Fibrin Sealant in Corneal Stem Cell Transplantation

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Purpose: To determine if transplanted corneal epithelial stem cells are safely and efficiently attached to the deficient limbal niche with use of fibrin sealant. The primary outcome is measured with respect to the stability of the transplant, with secondary qualitative evaluations of inflammation, patient comfort, speed of operation, and incidence of complications.

Methods: This retrospective case study examined a total of 114 corneal stem cell reconstructions performed in 95 patients from 1996 to 2004 using corneal stem cells primarily, with a minority of amnion alone, or both. Fibrin sealant was used as the only technique of stem cell adhesion for limbal reconstruction for primary or recurrent pterygia and various stem cell–deficient diseases from 2000 to 2004.

Results: The fibrin sealant group showed 1 small recurrence of pterygium but no complications. With sutures, there were 3 recurrences in the pterygia group. After completion of all surgical procedures, all patients were free of pterygia. Miscellaneous stem cell deficiencies were included to demonstrate that corneal stem cell transplants can be used in other corneal procedures in addition to pterygia.

Conclusions: Fibrin sealant alone effectively and safely attached corneal stem cell transplants to the limbal niche. The additional qualitative observations of a reduction in operation time, postoperative pain, and inflammation augurs for more extensive use of fibrin sealants in ophthalmology.

Key Words: fibrin sealant, pterygium, reconstruction, stem cell transplant

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Corneal stem cell deficiency is now a recognized clinical entity leading to a host of corneal diseases roundly referred to as ocular surface disease. That such a deficiency can significantly reduce vision and create severe ocular discomfort or pain is well documented.[1,2] Deficiency states develop from such disparate tissue injury classifications as traumatic, immunologic, and iatrogenic.[3–5]

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The recognition that corneal stem cell deficiency exists as an entity has stimulated attempts to restore this immensely important tissue to deficient eyes.[6] A variety of surgical approaches have evolved to select, harvest, and transport these tissues to their appropriate, natural anatomic niche at the recipient limbus.[2,7–10] A wide array of sutures and suturing techniques are used to affix these tissues to the episcleral tissues. Although generally successful, suturing thin tissues to the ocular tissues creates special problems related to tissue-to-tissue apposition, hemorrhage, inflammation, vascularization, and the discomfort to the patient from exposed sutures on the ocular surface.[11–13]

The goal of achieving optimal tissue apposition, using biologically suitable products, without sutures is an ideal to be sought. A variety of fibrin sealant products have been used experimentally, but only 1 commercial product, Tisseel, is FDA approved and generally available in most operating rooms.[14]

Herein, a series of patients are reported who underwent corneal stem cell transplant for a variety of diseases, principally pterygia. The fixation of these stem cell transplants, with sutures, from 1996 to 2000, is compared with a series fixated with a commercial fibrin tissue sealant (from 2000 to 2004) used as the only method of attachment to the underlying tissues.

The stability of the transplant, remarkable patient comfort, increase in operating speed, minimal inflammatory reaction, and lack of complications augur for conversion to this approach for corneal stem cell or other surface transplant surgery.

MATERIALS AND METHODS

Preparation of Fibrin Sealant

The fibrin sealant (Tisseel VH, Baxter Healthcare Corp, Deerfield, IL) was prepared according to the manufacturer's directions with 1 exception. In brief, color-coded vials are warmed for several minutes in a patented fibrotherm device. The procedure requires that the fibrinolysis inhibitor be added to the sealer protein concentrate vial and warmed. While this solution is stirring, the second component is prepared by injecting the contents of the of calcium chloride vial into the thrombin vial, which is then warmed. An exception to the manufacturer's instructions is then initiated. Only a small amount of the thrombin–calcium chloride solution is required to drive the reaction to fibrin formation. To slow the process of fibrin formation, only 0.1 mL of the thrombin–calcium chloride solution was withdrawn into a disposable syringe to which 0.9 mL of balanced salt solution (Acorn Inc, Decatur, IL) was added to achieve a 1:10 dilution. This syringe is placed into the diploject injector along with a parallel disposable syringe containing the fibrin sealer protein and fibrinolysis inhibitor. A mixer nosecone, topped by a blunt applicator needle, is attached

to the 2-syringe nozzle to facilitate mixing of the 2 syringe components. When the common plunger is depressed, the fibrin sealer solution and the thrombin solution are combined in the nosecone, in equal volumes, to form the resulting fibrin sealant that is directly applied to the designated tissues.

Corneal Stem Cell Surgical Technique for Pterygium

Under topical 4% Xylocaine (lidocaine hydrochloride, AstraZeneca LP, Wilmington, DE) anesthesia, the patient was prepared and draped in the usual fashion. Two percent Xylocaine was injected under the base of the pterygium and massaged under the pterygium to the limbus with a cotton-tipped applicator. The pterygium head was dissected from the cornea by slipping a spatula under its attachment at the limbus, above and below, bluntly separating the pterygium from the cornea and extending this natural separation plane toward the central portion of the attachment until resistance was met. Minimal superficial dissection of residual adhesions with a scalpel blade completed the removal from the cornea. The pterygium head was excised, the conjunctiva allowed to retract, and the subconjunctival scar excised en bloc. Bleeding points were cauterized with a bipolar eraser, and the denuded corneal surface and limbus smoothed with a fine diamond-studded ball.

In the opposite upper quadrant of the same eye a subconjunctival injection of 2% Xylocaine was placed about 7 mm from the limbus. The size of the episcleral defect was noted, and a similar sized limbal and associated conjunctival patch was demarcated with cautery points made with a bipolar eraser. The conjunctival patch was incised along the cautery points using sharp scissors, with the dissection extending to the limbus, while minimizing the amount of Tenon capsule attached to the graft. This conjunctiva was reflected over the cornea, and the limbal attachment was then cleaned with a Tooke knife and then laid back into its normal anatomic position. A superficial incision was made with a #15 Bard Parker blade parallel to the limbus just ahead of the palisades of Vogt, and the entire graft was excised from its limbal attachment with scissors held almost parallel to the insertion. The graft was then rotated into the defect site, spreading the conjunctiva out, being mindful of orienting the stem cell population toward the limbus.

Depositing the Tisseel under the graft requires that the graft be anchored superiorly with Colibri forceps and the tip of the applicator needle be inserted under the graft, injecting a thin layer of Tisseel. The graft is rapidly smoothed out against the episclera with a spatula while the Colibri forceps is used to pinch the donor and recipient conjunctiva together around the graft, starting at the limbus. Where necessary, some Tisseel can be injected under the recipient edge. Three minutes is allowed to elapse for adequate adhesive strength, an Ocuflox-soaked corneal shield is placed over the cornea, and the eye is patched and shielded.

In those cases in which the graft was anchored with sutures, interrupted 9-0 Vicryl (Ethicon, Inc, Johnson & Johnson Co, Somerville, NJ) was used to attach the graft at the limbus superiorly and inferiorly. Additional sutures were placed to appose the conjunctival wound, usually as few as 4 to as many as 6. In each case it was attempted to obtain episcleral bites to provide for optimal graft apposition. Any bleeding from suturing was controlled before completion of the surgery.

Amnion was attached to the recipient site in the same manner as the corneal stem cell transplant with sutures or Tisseel. Amnion was acquired in a frozen state from Bio-Tissue (Miami, FL).

The techniques for surgeries other than pterygia are identical to the pterygium procedure in the preparation of the fibrin sealant and recovery of the donor stem cell tissue. A variety of approaches are required here to prepare the recipient site, often requiring lamellar dissection of abnormal epithelia or pannus to create a suitable substrate surface. The stem cell donor tissue is obtained from the same eye, the fellow eye, or a suitable related or nonrelated donor. The principles of tissue adherence, as detailed above, remain the same.

Postoperative Treatment

Patients were instructed to keep the eye patched for 1 to 3 days but to place 1 drop of topical 0.3% ofloxacin antibiotic (Ocuflox®, Allergan Optical Inc, Irving, CA) and, in some cases, an antiinflammatory topical glucocorticoid, 1.0% prednisolone acetate (Pred Forte®, Allergan Optical Inc, Irving, CA) in the eye 4 times a day including the day of surgery. The eye was examined at 1 day, 1 week, and 1 month after surgery. Antibiotic was continued for approximately 2 weeks and then stopped in the absence of an epithelial defect. When prescribed, prednisolone acetate was continued for 1 to 2 months, in a tapering dose from 4 to 2 times a day.

RESULTS

A total of 114 corneal stem cell reconstructions were performed in 95 patients from July 1996 to January 2004 using corneal stem cells primarily but also amnion or both. The age range was from 13 to 84 years with a mean of 54 years. Men outnumbered women 70 to 25. No transplants from either group were lost, slipped, or in any position other than where they were placed at the time of surgery, as judged by direct observation at each time interval. Viability of the stem cell transplants was suggested by the absence of fluorescein staining on any of the stem cell transplants. Epithelial cells were observed to spread from the stem cell transplant on to the denuded cornea. A representative group of photographs illustrating the appearance of Tisseel stem cell transplants in pterygia are presented in Figure 1.

Tisseel fibrin sealant was used as the only method of stem cell adhesion in 41 surgeries completed on 35 patients from October 2000 to January 2004. Limbal reconstruction was accomplished using stem cell transplantation or amnion, of which 31 were for primary pterygium, 4 were for recurrent pterygium, and the remaining 6 were for an assortment of corneal stem cell deficient diseases (Table 1). The tissue fibrin sealant group showed 1 recurrence of the original disease process (detailed below) but no complications of the procedure in any patient. At the corneoscleral junction the donor tissue was well apposed to the recipient bed with either no gap or a minimal gap of less than 0.3 mm. On the conjunctival side most patients showed no gap, but some did have retraction of the conjunctival host bed revealing a gap of up to 0.6 mm. The presence

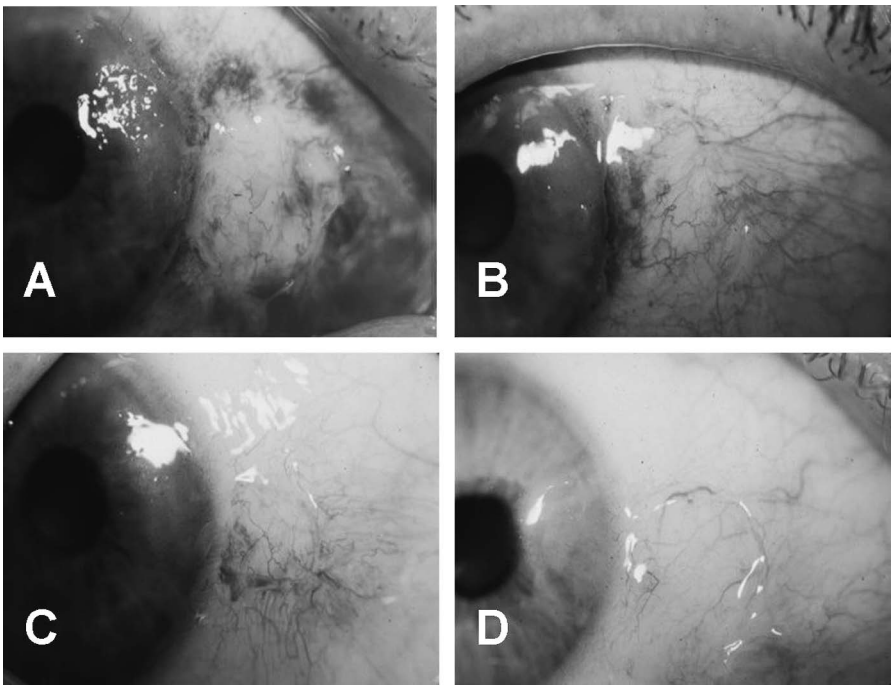

FIGURE 1. Corneal stem cell transplants for pterygia using Tisseel adhesive are displayed on a time course: A, 1 day; B, 7 days; C, 1 month; D, 6 weeks. Note the relatively noninflammatory appearance of the eye, good transplanted tissue apposition, and reestablishment of the normal limbal architecture.

of a gap in tissue apposition did not have any influence on the outcome. These gaps covered with epithelium without event, smoothing out as time elapsed. In the Tisseel group 4 patients presenting with recurrent pterygium showed no recurrence after surgery.

Sutures were used exclusively to affix the transplant to the recipient bed in 73 surgeries on 60 patients. Of these, 43 were primary pterygia, 19 recurrent pterygia, and 11 miscellaneous corneal stem cell deficiencies (Table 2). There was 1 recurrence in the primary pterygia group, 2 recurrences in the recurrent group, and none in the miscellaneous group. There were no discernable complications.

In the sutured group, 13 patients who presented with recurrent pterygia underwent 19 procedures, 2 of which recurred. One patient underwent 3 surgeries; the remaining patient had 2 surgeries. After the completion of all surgical procedures, all patients were free of pterygia. Of all of these patients only 1 received topical prednisolone acetate.

Miscellaneous stem cell deficiencies were included to demonstrate that corneal stem cell transplants can be used in other corneal procedures in addition to pterygia. For all corneal stem cell procedures, the length of operation and postoperative pain can be reduced when Tisseel is used as the sealant. In this category 6 stem cell transplants using Tisseel were successful. In the sutured group, 10 of 11 cases were successful; the 1 exception was in a case of pseudopterygium caused by a BB gun injury. This recurrence was subsequently treated successfully by amniotic membrane fibrin sealed to the surface with Tisseel.

Amniotic membrane was used more frequently in patients with pterygium recurrence or serious ocular surface disease. Fifteen of 114 procedures were performed with amnion, 10 with sutures, and 5 with Tisseel. Of the 10 sutured amnion grafts, 6 were recurrent pterygia, 2 primary, and 2 pseudopterygia. Of the 5 in the Tisseel with amnion group there were 2 primary, 1 recurrent, 1 pseudopterygium, and 1 corneal intraepithelial neoplasia. Amniotic membrane and cadaveric corneal epithelial stem cells were used in 1 very severe chemical injury and 1 ocular cicatricial pemphigoid. These 2 cases were the only failures occurring using amnion.

Prednisolone acetate was used as topical drops qid in 34 of the 41 Tisseel surgeries for a period of 1 to 2 months. All 4 of the recurrent pterygia in the Tisseel group were treated with prednisolone acetate topically. In the surgeries where tissues were sutured, prednisolone acetate was used in only 4 of the 71 procedures; the postoperative treatment employed before the use of Tisseel. This routine use of prednisone represents a

TABLE 1. Fibrin Sealant Patients

	Male	Female	Total
Number of patients	26 (74%)	9 (26%)	60
Age, mean ± SEM	52 ± 3	59 ± 5	
Max	78	77	
Min	13	40	
Diagnosis/number of procedures (41 total procedures in 35 patients)			
Primary pterygium	22	9	31
Recurrent pterygium	4	0	4
Injury	1	0	1
Chemical injury	1	0	1
CIN	2	1	3
Salzman nodular	1	0	1
PED	1	0	1
Medications used			
Ocuflox only	2 patients	3 patients	5 patients
Ocuflox plus pred forte	24 patients	6 patients	30 patients

TABLE 2. Suture Patients

	Male	Female	Total
Number of patients	44 (73%)	16 (27%)	60
Age, mean ± SEM	54 ± 3	53 ± 5	
Max	84	76	
Min	13	38	
Diagnosis/number of procedures (73 total procedures in 60 patients)			
Primary pterygium	31	12	43
Recurrent pterygium	13 (9 Patients)	6	19
Injury	1	0	1
Chemical injury	3	0	3
CIN	4	0	4
Salzman nodular	0	1	1
PED	1	1	2
Medications used			
Ocuflox only	41 patients	15 patients	56 patients
Ocuflox plus pred forte	3 patients	1 patients	4 patients

change in the operating surgeon's protocol for the postoperative treatment of pterygium. None of the recurrences in the sutured group had been treated with prednisolone acetate.

History of Recurrent Pterygium

This 40-year-old white woman developed a primary pterygium on the right cornea over a period of 2 years. It was excised on February 11, 2003 with Tisseel used to seal the donor stem cells to the recipient bed. Prednisolone acetate 1% was used QID for a period of 15 days. Three months later the cornea was clear and free of pterygia. At this time, Schirmer test strip result of 0 mm wetting in both eyes was uncovered. She was placed on artificial tears every 2 hours during the day, a gel at night, and both lower punctae were cauterized. Contrary to instructions she subsequently sunbathed frequently and over long periods during the summer months. Her use of ultraviolet protective sunglasses was erratic. Nine months after surgery a diminutive recurrence of the pterygium was noted.

DISCUSSION

A commercially available fibrin biosealant, Tisseel (Baxter Healthcare Corp, Inc, Deerfield, IL, or Tissucol, Baxter Healthcare Corp, Inc, Belgium), has been used in Europe for more than 25 years in over 9.5 million surgical procedures.[15] A Medline search of fibrin sealants was performed indicating there are thousands of references detailing the great diversity of applications spanning virtually all surgical procedures. In the United States the FDA approved Tisseel in 1998 to be used as an adjunct in cardiopulmonary bypass surgery, splenic injuries, and where control of bleeding by conventional techniques is ineffective or impractical. Its remarkable utility has extended its usage well beyond these applications to other surgical disciplines as a hemostatic adjunct, sealing of tissues, sealing of leaks, tissue gluing, and to provide a delivery system for other biologic agents.

Fibrin biosealants have been used in a wide array of ophthalmic procedures, but on a very limited or investigational status. Conjunctival closure in strabismus,[16,17] closure of leaking glaucoma filtering blebs,[18–20] bleb leaks,[21,22] vitreoretinal and macular hole surgery,[23–25] and drug delivery[26,27] are some of these procedures. Application of fibrin sealant to corneal disease has been broadly tested, but clinical implementation has been minimal. Among these are persistent epithelial diseases,[28] perforation,[29,30] and keratotomy.[31]

The natural formation of fibrin between cut tissues forms the first stage of healing by creating scaffolding on which repair fibroblasts and inflammatory cells can move into the injury site. Transplanted tissue adheres to the subjacent and adjacent tissues by components of the blood, converting fibrinogen to fibrin via plasmin interaction. In brief, thrombin converts fibrinogen molecules to long-chain fibrinogen monomers. Under the influence of factor XIIIa and Ca^{2+}, this soluble fibrinogen polymer is cross-linked to create an insoluble clot. Fibrin sealant mimics the last steps of the coagulation cascade through the activation of fibrinogen by thrombin, leading to a semirigid fibrin clot.[32]

Transplantation of corneal stem cells and associated conjunctiva from one area of an eye to another, or from the companion or a donor eye, with the use of fibrin biosealants has not been reported. By using a commercially available product, it is possible to accelerate the normal process of fibrin formation by creating strong adherence of the graft to the underlying episclera within minutes of application. The rigidity and strength of the fibrin clot increase progressively over time as a result of the polymerization and cross-linking of fibrin fibrils. As a consequence, there is a buildup of structure within the fibrin sealant.[33,34] The strength of this adherence is maintained right up to the time that fibrous tissue replaces the bridge between the transplanted tissue and the host bed.[35]

Until recently, sutures have been the primary means of injury/surgical repair. But sutures are not an ideal wound closure system because they do not actively participate in wound healing and actually increase the invasiveness of the procedure by inflicting added trauma to the injury site and adjacent tissue. Sutures may additionally act as a portal for infection along the suture tract as well as acting as a nidus of inflammation itself. Loose or broken sutures require removal and hence additional attention of the surgeon and office time. In any corneal surgery, precision suturing is an acquired technical skill involving prolonged OR time. Corneal suturing techniques vary among physicians as a result of the surgeon's technological experience as well as the nature and number of surgeries preformed.

In ocular surface surgery, sutures are normally used to fix the tissue edges to the underlying episclera and adjacent host conjunctiva. Sutures can only fix the edges of the tissue to adjacent tissues, leaving the entire undersurface of the graft in proximity to the underlying episclera, but not in direct apposition or adherence over its entirety. Biosealants provide extensive contact with the underlying tissues, encouraging earlier vascularization of the graft, hence its viability, and offering a smooth surface to the resurfacing eyelids.

In the Tisseel-treated group the entire undersurface of the transplant is adhered to the episclera as well as the edges. The presence of occasional surface gaps between the donor

and recipient conjunctiva did not prove to constitute any problem, the tissue smoothing out as time elapsed. Cutting the donor tissue slightly larger than the recipient and then pinching the donor and recipient conjunctival edges together improved correction of this technical detail.

Patients undergoing pterygium surgery with corneal stem cell transplant utilizing fibrin sealant note greater comfort postoperatively.[36] Patient comments on discomfort after surgery reveal considerably more comfort in the eye receiving the fibrin sealant compared with similar procedures performed with a sutured graft. The absence of sutures eliminates the rubbing, foreign body sensation, and irregularity of the edges of the graft, minimizing pain and irritation. Fibrin sealant avoids problems of monitoring or removing loose sutures, thus reducing the frequency of office visits and the cost thereof. Epithelium readily heals over the cut edges when apposition is good, reducing the opportunity for sterile or opportunistic organisms to invade irregularly apposed edges or travel along open suture tracts. Fibrin clots formed by the application of Tisseel are a less favorable environment for bacterial growth than clots formed under physiological conditions after trauma or surgery. Bacterial growth in solidified fibrin sealant is retarded by its natural properties.[37] The reduced inflammation in and around the transplant is likely to minimize the frequency of recurrence by encouraging rapid epithelial closure, vascularization of the transplant and return to a normal and functional stem cell barrier to future pterygium invasion. When allografted stem cells are employed, the lessened inflammation might reduce the incidence of rejection.

In this retrospective study the use of topical prednisolone in many of the fibrin sealant group but few of the sutured group is a confounding feature. This change was not driven by any specific feature of either group but by an effort to achieve the most successful result in the transplantation process. This difference in the protocol between the 2 groups does raise questions about what specifically is responsible for the reduction in inflammation noted qualitatively in the sealant-treated group. This fact in no way changes the result that the graft is stable, comfort is greater, and operative time is reduced in the fibrin sealant–treated group.

The single recurrence of a primary pterygium after surgery, utilizing Tisseel, holds an instructive note. Repeated and prolonged exposure to sunlight after pterygium surgery immediately duplicates the noxious conditions leading to the initial pterygium development. That ultraviolet exposure is the culprit in pterygium formation has been theorized for years, a conclusion bolstered by a number of reports.[37–40] Significant among these is the finding that proinflammatory cytokines, interleukins 6 and 8, are significantly increased with UVB exposure of pterygia, leading to the conclusion that UVB radiation plays a key role in the development of pterygia by initiating blood vessel formation, cellular proliferation, tissue invasion, and inflammation.[41]

The safety record of Tisseel is of considerable importance. Despite the application of 9.5 million doses, there is not a single report or suggestion of viral contamination traceable to Tisseel.[42] This clearly points to the company's successful 2-barreled approach to safety by careful donor selection and 2-step vapor heating treatment of serum.

A further measure of safety is the low incidence of allergic reactions attributable to Tisseel ie, 0.5/100,000 for all reactions and 0.3/100,000 for serious reactions. From 1990 to 1998, 5 cases of allergic reaction, characterized by skin rash, were reported to the manufacturer during a period of time that 1 million doses of Tisseel were administered.[43,44] In the complete history of the use of fibrin sealant there are 3 cases of anaphylactic reaction, 1 resulting in death.[45] The allergen was believed to be the bovine protein aprotinin, included in the product as an antifibrinolytic to inhibit clot breakdown. In all cases, fibrin sealant was used more than once over a short period; the greater the volume of sealant the greater the risk. To guard against the risk of such a catastrophic pitfall, a prior medical history of cardiac, neurosurgical, or other major surgery, less than 6 months from the time of anticipated surgery, would dictate caution. If surgery is required during that interval of time, determination of the IgG and IgE levels of antiaprotinin in patient's serum might be important.[46] Rare as these complications are, it is wise to investigate prior aprotinin exposure.[47]

There are substantial cost savings built into the use of Tisseel.[48,49] Correct suture placement incorporating episclera and adjacent conjunctiva is a time-consuming effort,[50] which is eliminated with Tisseel application. The reduced time in surgery translates to less facility cost. The cost of sutures is comparable to the cost of Tisseel. Furthermore, 1 unit of Tisseel can be used for multiple cases, further lowering the cost per patient. Rapid and efficient surgery reduces the chance for infection and saves surgeon and facility valuable operating room time. From the patient's standpoint greater comfort allows a more rapid return to their normal life style and productivity.

Fibrin sealants have not made great inroads into surgical techniques in ophthalmology. There is a need to reduce or replace sutures in a variety of ophthalmic procedures to improve outcomes, minimize complications, provide patient comfort, and shorten procedure lengths. Fibrin sealant use in this study is but one application for a wide range of possibilities in eye surgery.

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