

Repeated use of fibrin sealants kept at room temperature in conjunctival autografting: An animal study

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Purpose: In this study, our aim was to investigate if fibrin adhesives used in conjunctival wound surgery with autologous conjunctival grafts could be used repeatedly at different times after surgical opening. **Methods:** 40 New Zealand rabbits were used in the study. These animals were divided into four groups, each consisting of 10 rabbits, and hence 20 eyes. In the first group (control group), Tisseel fibrin sealant was used on the day the eye was first opened surgically; in the second group, it was used on the seventh day; on the third group, it was used on the 14th day; and in the fourth group, it was used 28 days after surgical opening. The graft from the inferior bulbar conjunctiva was attached using Tisseel fibrin glue to the superior scleral bed at the location where the superior bulbar conjunctiva was excised in the same eye. **Results:** No microbial growth was detected in the cultures of the samples tested. There were two partial graft loss in group 2 and there was one partial graft loss in each group of the other groups, and further total graft loss was present in one rabbit in group 3. None of the rabbits had any complications like granuloma, Corneal dellen or infection. **Conclusion:** Based on these results, fibrin sealants can be used repeatedly by storing them at room temperature. Repeated use of fibrin adhesives will reduce the cost of ophthalmologic surgeries and non-ophthalmologic surgeries.

Key words: Bulbar conjunctiva, conjunctiva, conjunctival autografting, fibrin sealants, pterygium

Pterygium is an abnormal fibrovascular proliferation that progresses from the conjunctiva to the cornea. The combined prevalence of pterygium in the world is 12%.^[1] The definitive treatment of this common disease of the ocular surface is surgical intervention. The most important complication of surgical intervention is postoperative recurrence. In addition to primary excision, many different surgical methods and adjuvants, including beta-irradiation, mitomycin C, 5-fluorouracil, amniotic membrane, and autologous conjunctival graft have been tried to solve this problem, which is bothersome for both the patient and the physician.^[2] There are many studies in literature which showed that autologous conjunctival and limbal-conjunctival graft method has the lowest recurrence.^[2-4]

In the autologous conjunctival graft technique, the graft tissue from the superior bulbar conjunctiva is attached to the scleral bed where the pterygium tissue is excised with suture or fibrin glue.^[5] The most important problem for pterygium surgery is recurrence. Although the issue of which method is advantageous regarding recurrence is controversial, many studies report that fibrin sealants are more advantageous.^[6-8] In a large series of 2,356 cases from South India, where pterygium surgery was performed with autologous conjunctival graft method using fibrin glue, the recurrence rate was reported as only 1.44%.^[9] Dehiscence and loss of the graft in the fibrin sealant-used patients, and granuloma formation in the suture-used patients are the prominent complications after pterygium surgery.^[5,7] However, in a study conducted in

South India, graft loss was reported as low as 0.93% in fibrin glue-used cases.^[9] The advantage of using fibrin glue is shorter operation time and better postoperative patient comfort, but the most important disadvantage is that the price is higher than sutures.^[4,5] In our study, we used fibrin glues on rabbit eyes at different times, and we evaluated the effectiveness and complications of the repeated use of fibrin glue kept at room temperature. Our study was the first to examine the effectiveness and complications of using the same fibrin glue at different times.

Methods

All the animal experiments were conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals.

In this study, 40 New Zealand rabbits, 6–12 months old, (weights between 2 to 3.2 kg from both sexes) obtained from Erciyes University Experimental Research Practice and Research Center were used [Table 1]. Ethics committee approval of this study was obtained from Erciyes University Animal Experiments Ethics Committee (Decision no: 19/142). These animals were divided into four groups, consisting of 10 rabbits each, and hence 20 eyes in each group, to demonstrate the use of fibrin glue long-term after surgical opening. Tisseel (Baxter,

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Vienna, Austria) was used as fibrin glue in the study. Tisseel fibrin glue consists of two components: The first component contains 91 mg/mL human fibrinogen, and 3000 KIU/mL synthetic aprotinin; the second component contains 500 IU/mL human thrombin and 400 μ mol/mL calcium. Tisseel fibrin sealant should be stored below -20°C . However, after thawing, it should wait at room temperature and should not be frozen again. In the first group (control group), Tisseel fibrin sealant was used on the day the eye was first opened surgically (day 0); in the second group, it was used on the seventh day (day 7); on the third group, it was used on the 14th day (day 14); and in the fourth group, it was used 28 days after opening (day 28). 0.1 ml of the drug was withdrawn from the vials for each use. The vials were stored at room temperature (lower than 25°C , as suggested by the manufacturer) after each use. The stored vials were cleaned with a 5% baticon before reuse.

For microbiological analysis, 50 μ L of each solution was taken and inoculation to 5% sheep blood agar (HiMedia, India) and MacConkey agar (HiMedia, India) were performed for bacterial growth, and inoculation to Sabouraud dextrose agar (Oxoid, UK) was performed for fungal growth (yeast and mold). Bacterial cultures were incubated for 24 hours at 37°C , yeast cultures for 24–48 hours at 37°C , and mold cultures at 25 – 27°C for one week under aerobic conditions. In addition, for the growth of anaerobic bacteria blood agar medium, they were incubated for 24–48 hours in an anaerobic jar. After incubation, petri plates were evaluated for bacterial and fungal colonies. Culture media without growth were incubated at 37°C for a further 24 hours. At least two cultures were made for each microorganism.

Rabbits were anesthetized with the combination of ketamine (Ketasol, Richter Pharma, Austria) + xylazine (Rompun, Bayer, Germany) (35 mg/kg + 5 g/kg). In addition to general anesthesia, topical anesthesia using 5% proparacaine was also performed. 5% povidone iodine was applied to the conjunctival fornices and it was washed with sterile saline prior to operation. An area of 0.5×0.5 cm beginning from the limbus was removed from both the superior bulbar conjunctiva and the inferior bulbar conjunctiva of the rabbits, including the tenon tissue under anesthesia [Fig. 1]. The graft from the inferior bulbar conjunctiva was attached using Tisseel fibrin glue to the superior scleral bed where the superior bulbar conjunctiva was excised in the same eye. As stated by the drug manufacturer, we waited for at least two minutes for the adhesion to fully occur after the application. Then with the help of forceps, it was checked whether the adhesion had occurred. Subconjunctival gentamicin (Gentrex, Bilim, Türkiye) + dexamethasone (Dekort, Deva, Türkiye) was injected postoperatively; tobramycin antibiotic ointment (Tobrex, Alcon, Tx, USA) was applied and the eye was closed with a bandage. The bandage was removed after 24 hours. Following removal of the bandage, rabbits were treated with a topical mixture of moxifloxacin (0.5%) and dexamethasone (0.1%) (Moxidexa, Abdi İbrahim, Türkiye) three times a day for 10 days. For the operation of the other eyes, the operated eyes were allowed to heal for at least 15 days.

Table 1: Characteristics of the rabbits

Genus	New Zealand rabbits
Gender	Both sexes
Weight	Between 2-3.2 kg
Age	6-12 months

The operated animals were evaluated using a pen light for several days (day 1, day 7, day 14, and, day 28) for about 1 month and the changes were recorded using a Canon EOS 5D Mark III Body DSLR camera with a macro lens [Figs. 2-5]. The eyes were examined for complications like partial or complete graft loss, pyogenic granuloma, and dellen formation. Also, purulent and mucous discharge which can be caused by bacterial infection, and blepharospasm, which can be due to pain or photophobia, were evaluated. Subconjunctival hemorrhage, corneal epithelial integrity, and changes in corneal and anterior chamber clarity, which could be a sign of inflammation, were evaluated. Microbiological inoculation was performed from the samples taken on days 1, 7, 14, and 28 from the fibrin sealant, which was kept at room temperature from the time it was opened.

Results

No microbial growth was detected in the cultures of the samples tested. There were five partial graft losses in group 2, and there were four partial graft losses in each of the other groups. Total graft loss was detected in two cases in group 2, and in one case for each of the other groups. The difference in terms of total and partial graft loss between the first day (0 days) and the other days (7, 14, and 28) for the Tisseel adhesive use was not statistically significant [Table 2]. None of the rabbits had any complications like granuloma, dellen, or infection. None of the rabbits had blepharospasm, purulent, or prominent mucous discharge during examinations. Subconjunctival hemorrhage, corneal epithelial defect, and a change in corneal and anterior chamber clarity, which could be a sign of inflammation, were not detected in any of the rabbits.

Discussion

In this study, to overcome the price disadvantage, we aimed to demonstrate whether fibrin glue could be used repeatedly at different times in pterygium surgery with a conjunctival autograft. For this purpose, fibrin glue was stored at room temperature after opening and used on days 7, 14, and 28. There was no statistically significant difference between the groups in terms of graft loss; no complications were encountered, and no growth was observed in the cultures in all groups. These results suggest that fibrin sealants can be used repeatedly by storing at room temperature. In pterygium surgery, excision



Figure 1: Harvesting of conjunctival graft from inferior conjunctiva



Figure 2: 7th day of operation in group 2 rabbits



Figure 3: 14th day of operation in group 3 rabbits

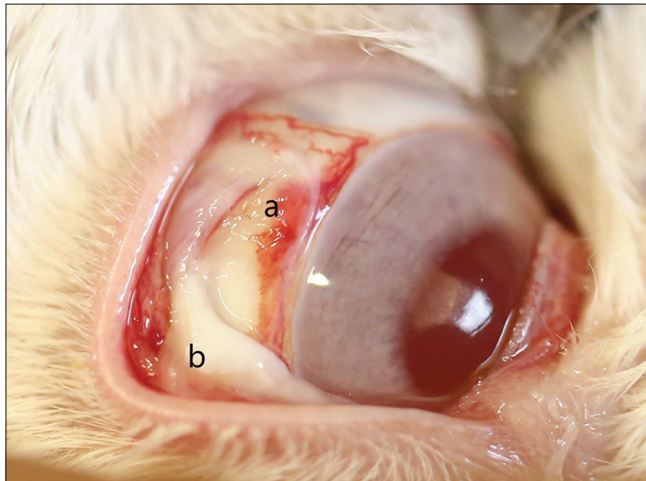


Figure 4: Example of partial graft loss from group 2 (day 7) rabbits; (a) Graft site (b) Graft



Figure 5: 21st day of operation in group 3 rabbits

Table 2: Comparison of total and partial graft loss of days

Days	n	Mean	Std. Err.	Std. Dev.	P
Day 0	20	1.315	0.133	0.582	0.453
Day 7	20	1.473	0.159	0.696	
Day 0	20	1.315	0.133	0.582	1.000
Day 14	20	1.315	0.133	0.582	
Day 0	20	1.315	0.133	0.582	1.000
Day 28	20	1.315	0.133	0.582	

Paired sample t-test

with conjunctival autografting is accepted as the best surgical method, especially in terms of the lowest recurrence rate.^[5] The most important debate at this point is whether the autograft will be attached to the scleral bed with fibrin glue or suture. Karalezli *et al.*^[6] found that recurrence rate was 4% in the fibrin glue group and 12% in the suture group. Similarly, Yüksel *et al.*^[7] reported recurrence rate in the fibrin group as 6.8% and in the suture group as 13.7%. There are studies that detect recurrence rate higher in the fibrin group, albeit in a smaller number.^[10]

Another important advantage of fibrin sealants is the low postoperative patient discomfort such as foreign body sensation, irritation, epiphora, pruritus, and dry eye sensation. Bahar *et al.*^[10] reported that pain complaints were significantly less in the fibrin sealant group and patients in this group reported more satisfaction.^[11] Zloto *et al.*^[5] reported that patients who were operated on using fibrin sealant suffered less postoperative discomfort compared to the suture group. Moreover, in the same study, patients who had undergone a pterygium operation with a different method previously in the other eye reported very low postoperative discomfort in the eyes operated with fibrin sealant. These results are consistent with the findings that we did not detect purulent, mucous secretion, and blepharospasm in the postoperative period in our study. Another advantage of fibrin sealants is that the operation time was found to be significantly shorter in cases operated using fibrin glue compared to the suture group.^[5,7,10]

A meta-analysis by Romano *et al.*^[12] found 7 grafts dehiscence, 3 graft loss, 4 granuloma formation, and 1 dellen in the fibrin group consisting of 3,72 patients, while they detected 2 dehiscence, 11 granuloma formation, and 3 dellen

in the suture group of 439 patients. Yüksel *et al.*^[7] detected hemorrhage in one eye, dellen formation in one eye, and conjunctival cyst formation in one eye postoperatively in the fibrin sealant group of 29 patients. They also reported granulation formation in seven patients in the suture group, which also consisted of 29 patients. There were three graft losses in the fibrin group in their study. Zloto *et al.*^[5] detected graft loss in five patients in the Evicel group of 29 patients, and detected pyogenic granuloma in one patient in the Tisseel group of 25 patients in their study. No complications were detected in the suture group. They attributed the absence of graft loss in the Tisseel group and the loss of five graft losses in the Evicel group to the aprotinin and factor 13, which were components of Tisseel. In the studies, dehiscence, loss of the graft in the fibrin group and granuloma formation in the suture group are the prominent complications.

Although the use of fibrin glue and autologous conjunctival grafts in pterygium surgery have advantages, such as lower recurrence rates, shorter operation time, and low postoperative patient discomfort compared to suture group, it has disadvantages in terms of economical cost.^[5,11,13] To eliminate this price disadvantage, it is necessary to use the same fibrin glue for more than one patient on the same day.^[5,11] However, gathering patients and performing multiple operations on the same day is not a practical solution. Autologous blood and self-made cryopreservative fibrin glue were used to overcome this obstacle.^[4,13]

In the study by Nadarajah *et al.*,^[13] autologous blood and fibrin glue in pterygium surgery with conjunctival autograft were compared; there was 24.2% total graft loss in the autologous blood group while they did not see total graft loss in the fibrin group. Partial graft loss was seen in seven patients in the autologous blood group and only in one patient in the fibrin group. Recurrence rate was 2% in the fibrin group, whereas this rate was 10.6% in the autologous blood group, although cases with total graft loss was excluded from the calculation. They found both graft loss and recurrence rates similar to other studies in literature. Moreover, tenon granuloma developed in three patients in the autologous blood group and no complications were seen in the fibrin group. Since it is necessary to wait for clot to form in the autologous blood group, the duration of the operation lasted longer than the fibrin group. Zeng *et al.*,^[14] in a meta-analysis study comparing autologous blood and fibrin glue, stated that autologous blood was lower than fibrin glue in terms of surgical time, graft retraction, and graft displacement; but there was no statistical difference between the two groups in terms of recurrence rate.

In their study to compare the self-made cryopreservative fibrin glue and commercial fibrin glue, Gong *et al.*^[4] did not observe a statistically significant difference between the two groups in terms of operation time, postoperative patient discomfort, and recurrence. They reported that the most important disadvantage of self-made cryopreservative fibrin glue was that it must be stored at -20°C.

The strength of our study is that this is the first study to report that fibrin adhesives can be used repeatedly. A limitation of our study is that we did not use our protocol on human participants. Further studies especially involving human participants are necessary on this subject.

Conclusion

The results of this study indicate that fibrin sealants can be used repeatedly at different times by storing at room temperature. These results, which we have obtained on rabbits, need to be investigated and proven by studies with human participants. This will reduce the cost of ophthalmologic surgeries such as conjunctival injury, filtering bleb dehiscence, and lamellar keratoplasty, and also non-ophthalmologic surgeries using fibrin adhesives.

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Conflicts of interest

There are no conflicts of interest.

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