Solid Hyaluronic Acid Film and the Prevention of Postoperative Fibrous Scar Formation in Experimental Animal Eyes

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Objective: To investigate the inhibitory effect of solid hyaluronic acid–carboxymethyl cellulose film (hyaluronic acid film) on the formation of postoperative wound adhesion on rabbit eyes.

Methods: We first created a conjunctival flap under which hyaluronic acid film was inserted. Then, we performed trabeculectomy on other rabbit eyes with hyaluronic acid film applied under and above the scleral flaps. Expression of proliferative cell nuclear antigen and α-smooth muscle actin (α-SMA) were histologically and immunohistochemically examined.

Results: Hyaluronic acid film significantly prevented adhesions after both kinds of surgery. Particularly, subconjunctival scar formation was significantly inhibited when the film was simply inserted under the wound. Furthermore, the adhesion around the scleral flap of trabeculectomy was less formed in eyes treated with hyaluronic acid film than in control eyes. Immunoreactivity to proliferative cell nuclear antigen almost disappeared after 28 days postoperatively in both treated and control groups. The α-SMA–positive cells appeared much less around the film-treated wound than the control eye.

Conclusion: The present results indicate that hyaluronic acid film can inhibit the formation of postoperative adhesion around the conjunctiva and sclera.

Clinical Relevance: The results of this study indicate that this substance has potential benefits for improving ophthalmic surgery, such as filtering surgery for glaucoma.


Both general surgeons and ophthalmologists are aware that adhesion formation after surgical operations sometimes causes serious problems. Ophthalmic surgeons sometimes have a hard time overcoming postoperative scar formation at the time of reoperation for strabismus, retinal detachment, or glaucoma, particularly filtering surgery.

A film consisting of solid hyaluronic acid and carboxymethyl cellulose (hyaluronic acid film, Seprafilm; Genzyme Corp, Cambridge, Massachusetts) has been reported to prevent the formation of adhesive tissues in surgical wounds in the fields of abdominal and respiratory surgery.1-3 Especially for glaucoma surgery, surgical prognosis after filtering surgery is well known to largely depend on wound adhesion. Various studies have suggested that the use of antimetabolic drugs, such as 5-fluorouracil and mitomycin C (MMC), is effective for maintaining the bleb.4-10 However, serious postoperative complications can arise from the antimetabolic effects of these agents, such as corneal erosion and infection from buttonholes of a thin filtering bleb.11-16

In recent years, various new methods for maintaining a filtering bleb have been examined by other researchers. Surgery with implants has been reported to allow effective control of intraocular pressure as a means of maintaining the physical space.17-20 In addition, as another approach, the CAT-152 Trabeculectomy Study Group21,22 attempted to use anti–transforming growth factor eye drops to restrain wound healing.

To the best of our knowledge, no basic research has yet been performed using hyaluronic acid film in ophthalmic surgery. This film mainly consists of hyaluronic acid, which has long been safely used for intraocular surgery. The present study evaluated and examined the effects of hyaluronic acid film on postoperative scar formation in rabbit ocular tissues to clarify the potential benefits of hyaluronic acid film in ophthalmic surgery.

METHODS

MATERIALS AND SURGICAL TECHNIQUES

Hyaluronic acid film (Seprafilm, 80 µm thickness) was kindly provided by the manufacturer. All experimental procedures were designed to conform to both the ARVO Statement for the Use of Animals in Ophthalmic Vision Research and the guidelines of our own institution.
A total of 20 Japanese white rabbits (body weight, 1 kg) were used. For all procedures, rabbits were anesthetized using 25 mg/kg of body weight of pentobarbital sodium and all surgical manipulations described later were performed under additional topical anesthesia with 0.4% oxybuprocaine hydrochloride eye drops. All experimental procedures were carried out in duplicate.

**EXPERIMENT 1: SIMPLE CONJUNCTIVAL INCISION**

Eight rabbits were used for this experiment. Conjunctival incision was made along the corneal limbus at an angle of about 45° to expose the sclera. The wound was then sutured with 8-0 polyglactin with (film-treated eyes) and without (control eyes) subconjunctival insertion of hyaluronic acid film (Figure 1A). To examine the strength of adhesion, surgical wounds were re-opened in 2 eyes of 2 rabbits in each group 60 days after initial surgery by the same surgical instruments used before, and also, 2 film-treated and 2 control eyes of 4 rabbits at 60 days after surgery were histologically prepared as described later in the “Histologic Preparation” subsection.

**EXPERIMENT 2: TRABECULECTOMY**

We conducted trabeculectomy by means of ordinary methods in other rabbit eyes (12 eyes of 12 rabbits). After creating a fornix-based conjunctival incision, a limbal-based square scleral flap (3×3 mm) was made (Figure 1B). After trabeculectomy was performed, the scleral flap was closed by suturing with 10-0 nylon at 5 points. During the surgical procedure, sheets of hyaluronic acid film were inserted above (3×6 mm) and below (4×5 mm) the scleral flap. The conjunctival wound was then closed by suturing with both 8-0 polyglactin and 10-0 nylon. Histologic and immunohistologic examinations were performed at 7, 14, and 28 days after surgery, respectively.

**HISTOLOGIC PREPARATION**

Rabbit eyes were histopathologically studied to examine the effect of hyaluronic acid film on inhibition of postoperative fibrous scar formation. At 60 days after experiment 1 and at 7, 14, and 28 days after experiment 2, each rabbit was rapidly perfused through the auricular vein with a total of 250 mg of pentobarbital sodium. Eyeballs (2 eyes of 2 rabbits at each postoperative date) were then enucleated, fixed in 4% paraformaldehyde solution overnight, dissected at the equator, and embedded in paraffin. Anterior segments of eyes containing the cornea, sclera, and conjunctiva were cut vertically at 4 µm thickness, mounted on subbed slides, and dried.

**MASSON TRICROME STAINING**

After deparaffinization with serially graded ethanol and xylene solutions, these sections were processed with Masson trichrome stain to evaluate the formation of fibrous tissues.

**IMMUNOHISTOCHEMICAL EXAMINATION**

To examine the proliferative activity of cells adjacent to the surgical wounds, we immunohistochemically measured the expressions of proliferative cell nuclear antigen (PCNA) and α-smooth muscle actin (α-SMA) after experiment 2. Tissue sections from eyes at 7, 14, and 28 days after surgery were blocked with 3% bovine serum albumin in phosphate-buffered saline for 1 hour and then incubated with either mouse antirabbit PCNA (1:20) or mouse antihuman α-SMA (1:100) monoclonal antibody as the primary antibody overnight at 4°C. Sections were then further incubated with horseradish peroxidase-conjugated rabbit antimouse IgG as the secondary antibody overnight at 4°C. Proliferative cell nuclear antigen and α-SMA were visualized by dinitro-
amino bentizine. To semiquantitatively measure expression of PCNA in proliferating cells around the space in which hyaluronic acid film had been embedded, PCNA-positive cells were counted in 4 randomly selected square areas (400 m × 400 m) in the subconjunctival space and 3 rectangular areas (200 m × 400 m) under the scleral flap in each eye. Counted numbers were then compared statistically. The t test was used to calculate the level of statistical significance (P < .05). To examine the level of expression of α-SMA, we qualitatively observed immunohistologic sections of scleral flap areas.

**RESULTS**

When hyaluronic acid film was not used in experiment 1, subconjunctival soft tissue was almost completely replaced by fibrous proliferation and scar tissue by 60 days after surgery (Figure 2A), whereas abundant subconjunctival soft tissue was preserved when hyaluronic acid film was embedded in the subconjunctival space (Figure 2B). These histopathologic differences were confirmed by reoperation. When the initial conjunctival wound was reopened, the conjunctival incision was much easier to make in film-treated eyes than in control eyes under a surgical microscope.

As for experiment 2, histopathologic examination revealed that spaces between the conjunctiva and scleral flap and between the scleral flap and scleral bed remained wide even 7 days after surgery in film-treated eyes (Figure 3A). At 14 days postoperatively, formation of subconjunctival fibrous scar appeared retarded in film-treated eyes compared with control eyes (Figure 3B and C). In addition, a conjunctival filtering bleblike space was observed in film-treated eyes but not in control eyes at 28 days postoperatively (Figure 3D and E). Under surgical microscopic examinations, diffuse but shallow conjunctival filtering blebs were maintained over the scleral flap in film-treated eyes at 7, 14,
and 28 days after surgery, but no filtering bleblike tissue was observed in control eyes at any date after surgery.

IMMUNOHISTOCHEMICAL FINDINGS

**Figure 4** shows the numbers of PCNA-positive cells per 1-mm² area in the space under the conjunctival flap (Figure 4A) and under the scleral flap (Figure 4B). Results obtained in this study indicate that overall numbers and course of PCNA-positive cells show a similar tendency between film-treated and control eyes in both areas, although the peak of PCNA-positive cells was seen at 14 days postoperatively in film-treated eyes, compared with 7 days postoperatively in control eyes (P < .05). **Figure 5** shows α-SMA-positive cells around a scleral flap at 7 and 28 days after surgery. In film-treated eyes, multiple layers of densely α-SMA-positive cells were found in subscleral flap tissue as well as a border area between the sclera and subconjunctiva at 7 days after surgery (Figure 5C). Although these positive cells also disappeared by 28 days after surgery in a control eye, no filtering space was recognized both under and over the scleral flap (Figure 5D).

**COMMENT**

In terms of physical characteristics, hyaluronic acid film changes from solid form into gel form within 24 to 48 hours in the tissue and stays within the tissue for about 7 to 14 days. This material functions as a barrier to contact between separated tissues by which the postoperative formation of adhesions is reduced and delayed. Furthermore, because the film comprises hyaluronic acid and sodium carboxymethylcellulose, no subsequent removal procedure is required, as the materials are eventually absorbed.
into the body. Burns et al\textsuperscript{23} reported that this film has no toxicity, at least according to the Ames mutagenicity test, rabbit pyrogen test, intracutaneous toxicity test, and intraperitoneal and systemic toxicity tests.

In the field of abdominal and obstetric surgeries, hyaluronic acid film has already been reported to effectively prevent postoperative scar formation in abdominal wounds and to remain safe for a long time.\textsuperscript{24} However, the possibility of infection must be considered as the film remains in gel form between tissues. Although we encountered no sign of infection in our present short-term study, such complications can be prevented by thorough sterilization beforehand.

Given the present results, we found that hyaluronic acid film was easily inserted into the conjunctival space. Ophthalmic surgeons are thus able to be easily inserted hyaluronic acid film under the scleral flap in glaucoma filtering surgery, into the subconjunctival space without interfering with the use of MMC, and around the extraocular muscles during ophthalmic surgeries.

Proliferative cell nuclear antigen–positive cells can be considered to mainly comprise fibroblasts. Generally, fibroblasts increase the most at 4 to 7 days postoperatively. Numbers subsequently decrease gradually until about 28 days postoperatively. This duration almost corresponds to the point at which hyaluronic acid film is completely absorbed into the body. The activity of fibroblasts has largely finished by 28 days postoperatively, also meaning that wound healing is almost completed. Figure 4 shows that PCNA-positive cells were almost equal between the 2 groups, indicating that hyaluronic acid film does not inhibit cellular reactions but delays the initiation of cellular proliferation to some extent. This result also suggests that hyaluronic acid film has no toxic effects on fibrous cells around surgical wounds and that the effects are due solely to physical separation of tissues. Figure 5 shows that expression of \(\alpha\)-SMA in myofibroblasts was much less abundant in film-treated eyes than in control eyes. Because conversion of fibroblasts into myofibroblasts expressing \(\alpha\)-SMA indicates enhancement of scar formation by contraction of the collagen matrix,\textsuperscript{26} these findings indicate that hyaluronic acid film inhibits fibrous contraction in the surgical wound.

The present results show that hyaluronic acid film effectively inhibits excessive scar formation after conjunctival incision in glaucoma filtering surgery (Figure 3B and D and Figure 5C). They also suggest that hyaluronic acid film can be used in combination with MMC treatment or even reduce the dosage of MMC. In addition, use of hyaluronic acid film may further provide easy preparation of subconjunctival tissues in the case of reoperation for pars plana vitrectomy and buckling surgery, allowing creation of a long-term effective filtering bleb after glaucoma surgery if necessary.

As for complications, rates of shallow anterior chamber and hypotensive maculopathy by overflow from the wound seem likely to decrease. Slow changes from solid to gel form can slowly decrease intraocular pressure, and postoperative management is easier.

We conclude that the use of hyaluronic acid film in the outer segments of the eye seems to offer a safe and effective means of inhibiting the formation of postoperative adhesions. Although further studies are warranted to clarify long-term safety and efficacy, this approach offers potential benefits to various types of ophthalmic surgery.

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REFERENCES