A Modified Chondroitin Sulfate Aldehyde Adhesive for Sealing Corneal Incisions

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Purpose. To compare a modified chondroitin sulfate aldehyde adhesive with standard sutures for sealing corneal incisions.

Methods. A keratome knife was used to create non–self-sealing, uniplanar, 3-mm, clear corneal incisions in enucleated rabbit eyes ($n = 18$). The wounds were sealed with either a chondroitin sulfate-aldehyde adhesive ($n = 8$), three 10-0 nylon sutures ($n = 5$), or one 10-0 nylon suture ($n = 5$). Wound stability was tested by filling the globes with balanced salt solution through an anterior chamber port and slowly increasing the IOP. The pressure changes were monitored with a digital manometer connected to the anterior chamber, and leak pressure was recorded for each eye. Confocal microscopy was performed on the glued eyes, to document the glue distribution along the wound.

Results. The mean leak pressures in the single-suture and three-suture subgroups were 26.4 ± 6.0 and 44.3 ± 8.2 mm Hg (SD), respectively. The maximum IOP achieved in eyes that received the glue was 104.7 mm Hg with a mean of 101.4 ± 3.2 mm Hg. None of the eyes in which glue was used showed leakage. At confocal microscopy, the glue was distributed inside the wound edges as a homogeneous thin layer of a less dense signal than that of the stroma.

Conclusions. A novel chondroitin sulfate-aldehyde adhesive was shown to be effective ex vivo for sealing corneal incisions in rabbit eyes and was superior to sutures for this purpose. (*Invest Ophthalmol Vis Sci.* 2005;46:1247–1250) DOI: 10.1167/iovs.04-1192

The repair of penetrating corneal wounds requires meticulous wound apposition to achieve a watertight seal. Sutures have customarily been used to repair corneal tissue breaks and the results of this method of wound closure have been successful. The use of sutures, however, is not without disadvantages. Suturing often entails prolonged surgical time and surgical skill, especially in the presence of extensive injuries. Because of the different levels of tension that the sutures produce, stress is placed on certain areas of the cornea and may lead to significant topographic distortions and high levels of astigmatism.[1] Loose sutures may harbor bacteria and cause local inflammation and tissue necrosis as a prelude to infection and possibly endophthalmitis.[2] When exposed, sutures bring forth significant patient discomfort and foreign-body sensation. Suture removal is also required when nonbiodegradable materials are used, with additional stress on the patient. Therefore, the number of patient visits is increased, and the follow-up periods are extended.

Despite recent advances in tissue adhesives, the search for the ideal biological glue continues. Chondroitin sulfate (CS) is a linear polysaccharide comprising glucuronic acid and C-acetyl-galactosamine, which is found in the extracellular matrix of tissues. CS can be modified to incorporate aldehyde groups, allowing application of CS to adhesive-forming chemistries. Because it is a natural component of many human tissues, we hypothesize that CS, if used as an adhesive, may cause less ocular tissue inflammation and foreign body reaction than do sutures. Furthermore, CS-based materials have demonstrated wound-healing benefits.[3]

Reaction of an aldehyde-amine is the basis for the design of this novel tissue-sealing combination. The glue is prepared from two components: CS-aldehyde and polyvinyl alcohol co-vinylamine (PVA-A), which is an amine provider that plays the role of bridging CS-aldehyde adhesive and two target layers of tissue at the corneal incisions. Both components are necessary to activate the adhesive properties and are applied one after the other. Polymerization occurs instantly without the need for external radiation or illumination source.

In the present study, the adhesive strength of CS-aldehyde glue was tested in uniplanar corneal incisions. Non–self-sealing corneal wounds were created in rabbit eyes and sealed with either CS-aldehyde glue or conventional nylon 10-0 sutures.

The purpose of the study was to detect the maximum intraocular pressure (IOP) achieved before wound leakage in these eyes as a means of evaluating the adhesive properties of the glue compared to the standard suture method.

Materials and Methods

Tissue Glue

Adhesive Components. The chemical substances used in this study did not require any light or laser activation to achieve polymerization. CS-aldehyde and the bridging reagent (amine provider) PVA-A were the two components of the glue. An amine-aldehyde interaction via a Schiff base mechanism, effective in high protein content tissues such as the cornea, was the basis for creating this adhesive (Fig. 1).

Synthesis of the Adhesive. The synthesis of the effective gluing reagent of the adhesive, CS-aldehyde, is based on oxidization of adjacent hydroxyls (on CS polysaccharide backbone) into aldehyde functional groups by periodate salt. Six hundred milligrams of chondroitin sulfate (CS; 0.8–1.2 mmol of adjacent diol, 70% CS-A; Sigma-Aldrich, St. Louis, MO) and 616 mg of sodium periodate (~2.88 mmol $NaIO_4$; Sigma-Aldrich) were dissolved together in 10 mL of deionized water and were protected from light. The reaction was allowed to continue for ~16 hours in the dark, with vigorous stirring.[4] The insoluble byproducts were removed with a 0.22-μm filter, and the

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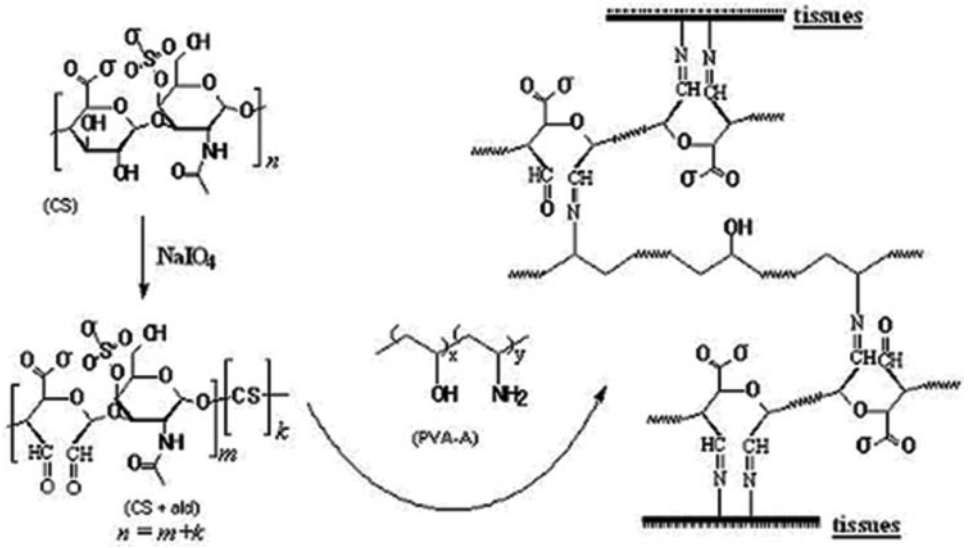

FIGURE 1. Schematic representation of tissue adhesive formulation. The adjacent hydroxyl groups of chondroitin sulfate (CS) backbone undergo oxidation into aldehyde groups by mixing it with sodium periodate ($NaIO_4$). PVA-A acts as the amine provider for bridging the CS-aldehyde adhesive and the tissues. On the tissue surface, a Schiff-base reaction occurs between the aldehydes of CS-aldehyde and the amines. The adhesive is formed and tissue integration occurs.

product was loaded into a Sephadex G-25 (Sigma-Aldrich) size-exclusion chromatography (SEC) column, by which the product was purified of water-soluble byproducts and unreacted small molecules. The product, CS-aldehyde, was obtained by lyophilization with a yielding rate of ~90%. The determination of the degree of aldehyde substitution was performed by hydroxylamine hydrochloride titration.[5] The gluing component of the adhesive was a 40% water solution of CS-aldehyde, which is transparent to light and slightly yellow.

The bridging component, 10% PVA-A, was used to overlay the CS-aldehyde once it was applied and component was intentionally stained blue with an albumin-specific and biocompatible dye (Cibacron Blue; Sigma-Aldrich). Staining the bridging component permitted direct observation of the polymerized glue relative to the incision and ensured that the glue did not gain entry into the anterior chamber.

Surgical Procedure

Experimental Setting. Nine adult New Zealand White rabbit heads were obtained from a local abattoir and were used for study. The heads were stored for 12 hours in a cool, moist chamber (4°C) before the eyes were enucleated. The globes were surgically removed, leaving sufficient conjunctival skirt as an aid for fixation. To minimize movement, which can otherwise affect pressure readings on the manometer, each globe was mounted on a metal base supporting a receptacle filled with polystyrene foam. The remaining conjunctiva was then secured in all four quadrants with pins (Fig. 2). A 27-gauge needle (BD Biosciences, Franklin Lakes, NJ) connected to an infusion system with a physiologic saline–filled bag (Balanced Salt Solution [BSS]; Abbott Laboratories, Abbott Park, IL) was inserted at the 3 o'clock position relative to the surgeon's view and in a parallel direction to the iris plane. A second 27-gauge needle attached to a digital manometer (Digimano 1000; Netech Corp., Hicksville, NY) was introduced into the anterior chamber in a position 180° away from the first needle. The BSS bag height was adjusted accordingly, with a modified pulley system to maintain an IOP of 18 to 22 mm Hg.

IOP was subsequently increased in a stepwise fashion by raising the infusion bag height and allowing the pressure to stabilize at four different levels: 4 (level 1), 80 (level 2), 120 (level 3), and 160 (level 4) cm above the eye. By using this maneuver, we determined the maximum IOP achievable with the experimental setup.

Under microscopic visualization (Möller Ophthamic 900; Haag-Streit AG, Wedel, Germany), a 3-mm-wide uniplanar stab incision was made parallel to the iris with a disposable 45° angle keratome (Beaver; BD Surgical Systems, Franklin Lakes, NJ). The incision was performed at 90° away from the needle ports and was designed to leak spontaneously. The leak pressures were read from the digital manometer and subsequently recorded. The incision site was dried with a sponge (Weck-Cel; Edward Weck, Inc., Research Triangle Park, NC), and an air bubble was used to reform the anterior chamber and displace the remaining fluid.

Study Group. Next, a 2.5-mm, straight, rounded-tip, crescent knife (Beaver; BD Surgical Systems) was used to apply the bridging component of the adhesive (PVA-A) to the wound margins. A thin layer was used to coat the surface of the incision and the internal wound lip, approximately 0.5 mm in from the outer wound edge. With a second crescent knife, a thin layer of CS-aldehyde was then applied over the first layer. The two components were allowed to polymerize for 30 seconds. Once the glue solidified, saline was infused to displace the air bubble. The previously described stepwise increments in IOP were attempted, and the maximum pressure achieved before wound leakage was subsequently recorded.

Control Group. The control group was composed of 10 eyes, further divided into two subgroups, consisting of five eyes each. All the eyes in the control group were sealed with 10-0 nylon sutures (Shar-

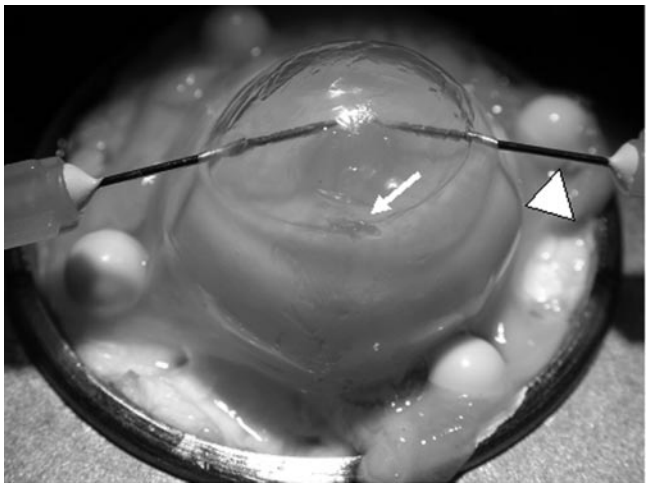

FIGURE 2. Experimental setup. Rabbit eyes were mounted to a metal base. The conjunctival skirt was secured with four pins to a polystyrene base, to minimize movement of the globe (*arrowhead*). Two 27-gauge needles, attached to a bottle of saline and a digital manometer, were inserted into the anterior chamber 180° away from each other. An air bubble was used to reform the anterior chamber before application of the glue (*arrow*, darker area).

point; Surgical Specialties Corporation, Reading, PA). A single interrupted suture was placed in the eyes of one subgroup, whereas the other subgroup received three interrupted sutures. These control eyes were all subjected to the same trials as the study group, to detect leak pressure. The maximum pressure achieved without producing leakage was recorded from the manometer.

Confocal Microscopy

The glued eyes mounted on polystyrene foam were examined with a tandem, white-light, scanning confocal microscope (Confoscan III; Nidek Technologies America, Inc., Greensboro, NC) to document the morphology of the corneal wound after application of the glue. To image the corneal tissue, sections at approximately 10-μm thickness intervals were used. Photographs were taken at a magnification of 40×, using the standard focusing objective lens (Carl Zeiss Meditec, Dublin, CA) and were then analyzed on computer (Nidek Advanced Vision Information Software [NAVIS]).

Statistical Analysis

Comparisons between groups were made on computer (StatsDirect, ver .2.4.1; Cheshire, UK). Comparisons between maximum resisted IOPs before leakage were performed with the nonparametric Mann-Whitney test.

Results

Strength Testing of Corneal Incisions

The mean baseline IOP measurement in the intact globe (before the incision was created) was 102.2 ± 2.6 mm Hg (SD) at a maximum height of 160 cm above the level of the eye. This pressure and height was considered sufficient to achieve a watertight sealing effect of the tissue adhesive for corneal wounds.

The nonsealed incision IOP was measured in all eyes immediately after the corneal incisions were performed in all groups. The mean IOP was 5.6 ± 1.1 mm Hg (SD) in globes under these circumstances, when all eyes spontaneously leaked.

In the control groups, the corneal incisions were considered sealed with either one or three sutures in place. The mean nonsealed incision IOP for this group was 5.3 ± 2.2 mm Hg. The mean maximum IOP resisted in the single interrupted suture subgroup was 26.4 ± 6.0 mm Hg (SD), with a range from 21 to 35.6 mm Hg. In eyes that were sealed with three sutures, the mean IOP was 44.3 ± 8.2 mm Hg, with a range from 32 to 53.4 mm Hg. The difference between the maximum resisted IOP was significant between these two groups (P = 0.008).

None of the eyes in the glue group leaked when subjected to the maximum attainable IOP permitted by the experimental setup. The maximum IOP achieved in eyes that received the glue was 104.7 mm Hg with a mean of 101.4 ± 3.2 mm Hg. This pressure was more than four times higher than that obtained in the single-suture group and was more than twice that in the three-suture group.

Confocal Microscopy Findings

Confocal microscopy was used to examine the glue at the incision site. The presence of the glue was demonstrated between the wound edges, present only in one third of the external aspect of the wound. A thin amorphous layer of adhesive served to bond the two stromal layers together. The glue was observed as a homogeneous substance with a less dense signal than stroma inside the wound edges (Fig. 3).

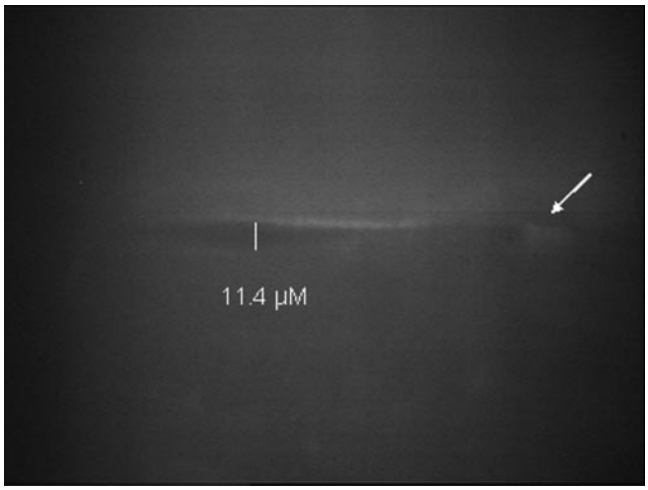

Figure 3. Confocal microscopy. Wound gape is demonstrated in areas without glue (*vertical line*). In areas that have been adequately sealed, the adhesive can be seen in the intrastromal area bridging the gap between the two wound edges (*arrow*).

Discussion

The goal of corneal wound repair is to achieve a watertight seal and an adequately formed anterior chamber devoid of synechia. In addition, correct apposition of the two separated surfaces is crucial to the wound-healing process. Presumed self-sealing corneal incisions, currently popular among surgeons, have been shown to gape in response to fluctuating IOPs.[6–8] This transient gaping may explain the increase in the rate of endophthalmitis associated with phacoemulsification and clear corneal incisions.[9–11]

Tissue adhesives have evolved and are continuously being improved to surpass the problems that are encountered with sutures. As early as 1968, adhesives for ophthalmic use had already been reported.[12] Fibrin-based adhesives and cyanoacrylate glues, although both far from being ideal, have been extensively used in ophthalmic surgery.[13–15] Photocured glues have the advantage of controlled polymerization through an external source of energy and have also been studied and used for ophthalmic procedures.[16–18] Temperature-controlled photothermal welding, in which collagen denaturation is used to cause tissue adhesion, has also been used experimentally on aortic,[19] gall bladder,[20,21] and corneal tissue.[22,23] Photosensitizers with laser irradiation have likewise shown success in corneal repair.[24] However, in our work, a novel self-polymerizing ophthalmic tissue adhesive was evaluated in corneal incisions, with the advantage of eliminating secondary sources of activation and possibly providing a more biocompatible effect.

Rabbit corneas are much thinner than human, making it difficult to create a valved, self-sealing incision. To test the sealing capacity of the CS-aldehyde adhesive fully, the intention was to fashion a wound that would tend to leak continuously and not seal spontaneously. Our results show that when compared with sutures, CS-aldehyde was able to attain much higher IOPs.

Although our results are not strictly comparable to those in other experimental models using different bioadhesives, we were able to demonstrate a superior sealing effect of the CS-aldehyde glue compared with standard suturing, which is the current method for the regular synthesis of corneal wounds. This may represent a significant improvement in corneal surgery if other aspects of safety and toxicity of this adhesive are found to be compatible with the living eye.

One of the major limitations of this study, however, was the experimental setting. Using a modified pulley system to lift the saline infusion (BSS; Alcon Laboratories) at regulated intervals, the maximum pressure achieved, although well above the normal IOP, was not enough to cause leakage in the glued eyes. Therefore, we did not include the tissue glue group in the statistical analysis, as none of these eyes leaked at the maximum achievable pressure in our trials. Further studies are needed to determine the true leak pressure of this adhesive with higher pressures (i.e., using a constant-flow mechanical pump to generate more extreme levels of IOP).

Although the toxicity of the adhesive cannot be addressed by this experimental design, studies with fibrin glue have shown that a biological substrate may promote faster healing and would be better tolerated by ocular tissues.[25] CS has already been studied in rabbit maxillary sinus mucosa where CS-modified hydrogel induced epithelial regeneration and served as a repository for cytokines and growth factors to promote wound healing.[26] To our knowledge, this is the first report using CS-based adhesive for ophthalmic purposes.

In conclusion, a non–energy-requiring, modified CS-aldehyde adhesive was shown to be effective ex vivo for sealing corneal incisions in rabbit eyes and may be superior to sutures for this purpose. Before clinical applications of this adhesive are proposed, further in vivo studies are needed to determine its toxicity and biodegradation in models of corneal wound healing.

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