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New thin-film adhesive for sealing full-thickness corneal incisions in rabbits

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Purpose: To compare the repair of penetrating corneal incisions in an in vivo rabbit model using a laser-activated thin-film adhesive, sutures, or self-seal.

Setting: The University of Sydney, Camperdown, Australia.

Design: Animal studies.

Methods: Under an operating microscope, 2.0 mm penetrating incisions were created in 162 right eyes. Incisions in one group were repaired with the adhesive, the second group received a single 10-0 nylon suture, and the third group was left to self-seal. Rabbits were killed humanely at predetermined timepoints over 2 weeks, and wound healing was assessed using burst pressure and immunohistological studies. A modified McDonald-Shadduck scoring was used to assess eyes.

Results: The mean burst pressure of the adhesive group was significantly higher than the sutured or self-sealed groups at all timepoints within the first 72 hours. At 0 hour, the burst pressure was 98.0 (±17.0) mm Hg, 30.6 (±2.1) mm Hg, and 3.8 (±0.6) mm Hg ($P < .00001$) for adhesive-treated (n = 5), sutured (n = 5), and self-sealed wounds (n = 5), respectively. These increased to 229.0 (±53.7) mm Hg, 12.4 (±2.9) mm Hg, and 27.3 (±4.0) mm Hg ($P = .0011$) at 72 hours. The modified McDonald-Shadduck score was significantly higher for eyes repaired using the adhesive than those sutured or left to self-seal for the first 72 hours. On histology and immunofluorescence, adhesive treatment demonstrated better wound approximation and higher myofibroblastic activation than the other groups.

Conclusions: The adhesive was efficacious in sealing penetrating corneal incisions and tolerated higher burst pressures than sutures or self-seal. The adhesive was biocompatible in rabbits, and incisions demonstrated a rapid gain in wound strength that sustained over the study period.

J Cataract Refract Surg 2022; 48:355–362 Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of ASCRS and ESCRS

Full-thickness corneal wounds disrupt the integrity of the eye and allow ingress of pathogenic organisms that may be present on the ocular surface. Entry of significant pathogen load into the sterile intraocular environment was postulated to cause sight-threatening endophthalmitis.[1] In the United States, there was a rise in the incidence of postoperative endophthalmitis from 1.79 per 1000 prior to 1994 to 2.47 per 1000 in 2001 for routine cataract surgeries.[2] This trend coincided with the popularization of self-sealing clear corneal incisions (CCIs) in 1994, reported by Fine.[3] In 2002, self-sealing CCIs were the preferred method for 57% to 66% of respondents performing routine cataract surgery at the American Society of Cataract and Refractive Surgery conference, whereas 92% preferred sutureless wound closure for cataract surgeries.[4] However, a leaking CCI 1 day postoperatively carries a higher risk (44 times) of endophthalmitis when compared with a CCI that did not leak.[5]

Sutures remain the gold standard in multiple medical specialties because of the lack of better alternatives.[6] Sutures inflict trauma to surrounding normal tissue, cause a foreign body reaction, and increase the risk for infection.[7] Uneven tension over the suture can cause corneal deformation resulting in irregular astigmatism.[8] Fibrin glue provides poor sealing, carries a risk for disease transmission, is time consuming to apply, and has strict storage and handling requirements.[9] Cyanoacrylate adhesives seal strongly but produce a rough surface on curing, necessitating the use of bandage contact lens for comfort.[10] Their rapid polymerization on the ocular surface makes accurate application a challenge.[11] They are also nonbiodegradable and cytotoxic and incite inflammatory reactions.[10]

Submitted: April 21, 2021 | Final revision submitted: July 2, 2021 | Accepted: July 12, 2021

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Funded by National Health and Medical Research Council (NHMRC) project grant (APP1067749 to L.J.R. Foster and S.L. Watson). S.L. Watson is a Sydney Medical School Foundation Fellow.

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0886-3350/$ - see frontmatter
https://doi.org/10.1097/j.jcrs.0000000000000751

ReSure and OcuSeal are polyethylene glycol (PEG)-based synthetic ocular surface sealants developed to close leaking corneal incisions after cataract surgeries. In a randomized clinical trial, ReSure was superior to sutures in sealing leaky corneal wounds with 1-ounce force provocation.[12] ReSure sealant remained in 31.3% of eyes 3 days postoperatively and in only 2.6% of eyes 7 days postoperativel.[12] It is unclear whether this is an adequate time for wound healing. Although OcuSeal was well tolerated on the ocular surface when compared with sutures and stromal hydration, the postoperative intraocular pressure differences did not demonstrate statistical significance.[13,14]

We developed a new chitosan-based, thin-film adhesive to seal full-thickness corneal incisions.[15] This investigational adhesive film uses indocyanine green (ICG) as a chromophore to harness laser energy. The natural tissue adhesive property of chitosan is significantly enhanced through irradiation with near infrared laser. It is postulated that the laser energy induced transient heat expansion and unweaving of the chitosan and collagen polymer chains, allowing them to interweave on subsequent cooling, increasing the interface adhesion.[9,15–19] Commercial chitosan is a polysaccharide often derived from deacetylated chitin, which is abundantly found in crustacean shells and does not carry any risk of transmitting infections.[19] Chitosan is a U.S. Food and Drug Administration (FDA)-approved product for dietary use and wound dressings.[20] Indocyanine green is also FDA-approved as an intravascularly injectable medical dye for retinal angiography, lymphangiography, and tumor imaging.[21,22]

Previous in vitro studies using this chitosan-based adhesive have demonstrated its ease of use, strong tissue adhesion, and cellular biocompatibility.[19] Subsequent in vivo studies showed its potential for the rapid repair and enhanced healing of transected sciatic nerves in rats.[17,23] In addition, we have reported on the efficacy of this technology for sealing penetrating corneal incisions up to 6.0 mm in an ex vivo bovine model.[9,24] This technology can also be adapted to deliver antimicrobial drugs and other pharmaceutical agents directly at the wound site.[25]

In this study, we evaluated the sealing and healing efficacy of this adhesive in an in vivo rabbit model. As the intended use for this technology was for closing leaking CCIs for routine cataract surgeries and a Cochrane review of cataract surgery corneal incision sizes by Jin et al. reported dimensions of <1.5 mm, 1.8 mm, 2.2 mm, and 3.0 mm, we chose 2 mm incisions in the relatively smaller rabbit eye to investigate this technology.[26] Stepped incisions were found to tolerate fluctuation of intraocular pressure better than uniplanar incisions.[27] The rabbit eye had been evaluated for cataract surgeries and corneal transplantation and was selected for its comparable sensitivity to injury and recovery times as their human counterparts.[28–30] The New Zealand white rabbit's cornea has anatomical features similar to those of a human eye.[31,32] Using the highest tolerable fluid pressure as a quantitative measure for wound strength, we compared corneal incisions repaired using this adhesive with the current practice of using sutures or left to self-seal. A modified McDonald-Shadduck (mMS) scoring system was used to grade ocular clinical findings at each timepoint for tolerability.[33–35] Further histological and immunofluorescence assessment was also conducted on corneal healing at each timepoint.

METHODS

Animals

Healthy New Zealand albino rabbits of mixed sex were obtained from Piper's Farm (Cowra, New South Wales, Australia), weighting between 1.5 kg and 2.5 kg and aged at least 6 weeks old to ensure head maturity.[28] One hundred sixty-two rabbits were acclimatized with unlimited access to standard chow and water for at least 1 week before any procedure. All procedures conformed to the Association for Research in Vision and Ophthalmology statement for humane use of animals in vision research. Institutional ethics review board approval was obtained prior to commencing any work (University of Sydney AEC 2018/1335).

Anesthesia and Wound Creation

Procedural sedation and anesthesia were provided by a specialized veterinary anesthetic team. All procedures were performed aseptically under an operating microscope. The region around each right eye was prepared with diluted povidone–iodine (1% wt/vol). A sterile eyelid speculum was used to open the right palpebral fissure. Topical oxybuprocaine hydrochloride (0.4%), atropine sulfate (1%), phenylephrine hydrochloride (2.5%), and chloramphenicol (0.5%) were instilled over the ocular surface. All eyedrops were single-use Minims from Bausch & Lomb, Inc.

A central horizontal 2 mm penetrating corneal incision was created using a sterile size 11 scalpel blade. Penetration was visually confirmed by the rapid efflux of aqueous fluid and collapse of the anterior chamber. Incision size was externally and internally confirmed using a Castroviejo caliper. To benchmark this technology, uniplanar incisions were used instead of stepped incisions to make the wounds more susceptible to leaking.

Postprocedure analgesia was topical buprenorphine (25 μg/h) for all animals, with additional subcutaneous meloxicam (1 mg/kg) given at the discretion of the veterinary team based on animal welfare.

Treatment Groups

Rabbits were randomly divided into 3 treatment groups: (1) adhesive film, (2) sutures, and (3) self-sealing wounds. For rabbits in group (1), the epithelium around the 2 mm incision was mechanically debrided to expose a 4 × 6 mm oval-shaped corneal stroma. The ocular surface was dried with sterile cellulose spears, and the adhesive was centrally applied over the incision. The adhesive was manipulated for fit prior to activation. Adhesives (4 × 6 mm oval films) were obtained from Repartech Pty Ltd. (Sydney), sealed in foil packs.[15] A continuous-wave near infrared gallium–aluminum–arsenide diode laser (810 nm, 125 mW, model: MDL-III-808-1W; CNI Optoelectronics Tech Co., Ltd.) was used to activate the adhesion. A 1 mm diameter beam spot was applied to the adhesive film using a handheld fiber optic probe moving at a speed of approximately 1 mm/s. Confirmation of adhesion was visualized by a gentle warping of the film onto the exposed stroma.

In treatment group (b), incisions were repaired using a single simple interrupted 10-0 nylon suture (Ethilon* CS140-6 6.5 mm 3/8 c Spatula). The needle was passed 0.75 mm from the wound edge, at 80% to 90% stromal depth, and tied using 2-1-1 knot architecture with the knot buried. In the third group (c), incisions were left to self-seal to simulate the natural history of full-thickness corneal laceration healing.

In all animals, a bandage contact lens (PureVision [balafilcon A]; Bausch & Lomb, Inc., BC 8.6 mm, 0 diopter) was applied over the wound repair and under the nictitating membrane. Tarsorrhaphy was performed for comfort and injury prevention for all treated eyes.

Timepoints and Eye Assessment

Six rabbits from each group were killed humanely at 0 hour, 3 hours, 6 hours, 9 hours, 12 hours, 24 hours, 72 hours, 168 hours, and 336 hours postoperatively with an intravenous overdose of sodium pentobarbitone. These timepoints were determined from the previous rodent studies (UNSW ACEC 10/49A).[36] Five eyes were used for burst pressure testing, and 1 for histological studies. An mMS scoring system and a portable slitlamp was used to assess the right eyes at killing.[33–35] In brief, mMS was scored by the total of conjunctival congestion (0 to +3), conjunctival swelling (0 to +4), conjunctival discharge (0 to +3), aqueous flare (0 to +3), iris involvement (0 to +4), corneal opacification by severity (0 to +3), corneal opacification by surface area (0 to +4), corneal vascularization (0 to +5), and corneal fluorescein staining type (0 to +4).[33–35]

Burst Pressure Testing

The right corneoscleral rim was gently dissected and mounted on a Barron artificial anterior chamber. The ciliary body and iris were removed to prevent obstruction to the internal wound lips. Saline (NaCl 0.9%) was infused at 10 mL/h into the chamber, while fluid pressure was continuously read by a digital manometer (Phillips IntelliVue X2). Burst pressure was defined as the highest tolerable intraocular pressure obtained at the first indication of wound leak by the observation of Seidel sign, using a portable slitlamp. The maximum recordable pressure by in this experimental setup was 360 mm Hg.

Histological Analysis

Dissected corneoscleral rims were immediately transferred into 10% neutral buffered formalin and fixed for 10 hours. Fixed corneal tissues were then serially dehydrated through increasing concentrations of ethanol (50% to 100%) and then cleared in xylene, using an automatic tissue processor (Excelsior ES tissue processor; Thermo Scientific), as previously decribed.[37] Corneal tissues were trimmed to localize the corneal incisions before embedding in paraffin wax. A manual microtome (Reichert-Jung Histocut 820, Leica) was used to produce 7 μm thick sections for the histological analysis.

Representative midsagittal tissue sections screened under dark-field illumination containing the corneal incision were deparaffinized and hydrated to distilled water. Periodic acid-Schiff staining was performed as per the study by Lovicu et al.[37] After staining, the sections were permanently mounted using DePex mounting medium, viewed under a Leica DMLB microscope, and photographed using a Leica camera (Leica DFC-280).

Alpha Smooth Muscle Actin Immunolabeling

Corneal sections were labeled for alpha smooth muscle actin (αSMA) reactivity as per the study by Lovicu et al.[37] In brief, tissue was deparaffinized and hydrated as described earlier, prior to two 5-minute washes with phosphate-buffered saline (PBS) supplemented with 0.1% bovine serum albumin (BSA). Blocking with 3% normal goat serum was directly applied to sections at room temperature for 30 minutes. A primary antibody against alpha smooth muscle (αSMA, Sigma-Aldrich Corp., mouse monoclone 1A4, catalog # A5228) was then applied (10 μg/mL) directly to sections and left in a humidified chamber at 4°C for at least 12 hours. Unbound antibody was removed by washing with PBS/BSA (3 × 5 minutes). Goat anti-mouse Alexa-Fluor 594 IgG (Abcam) was then applied as the secondary antibody (1:600 dilution in PBS/BSA) and left in the dark (2 hours, 22°C). Unbound secondary antibody was removed by washing with PBS (3 × 5 minutes). Sections were counterstained with 0.3 μg/mL bisbenzimide (Hoescht dye 33342; Sigma-Aldrich Corp.) for 5 minutes prior to mounting with 10% PBS (vol/vol) in glycerol. Sections were viewed using a Leica DMLB epifluorescent microscope and photographed using a Leica camera (Leica DFC-280). Composite photographs of αSMA immunohistochemistry was overlaid with periodic acid-Schiff histology from representative sections using computer software (Fiji, ImageJ) to show the spatial distribution of αSMA and cell nuclei.[38]

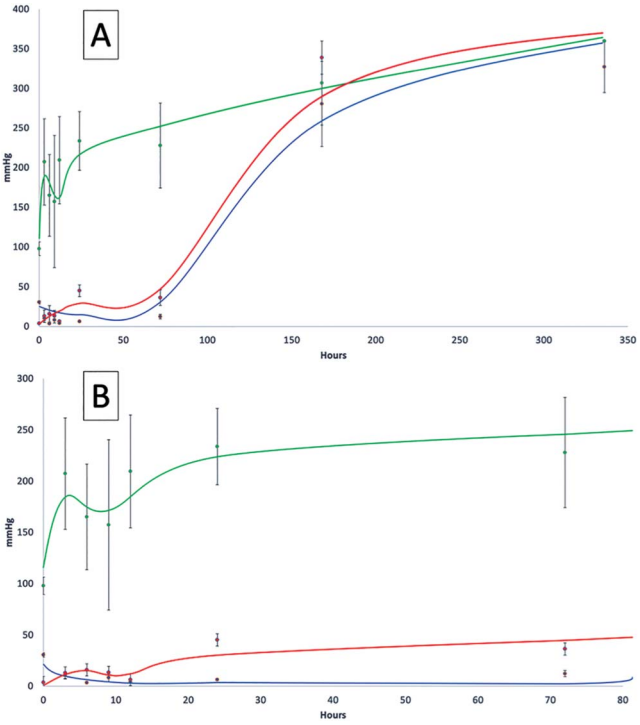

Figure 1. The mean burst pressure tolerated by 2 mm penetrating corneal incisions closed using adhesive (*green*), suture (*blue*), or left to self-seal (*red*) over 336 hours (14 days) (*A*) and during the first 72 hours (3 days) (*B*).

Statistical Analyses

The quantitative outcomes in this study were burst pressures and mMS scores with time, between adhesive treated, sutured, and self-sealed incisions. Standard error of the mean was reported after determination of the sample mean. Statistical significance was determined using analysis of variance at 95% CI ($P \leq .05$). All values were reported to 3 significant figures.

RESULTS

Burst Pressure and mMS

The adhesive was successfully applied in all 54 eyes of the treatment group (a). The mean duration of laser exposure required to activate the adhesive was 39 (±1.5) seconds. The rabbit's nictitating membrane was observed to prematurely dislodge the adhesive. The adhesive was adherent on 6 of 6 (100%) corneas at 0 hour, 5 of 6 (83.3%) corneas at 3 hours, but all dislodged from 6 hours onward. Despite the premature dislodgement, the burst pressure tolerated by the adhesive-treated incisions remained statistically higher than that by sutured or self-sealed incisions immediately after application and up to 72 hours postoperatively. No statistically significant differences between average burst pressures were observed at 9 hours, 168 hours, and 336 hours (P = .127, .686, and .397, respectively). Figure 1 demonstrates the trend of mean burst pressures for 2 mm penetrating corneal incisions repaired using the adhesive

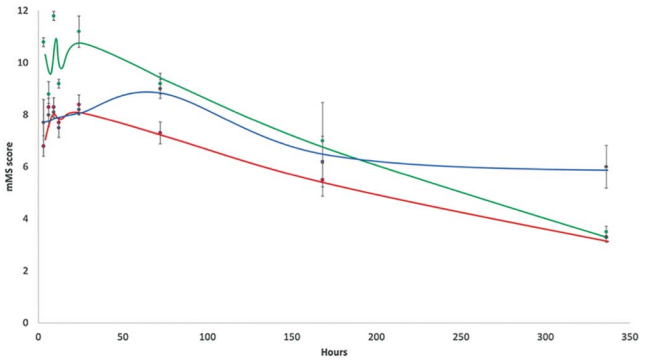

Figure 2. The trend of mean mMS score of eyes with 2 mm penetrating corneal incisions closed using adhesive (*green*), suture (*blue*), or self-seal (*red*) over 336 hours (14 days). mMS = modified McDonald-Shadduck

(a), suture (b), or left to self-seal (c). The large variation of recorded burst pressures at 9 hours was likely due to human error during tissue collection. At 168 hours and 336 hours, most the burst pressure readings exceeded the maximum recordable pressure of 360 mm Hg, resulting in no statistically significant difference observed.

Eyes treated with the adhesive had significantly higher mMS scores than those treated with sutures or self-sealed, up to 72 hours postoperatively. This trend is shown in Figure 2. At 336 hours, the mean mMS scores were comparable between eyes treated with the adhesive and those self-sealed, whereas the score remained elevated for eyes that received sutures, and this difference was statistically significant (P = .00297).

Histology

Full-thickness incision tracks were visible in all sections, with stromal edema immediately adjacent to the incision track evident by the widening of spaces between corneal lamellae up to 72 hours (Figure 3). Corneal edema was largely resolved beyond 168 hours (7 days; Figure 4). There was a strong fibrinous reaction for the first 72 hours after wound creation (Figure 3, A, C, D). The fibrin plug occluded and bridged the posterior wound edges. The histological sections from the first 72 hours suggest a degree of epithelial ingrowth from the surface epithelium along the anterior wound lip, and this terminated posteriorly at the fibrin plug (Figure 3, A, C, D, F).

In corneas treated with the adhesive, the fibrinous reaction seemed less intense than wounds closed by self-sealing or sutures, and wound edges appeared well approximated with a minimal stromal defect (Figure 3, B, E). Although denuded stroma was readily visible after epithelial debridement for placement of the adhesive at 24 hours (arrow in Figure 3, B), epithelia of these corneas had regenerated by 72 hours (arrow in Figure 3, E). Minimal epithelial ingrowth was observed in corneas treated with the adhesive at 72 hours (Figure 3, E).

From 72 hours, the anterior edge of all corneal incisions was covered with regenerated sheets of epithelium. Figure 4, H shows an exaggerated epithelial plug in a corneal wound treated with the adhesive at 168 hours. This was not observed in other similarly treated corneas and was most likely a consequence of wound dehiscence (arrows in Figure 4, H). For animals in the adhesive group, keratocyte accumulation alongside the incision was observed at 72 hours after repair (Figure 3, E). By contrast, keratocyte accumulation was comparatively limited in wounds of the self-sealed or sutured group at this timepoint (Figure 3, D, F). At 168 hours, keratocyte accumulation along incision track was observed in all 3 groups, and the regenerated epithelium was thicker over the incision than that at the periphery (Figure 4, G–I). At 336 hours, corneas in the adhesive and self-sealed groups showed healthy scar formation, but those repaired using sutures showed irregularities in the thickness of the overlying epithelium and stromal breakdown along the suture track (Figure 4, J, K, L).

Immunofluorescent Labeling of αSMA

Alpha Smooth Muscle Actin (UniProt: P62740) was abundantly expressed by the epithelial layer of all 3 groups throughout the study (Figures 5 and 6). At 24 hours, epithelia were not regenerated after debridement in the adhesive group; consequently, no αSMA expression was detected (Figure 5, B). In the sutured and self-sealed groups, αSMA-positive epithelial cells were observed to fill the anterior stromal defects (Figure 5, A, C). Epithelial cells were observed to fill the anterior stromal defect at 72 hours in the adhesive group (Figure 5, E).

Limited αSMA reactivity was detected in the corneal stroma of the sutured and self-sealed groups at 24 hours and 72 hours, with αSMA-labeling predominantly found immediately adjacent to the incision tracks (Figure 5, A, C, D, F). This distribution most likely represented quiescent keratocytes transforming into myofibroblasts.[39] In the adhesive group, tissue sections did not demonstrate any αSMA reactivity at 24 hours, but at 72 hours, limited αSMA labeling was observed within the stroma, alongside the incision track (Figure 5, B and E respectively).

At 168 hours, αSMA labeling was observed alongside the incision tracks of all 3 groups (Figure 6). In the adhesive-treated eye complicated by wound dehiscence, stromal αSMA labeling was similarly abundant along the incision track (Figure 6, H). At 336 hours, the density of stromal αSMA labeling appeared similar for incisions repaired using sutures and self-sealed; however, the density was noticeably greater in the adhesive group (Figure 6, J, K, L).

DISCUSSION

We developed a chitosan-based, thin-film infrared laser–activated adhesive to close penetrating corneal incisions.[9,15,24,25] This investigational adhesive sealed 2 mm penetrating corneal incisions in an in vivo rabbit model and tolerated significantly higher burst pressures than sutured or self-sealed repairs. The tolerated burst pressure in the adhesive group continued to rise over 72 hours and was maintained over the 2-week study period. Eyes repaired using the adhesive had higher mMS scores than sutured or

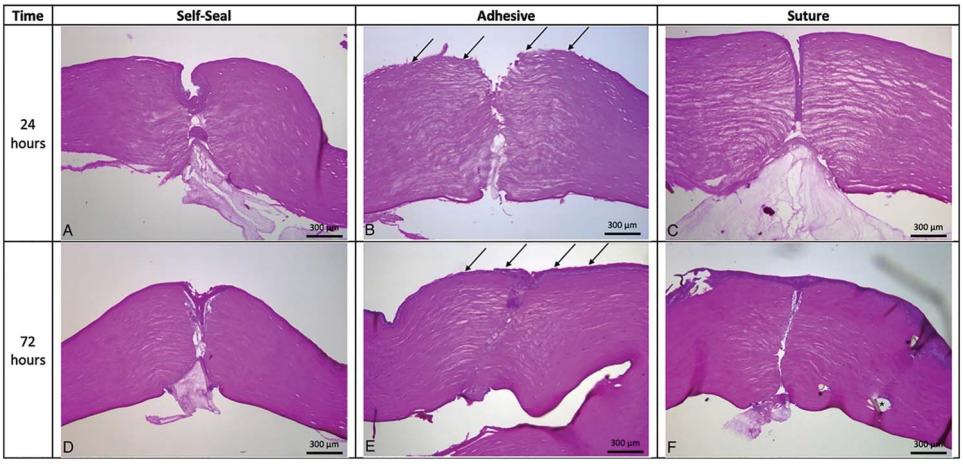

Figure 3. Representative periodic acid-Schiff light microscopy of penetrating corneal incisions in rabbits treated with self-sealing (*A*, *D*), adhesive (*B*, *E*), or suture (*C*, *F*) at 24 hours (day 1, *A-C*) and 72 hours (day 3, *D-F*). *Arrows* in *B* demonstrate denuded corneal stroma after epithelial debridement. *Arrows* in *E* demonstrate regenerated corneal epithelia after mechanical debridement. In *F*, a maturing but thickened epithelial sheet anteriorly, and good approximation of stromal lamellae, posterior wound gape persists with fibrin plug. The presence of cystic spaces around suture material is marked (*).

self-sealed for the first 72 hours. Our immunolabeling studies correlated with our burst pressure data, suggesting accelerated healing with rapid gain of wound strength.

In vivo studies reporting the burst pressure of corneal sealants/adhesives seems limited to the study by Khadem et al. who reported the use of 2 photodynamic laser-activated tissue glues to seal 6 mm corneal incisions in rats.[40] The mean burst pressure at day 1 for control, BSA/Chlorin e6, and BSA/Janus Green were 193 mm Hg, 357 mm Hg, and 430 mm Hg, but at 1 week, these were 232 mm Hg, 200 mm Hg, and 220 mm Hg, respectively.[40] They reported statistically higher burst pressures in incisions treated with their glue than control at 1 day, but no statistically significant difference was observed after 1 week and 2 weeks.[40]

In this study,, the mean burst pressure for the adhesive group was 3 times greater than that of the suture group. The mean burst pressure in the adhesive group gradually increased from 98.0 (±17.0) to 229 (±53.7) mm Hg over the first 72 hours. The mean burst pressures measured in the sutured and self-sealed treatment groups increased to similar levels as those in the adhesive group from 168 hours. No statistically significant difference between burst pressures were observed at 168 hours and 336 hours due to the ceiling (360 mm Hg) of our experimental setup. The immunolabeling findings correlated with our burst pressure data and revealed that corneal incisions repaired using the adhesive had closely approximated wound edges early after treatment and had higher myofibroblastic activity alongside the incision track at the end of the study.

The mMS score was used as a measure of tolerability. The mean mMS scores were significantly higher during the first 24 hours for eyes in the adhesive group than the sutured or self-sealed groups. From 72 hours, the mean mMS score for eyes in the adhesive group improved to that observed in the self-seal group, whereas the mMS scores in the suture group remained elevated. Epithelial debridement for adhesive application adversely affected the mMS score as the improvement of mMS scores coincided with epithelial healing, confirmed histologically at 72 hours.

The results reported in this study are consistent with our ex vivo bovine corneal model where penetrating 2.0 mm incisions repaired with the adhesive exhibited a mean burst pressure of 239.2 mm Hg.[24] This higher burst pressure was likely attributed to interspecies differences in corneal thickness, and the use of the same adhesive, but of a larger area (8.0 mm disc vs 4 × 6 mm oval).[24] Furthermore, we modified the adhesive application technique with the addition of epithelial debridement. This was performed to improve

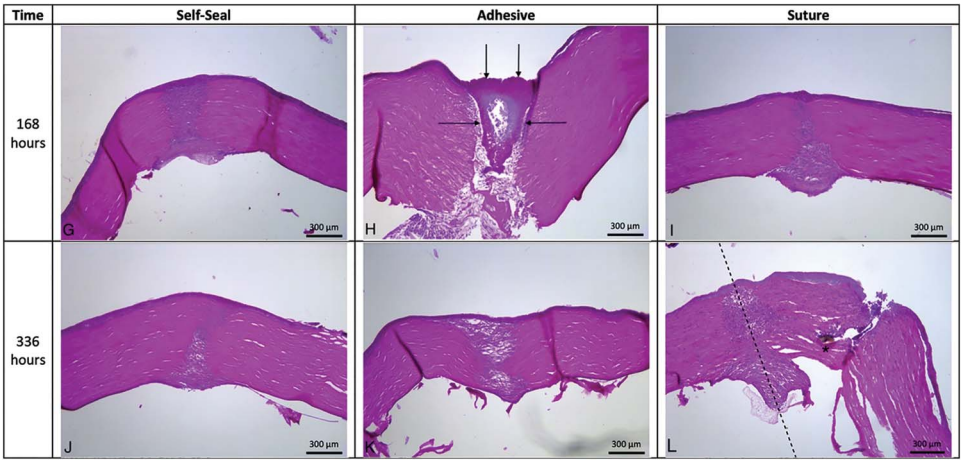

Figure 4. Representative periodic acid-Schiff light microscopy of penetrating corneal incisions in rabbits treated with self-sealing (*G*, *J*), adhesive (*H*, *K*), and suture (*I*, *L*) at 168 hours (day 7, *G-I*) and 336 hours (day 14, *J-L*). *Arrows* in *H* highlight the exaggerated epithelial plug that suggests wound dehiscence. In *L*, the incision axis is marked by a *dashed line* and suture material marked (*). There were irregularly thickened epithelial sheets, keratocyte accumulation along the incision track, and a strong inflammatory response within stroma, with stromal swelling and breakdown.

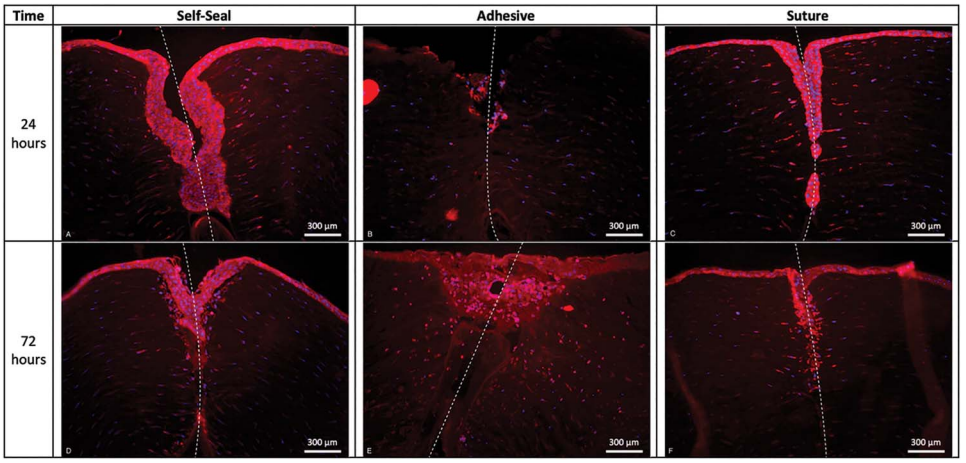

Figure 5. Representative composite images of immunofluorescence microscopy with alpha smooth muscle actin (*labeled red*) and Hoechst nuclear dye (*stained blue*) of penetrating corneal incisions in rabbits treated with self-sealing (*A*, *D*), adhesive (*B*, *E*), and suture (*C*, *F*) at 24 hours (day 1, *A-C*) and 72 hours (day 3, *D-F*). Incision tracks were labeled using a fine *dashed white line*.

adhesive retention on the cornea against the sweeping action of the nictitating membrane found in rabbits.

Shirzaei et al. investigated GelCORE, a gelatin-based corneal sealant, and compared it against ReSure, a PEG-based sealant, in an ex vivo rabbit model.[41] With 2.0 mm incisions, they reported burst pressures of 225.76 (±32.2) and 115.51 (±47.25) mm Hg, respectively.[41] In another ex vivo rabbit study, Kalayci et al. reported the application of SprayGel, another PEG-based sealant, to 2.0 mm incisions and determined a mean burst pressure of 128 mm Hg.[42] In contrast to these ex vivo corneal wound sealing studies that used fluid-based sealants, this study reported a solid, thin-film, laser-activated adhesive in an in vivo model.

The bonding of the adhesive to cornea was postulated to be laser-induced, transient thermal expansion of the chitosan and corneal collagen biopolymers at the contact interface, with subsequent entanglement on cooling.[9,15–19] ICG has been used as a chromophore for corneal welding.[43] Matteini et al. applied ICG 12% wt/wt water within the incision track to localize laser energy.[43] Because of the solid morphology of this adhesive, ICG was only in contact with the corneal surface, excluding internal wound edge welding as a possible mechanism of action. The interface temperature rise during the application of our adhesive film was approximately 54°C (±3.0), which was less than the irreversible denaturation temperature of collagen of 65°C.[25] With the 4.0 × 6.0 mm oval adhesive applied over 2.0 mm incisions, the interface temperature rise included healthy corneal tissue up to 2.0 mm from the incision edge. In this study, there were no clinical or histologic evidence of thermal coagulative necrosis to the anterior corneal tissue underneath the adhesive, immediately and up to 14 days postprocedure.

Quiescent keratocyte transformation into αSMA-positive myofibroblasts was largely dependent on the presence of growth factors from tear film, corneal epithelium, and endothelium, aided by the disruption to the epithelial or Descemet basement membrane from injury.[44] We observed a slight delay in labeling for αSMA alongside the incision track for the first 72 hours in the adhesive group, when compared with self-sealed and sutured groups. This observation could be attributed to the transient barrier effect by the adherent adhesive against signaling factors found within the tears and/or the loss of epithelium from mechanical debridement.[44] The adhesive was dislodged prematurely, and reestablishment of epithelial sheets was observed from

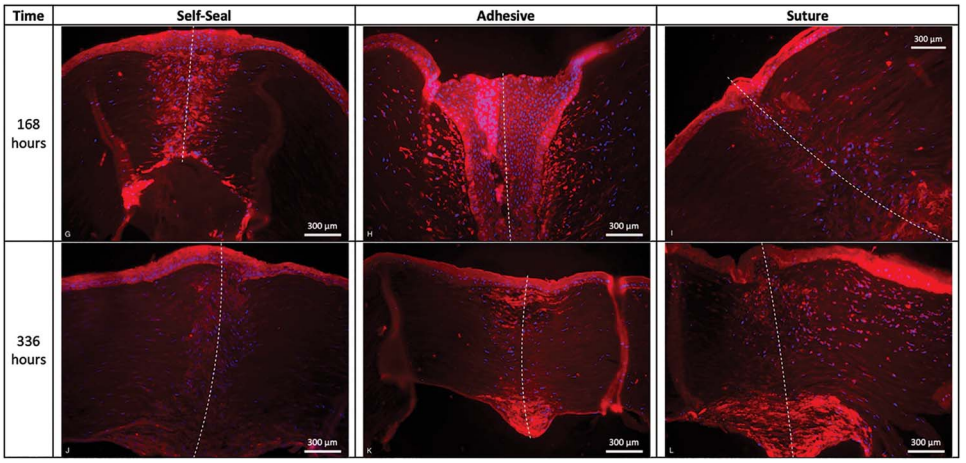

Figure 6. Representative composite images of immunofluorescence microscopy with alpha smooth muscle actin (*labeled red*) and Hoechst nuclear dye (*stained blue*) of penetrating corneal incisions in rabbits treated with self-sealing (*G*, *J*), adhesive (*H*, *K*), and suture (*I*, *L*) at 168 hours (day 7, *G-I*) and 336 hours (day 14, *J-L*). Incision tracks were labeled using a fine *dashed white line*.

72 hours after treatment and robust stromal αSMA labeling was observed from 168 hours. This delayed appearance of αSMA-positive myofibroblasts in the adhesive group did not affect the gain of wound strength with time.

This study demonstrated a quick, easy-to-use adhesive technology to effectively seal penetrating corneal incisions and contributes to the growing repertoire of corneal wound closure technologies in the preclinical phases.[45] The in vivo nature of this study assessed the sealing strength and the biocompatibility of this technology with an added healing dimension for assessing the efficacy of the corneal adhesive over time. This study design can be assimilated for future animal work evaluating corneal sealants or adhesives. Burst pressure was used as a quantitative measure of wound strength and resistance to fluid leak; however, because of differences in experimental setup, burst pressures were not comparable between studies. Finally, the presence of the nictitating membrane in the rabbit model was technically challenging for our film-type adhesive. Future studies could consider surgical removal of the nictitating membrane prior to treatment or the use of a primate model.

This study followed up rabbit corneal healing over 2 weeks. The chosen end points were extrapolated from our preliminary rodent study and to ensure sufficient time for healing.[36] Our histology study confirmed the obliteration of the incision track at 168 hours and correlated with a high mean burst pressure of 307.0 (±53.0) mm Hg. Our study was not designed to investigate long-term corneal scar maturation. To assess scar clarity at maturation, some authors had kept rabbits up to 6 months postprocedure.[46]

In translating this technology to the clinical setting, the need for localized epithelial debridement prior to adhesive application and measurement of burst pressure should be considered. Epithelial debridement was added to improve retention on the corneal surface against the rabbit's nictitating membrane. In humans, the absence of the nictitating membrane may not require epithelial debridement for use with this technology. If debridement is required, this could readily be performed at the slitlamp or in a procedure or operating room setting under local anesthesia. Debridement is envisaged to be similar to that performed for conditions such as recurrent corneal erosion.[47] Burst pressure testing is also not possible in clinical trials; however, a standardized provocation test as suggested by Masket et al. of using a calibrated force gauge delivering 1 ounce could compare the efficacy of this technology against other wound closure options.[48] Uniplanar incisions were used in our model. Stepped corneal incisions are designed to prevent postoperative wound leaks but are not universally successful.[49,50] Future studies could investigate the use of our device to further improve the performance of stepped incisions, particularly with limbal incisions where eyelid compression on opening and closing may have a greater role in leaks.

In conclusion, we have developed a laser-activated adhesive technology unique in its thin film morphology that provides uniform sealing and a quick and easy application. This adhesive sealed 2.0 mm penetrating corneal incisions immediately on application in an in vivo rabbit model. The burst pressures of incisions closed using this technology were significantly higher than incisions closed using sutures or self-sealed during the first 72 hours. The higher burst pressure in the adhesive group was maintained over the entire 2-weeks study period. This adhesive technology was also biocompatible, albeit with a higher modified McDonald-Shadduck score for the first 72 hours, when compared against the sutured and self-sealed incisions, because of epithelial debridement for adhesive application. Our immunolabeling studies correlated with the burst pressure data, suggesting an accelerated healing with rapid gain of wound strength in the adhesive group when compared with the standard techniques in current ophthalmic practice.

WHAT WAS KNOWN

- Leaking corneal incisions after routine cataract surgery had a 44-fold higher risk of infective endophthalmitis compared with incisions that did not leak.[5]
- To arrest wound leak, corneal sealants can be used. Currently available corneal sealants cure rapidly, and their precise application can be a challenge.
- Our new laser-activated corneal adhesive sealed 2 mm penetrating incisions in an ex vivo bovine model and tolerated a mean burst pressures of 239.2 mm Hg.[24]

WHAT THIS PAPER ADDS

- This new corneal adhesive sealed penetrating corneal incision immediately and tolerated higher burst pressures than sutured or self-sealed incisions.
- This adhesive was easily applied and biocompatible in an in vivo rabbit model, and wound strength, assessed by burst pressure measurement, increased over 7 days postapplication.
- Laser-activated corneal adhesives provide a potential new mode of sealing corneal wounds postcataract surgery and/or trauma.

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Disclosures: *L.J.R. Foster in an inventor on international patents regarding the adhesive: EP1796747, DK1796747T3US, and US9029349B2. The other authors report no financial or proprietary interests.*

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