	18.27	0.029
26% Poloxamer hydrogel Group (n=6)	Rabbit 7	2893	2876	17	0.59	
	Rabbit 8	2529	2428	101	3.99	
	Rabbit 9	2608	2480	128	4.91	
	Rabbit 10	2755	2601	154	5.59	
	Rabbit 11	2738	2731	7	0.26	
	Rabbit 12	2962	2480	128	16.3	
	Mean ± SD	2747.5±164	2599.3±174.2	148.2±173.9	5.27	

ECC = endothelial cell count; OVD = ophthalmic viscosurgical devices; SD = standard deviation.

Supplemental videos

Video 1 (available at http://jcrsjournal.org)

In vitro behavior of ophthalmic viscosurgical devices (OVDs) and 26% poloxamer hydrogels is described as follows: The measurement of retention time in the anterior chamber during phacoemulsification. Behavior of fluorescein-stained cohesive and dispersive OVDs, and 26% poloxamer hydrogel in porcine eyes. Removal of remaining poloxamer hydrogel using cold BSS through the main incision.

Video 2 (available at http://jcrsjournal.org)

In vivo behavior of 26% poloxamer hydrogels is shown in this video. Throughout the 5-min intermittent phacoemulsification, the anterior chamber stability was maintained under the poloxamer shell, and the poloxamer shell remained in place during the entire phacoemulsification period.

Application of thermoreversible hydrogel, poloxamer 407 for protection of the corneal endothelium during phacoemulsification: An experimental study in porcine and rabbit eyes

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Purpose : To evaluate the utility of thermoreversible (poloxamer) hydrogels as a substitute for ophthalmic viscosurgical devices (OVDs) during phacoemulsification, and to compare their endothelial protective effect with that of hyaluronic acid-based OVDs during phacoemulsification in porcine and rabbit eyes.
Methods : Fluorescein-stained poloxamer hydrogels (20,22,24, and 26% [weight/weight%]), and cohesive (sodium hyaluronate 1% [Provisc]) and dispersive (sodium hyaluronate 3.0%-chondroitin sulfate 4.0% [Viscoat] OVDs were injected into the anterior chamber of porcine eyes incubated at 32°C. In the in vitro study, the retention time was measured in 3 groups of 45 porcine eyes during continuous phacoemulsification. In the in vivo study, the endothelial cell count (ECC) was measured before and 3 days after intermittent phacoemulsification in 12 rabbit eyes randomized to a poloxamer hydrogel or a dispersive OVD group.
Results : The optimum concentration of thermosensitive hydrogel was 26%, at which no gel-to-sol phase transition occurred in the anterior chamber, with a 21°C irrigation solution. In the in vitro study, the mean retention times were 5.53 seconds ± 1.77, 125.00 ± 29.34 seconds, and 221.53 ± 42.48 seconds in the cohesive OVD, dispersive OVD, and 26% poloxamer hydrogel groups, respectively ($P < .001$). In the in vivo study, the mean decrease in ECC was significantly lower in the 26% poloxamer hydrogel group than in the dispersive OVD group ($P = .029$).
Conclusion : Thermoreversible hydrogels might be suitable substitutes for hyaluronic acid-based OVDs for corneal endothelial protection during phacoemulsification.

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keywords : Thermoreversible hydrogels, Poloxamer, corneal endothelium, phacoemulsification, ophthalmic viscosurgical device
***Student Number* : 2018-24065**