Experimental Epikeratophakia With Biological Adhesive

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• **Successful experimental epikeratophakia grafting was done with the use of a biological adhesive. The use of an adhesive eliminated the need for any suturing of the corneal lenticule to the host cornea, and this method reduced the length of the operative procedure to only one third of the time that is taken when sutures are used. Tisseel (Immuno AG, Vienna), a commercially available two-component adhesive system based on human fibrinogen, which is activated by thrombin, was used. The glue was used in combination with an antifibrinolytic agent. With alteration of the operative technique and lenticule design, 70% of glued epikeratophakia grafts in a rabbit model were retained, compared with a 50% success rate with grafts applied without the use of an adhesive.**

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The use of fibrin to seal wounds in ophthalmic surgery was first advocated in the 1940s when Katzin[1] reported the use of aqueous fibrin for the fixation of penetrating corneal grafts in rabbits. Tassman[2] subsequently reported the use of fibrin in clinical keratoplasty. A further refinement of the technique was put forward by Rosenthal et al[3] who used a platelet/fibrinogen/thrombin mixture to perform sutureless lamellar keratoplasty in the rabbit. Successful experimental lamellar keratoplasty has also been achieved by Klemen et al,[4] with the use of highly concentrated fibrinogen. A recent report from the Soviet Union has indicated that the use of fibrin with sutures can be successful for lamellar keratoplasty in humans.[5]

Epikeratophakia is a new type of lamellar keratoplasty procedure that is being increasingly carried out, and that could well benefit from the realization of a practical adhesive technique. Robin and Salazar[6] have glued experimental epikeratophakia grafts by using a mollusk-derived adhesive, but they found it necessary to use some sutures to stabilize the graft. We are not aware of any reports of the use of fibrin adhesive for sutureless epikeratophakia.

In the past, one factor that limited the use of fibrin-based adhesives was the need to obtain fresh plasma from the recipient, but now commercially prepared fibrin adhesives, such as Tisseel (Immuno AG, Vienna), eliminate the need to obtain fresh autologous blood products. Tisseel adhesive has been used successfully by Buschmann[7] in ophthalmic surgical practice to glue conjunctival wounds and also to seal perforations of the lens capsule.[8] It has also recently been tested for its bonding power in the closure of cataract surgery incisions.[9] We set out to evaluate Tisseel adhesive in a rabbit model of epikeratophakia that was originally developed to study tissue handling in the production of lenticules that were lathed at room temperature.[10]

MATERIALS AND METHODS

Biological Adhesive

Tisseel adhesive is based on a lyophilized pooled human plasma protein concentrate. In the production of Tisseel, the plasma donors are tested at every donation for hepatitis B surface antigen, anti-human immunodeficiency virus, and increased alanine aminotransferase levels; those donors who test positive are irrevocably excluded from the plasmapheresis program. As an additional precaution, the lyophilized Tisseel is subjected to heat treatment at 60°C for 30 hours. Heat treatment has been shown to reduce effectively viral titres of human immunodeficiency virus and other viruses in vitro.[11] Clinical studies of the use of Tisseel in general surgery have failed to show evidence of transmission of hepatitis,[12] and a recent clinical report of heat-treated pooled plasma products has shown no evidence of transmission of the acquired immunodeficiency syndrome virus.[13]

Tisseel is made up from lyophilized clottable protein (fibrinogen) and thrombin, which is used to induce fibrin formation. The lyophilized fibrinogen (75 g/L) is dissolved in a solution of bovine aprotinin (3000 kallidinogenase inactivator units/mL). Aprotinin is a broad-spectrum protease inhibitor that is incorporated to extend the duration of adhesive action by delaying fibrinolysis. The lyophilized bovine thrombin (500 IU/mL) is made up into solution with calcium chloride (40 mmol/L).

Lenticule Preparation

Lenticules were prepared using the technique developed by one of us (C.K.R.)[14]; this technique is also currently used for the preparation of lenticules for human epikeratophakia. For this experiment, donor rabbit corneas were preserved in liquid nitrogen for varying periods of time before use. The corneas were punched to a diameter of 9 mm, soaked in a solution of dimethyl sulfoxide, sucrose, and dextran 70, and then desiccated on polymethyl methacrylate bases for 48 hours. This allowed the buttons to become adherent to the bases and sufficiently dry to be lathed at room temperature. Lenticules were cut to an optic zone diameter of 6.5 mm, with an optic zone radius to correct high hyperopia. Lathed lenticules were rehydrated and removed from their bases and then lyophilized and stored in vacuum vials before surgery.

Surgical Series

Adult female New Zealand white rabbits (weight, approximately 3.5 kg) underwent epikeratophakia surgery to one eye only. Each animal underwent a preliminary excision of the nictitating membrane from the eye to be grafted, and epikeratophakia surgery was carried out a week or more later. Surgical procedures were carried out with the rabbits under general anesthesia, and full postoperative care was given, including analgesia as required.

Anesthesia

Animals that underwent the operation were premedicated with 5 to 10 mg of midazolam (Hypnovel) intravenously, and these animals were anesthetized with 4% topical cocaine hydrochloride, and 2% to 4% halothane, in oxygen (2 L/min), by mask with a scavenging apparatus. Postoperatively, buprenorphine (Temgesic) (0.05 mg/kg) was given subcutaneously and repeated if necessary, although the majority of animals made a rapid recovery and did not require further analgesia.

Surgical Technique

Group 1 (Grafts Fixed With Adhesive).—After fixation of the globe, a 6-mm trephine was used to mark the site for the graft. Epithelium was stripped only within the zone that was delimitated by the trephine mark. The trephine mark was then deepened with a diamond blade to approximately 0.1 mm, as gauged in relation to the small-end facet of the multifaceted knife blade. An angled lamellar dissector was used to create a lamellar split that extended to a minimum of 1.5 mm peripherally. A small quantity of the reconstituted fibrinogen was applied directly to the graft bed and spread into the peripheral split. The lenticule was applied to the bed.

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and the wing zone was tucked into the peripheral lamellar split with the use of an angled iris repositor. The reconstituted thrombin was then applied to the external surface of the graft. The setting of the adhesive was monitored by leaving a few small air bubbles trapped in the graft/host interface. After a few minutes, when the fibrinogen had clotted, the bubbles ceased to be mobile beneath the graft when external pressure was applied. The procedure was completed by the application of a bicurved Ledasoft 80% hydration bandage soft contact lens (David Thomas Contact Lenses, Northampton, England), topical gentamicin sulfate, and subconjunctival injection of 4 mg (0.1 mL) of methylprednisolone acetate (Depo-Medrone).

Group 2 (Controls Without Adhesive).—The animals in group 2 underwent an identical surgical procedure to those in group 1, but no adhesive was applied. The lenticule was simply tucked into the lamellar split, and the contact lens was placed on top.

Postoperative Management

Postoperatively, the animals in group 1 were treated with topical aprotinin (Trasylol), 10000 kallidinogenase inactivator units/mL, made up in 0.5% methylcellulose, four times daily. This was used to inhibit the fibrinolytic activity of the tears and to allow the great to stabilize. Animals 1 and 2 were treated with topical aprotinin four times each day for the first five postoperative days. For the remainder of the group, aprotinin drops were used for the first 24 hours only. For all animals, topical preservative free benoxinate hydrochloride (oxybuprocaine) was used daily before removal of the bandage contact lens for examination purposes. The animals were monitored with the use of a biomicroscope (Topcon, Tokyo Optical Co), and anterior segment photography was carried out daily until the graft was completely reepithelialized, and then at intervals thereafter. Animals were followed up for periods that ranged from one to five weeks.

At varying periods postoperatively, the animals were killed with an overdose of pentobarbital sodium. The eyes were immediately enucleated and placed in fixative. Corneas were subsequently embedded in glycol methacrylate resin, sectioned, stained with hematoxylin-eosin, and examined by light microscopy.

RESULTS

Clinical Findings

This report documents the clinical course of a series of 20 rabbits that underwent epikeratophakia. The first ten animals (group 1) received glued grafts, and in the subsequent ten control animals (group 2), the lenticules were grafted without glue. The Table shows a summary of the time in days to complete reepithelialization of the graft, follow-up time, and clinical outcome.

Figure 1 shows the clinical appearance of a glued graft (animal 9) at 16 days postoperatively. It can be noted that the graft and graft/host interface are perfectly clear.

Early in the experiment, it was found that excess fibrin at the graft/host interface could cause some degree of haziness in the initial postoperative phase, but this quickly disappeared when the aprotinin drops were discontinued and normal fibrinolytic activity resumed. The use of a large amount of fibrinogen, however, proved to be unnecessary, and adequate adhesion could be achieved with such a small quantity that there was no visible opacification of the interface zone at any stage postoperatively.

At the time of complete reepithelialization of the graft, 80% of the glued grafts were in place, but only 50% of the controls had been retained. All the animals in which the graft reepithelialized developed a secondary epithelial breakdown after their contact lens was removed. This complication could generally be resolved by treatment with topical betamethasone sodium phosphate/neomycin sulfate ointment one to three times each day, with clearing of the graft and

	Follow-up Data			
Animal No.	Time to Epithelialization, d	Follow-up Time, d	Graft Retained*	Comment
		Group 1 (Adhesive)		
1	9	29	+	Secondary epithelial breakdown
2	10	29	+	Late graft melt
3	8	9	+	Late inflammation
4	9	9	+	Late inflammation
5	9	16	−	Secondary epithelial breakdown
6	−	7	−	Graft lost on day 3
7	6	33	+	Secondary epithelial breakdown
8	3	13	+	Secondary epithelial breakdown
9	6	31	+	Secondary epithelial breakdown
10	−	9	−	Graft lost on day 7
		Group 2 (Controls—No Adhesive)		
11	−	6	−	Graft lost on day 5
12	−	5	−	Graft lost on day 5
13	8	35	+	Secondary epithelial breakdown
14	−	2	−	Graft lost on day 2
15	5	35	+	Secondary epithelial breakdown
16	5	5	+	Inflammation
17	4	26	+	Secondary epithelial breakdown
18	3	26	+	Secondary epithelial breakdown
19	−	1	−	Graft lost on day 1
20	−	1	−	Graft lost on day 1

*Plus sign indicates that the graft was retained for the duration of the follow-up period; minus sign, that the graft fell off.

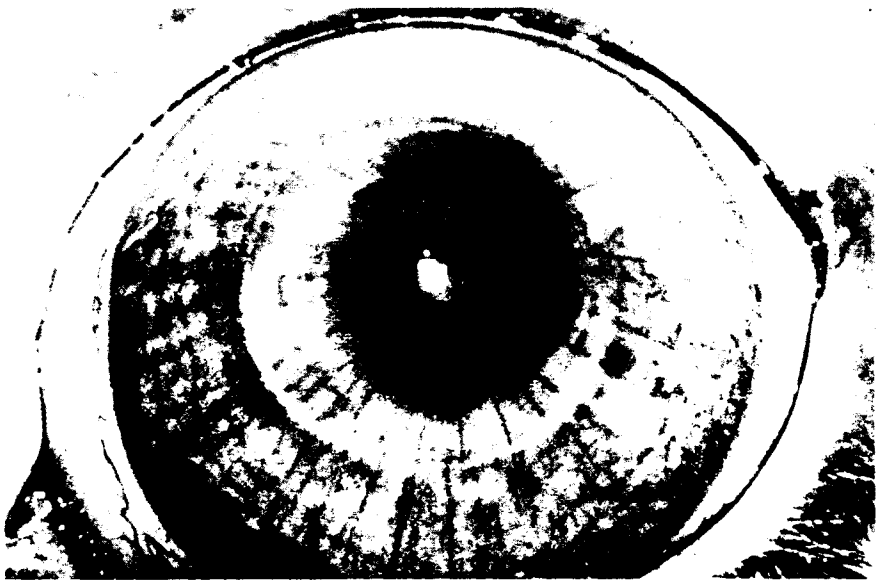

Fig 1.—Glued rabbit epikeratophakia graft at 16 days postoperatively with intact epithelial cover and clear graft (animal 9).

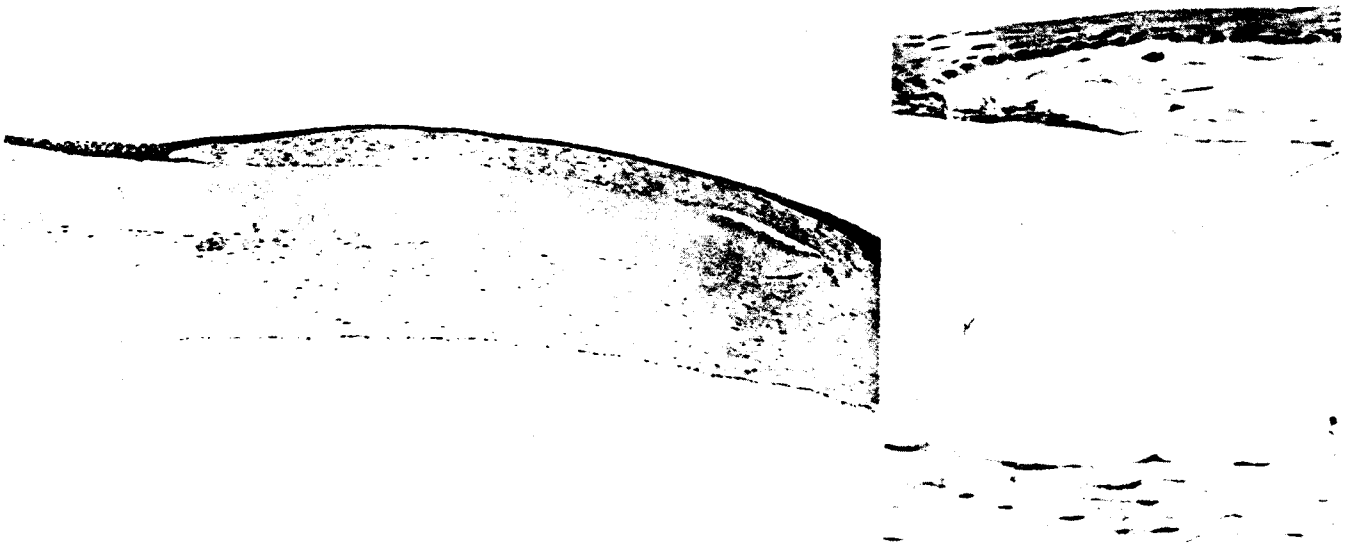

Fig 2.—Left, Glued graft at 13 days postoperatively. Low-power view of lenticule wing zone lying in peripheral lamellar split of host. Lenticule is epithelialized but devoid of keratocytes (hematoxylin-eosin, original magnification ×10). Right, High-power view of Fig 2, left, in region of external graft/host junction showing hyperplasia of epithelium. Graft bed clearly defined by active keratocytes (hematoxylin-eosin, original magnification ×25).

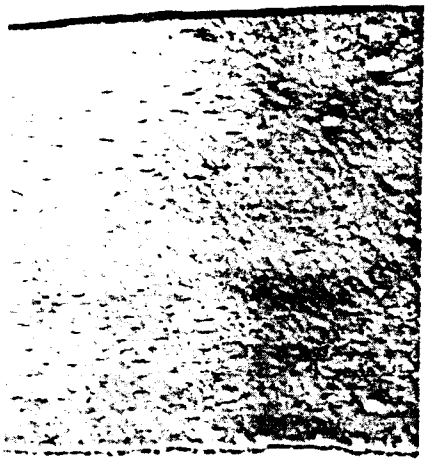

Fig 3.—Glued graft at 29 days postoperatively. Medium-power view of central graft zone showing moderate degree of population of lenticule with active keratocytes. Graft/host junction is no longer defined (hematoxylin-eosin, original magnification ×10).

reestablishment of graft epithelial cover, as seen in animal 9 (Fig 1). In animal 5, epithelial cover was not reestablished, and the graft was lost.

In group 1, the grafts of animals 6 and 10 were lost before epithelialization. The lenticule was inadvertently not well aligned on the visual axis in animal 6, and the eye could not be successfully fitted with a bandage contact lens. Following loss of the graft, the cornea reepithelialized and was clear apart from a slight annular scar at the trephination site. In the case of animal 10, the cause of graft loss was not immediately apparent although the Tisseel used in this case was not freshly reconstituted but had been made up and then frozen and thawed on two occasions.

Histopathologic Findings

In the first few days postoperatively, the fibrin glue could be seen on the graft bed as a homogeneous eosinophilic substance. However, the glue was fairly rapidly dispersed and, by two weeks, could no longer be seen. Figure 2, left, shows the wing zone of a graft (animal 8) at 13 days postoperatively. The lenticule has been reepithelialized, but there is no cellular repopulation of the graft tissue. The graft/host interface is highlighted by accumulation of active keratocytes. There has been slight artifactual separation of the tissue layers near the apex of the wing zone, but the section clearly demonstrates the large undertuck achieved with this graft/host-bed diameter disparity. Figure 2, right, shows, under higher power, the region of the external graft/host junction. The epithelium is thickened and fills out the slight irregularity of the junction; the graft bed is clearly defined by a line of keratocytes, but no fibrin adhesive is apparent.

Figure 3 shows the central graft zone (animal 1) at 29 days postoperatively. By this time, the lenticule has become significantly populated by active keratocytes. Since the rabbit cornea lacks Bowman's layer, the junction between the graft and host is no longer clearly defined once the lenticule has become cellularized.

Animals 8 and 9 (glued grafts) and 16 (control) showed inflammatory reactions with infiltration of the graft and host by polymorphonuclear leukocytes and macrophages. This reaction was thought probably to be due to infection, and there was no evidence of any allergic or immune type of reaction against the glue.

COMMENT

To our knowledge, successful sutureless epikeratophakia grafting has not previously been reported. In this animal model, the 70% success rate of glued graft retention is a significant improvement on previously obtained results with experimental lamellar keratoplasty. Rosenthal et al[3] achieved a 50% success rate of gluing lamellar grafts with a platelet/fibrinogen/thrombin mixture, and Cardarelli and Basu[15] achieved only about a 20% success rate using cyanoacrylate glue.

The differences in both surgical technique and adhesive material used in these studies may have influenced the varying success rates. From the surgical point of view, Rosenthal et al[3] and Cardarelli and Basu[15] both performed autokeratoplasty using viable donor lamellar tissue with an intact epithelial layer. We, however, used homologous-lathed lyophilized tissue

that was devoid of epithelium or living keratocytes. The use of donor tissue without viable epithelium might have been expected to give a poorer success rate due to the greater time required for an intact epithelial layer to be established over the graft. In this series, the time to complete disappearance of a graft epithelial defect ranged from three to ten days. No grafts were lost that had intact epithelium. This may be because once complete epithelial cover of the graft has been obtained, the graft stromal tissue undergoes a degree of deturgescence and is held in place by hydrostatic forces, as well as by the normal-healing process.

In lamellar autokeratoplasty, there is no change in corneal contour, with the only irregularity being at the graft/host junction. As the wound heals, this junction is filled with epithelial cells that migrate from the wound border. However, until the junction is watertight, the exposed stromal wound edges are edematous, and the edge of the graft may be traumatized by eyelid movement that can lead to poor wound edge apposition or wound dehiscence. In our epikeratophakia surgical technique, we created a peripheral lamellar split of 1.5 mm to incorporate the thin lenticule wing zone, and this protected the graft edge from external trauma. The epikeratophakia lenticules used for this series were designed to correct high hyperopia (typical change in keratometry = +15 diopters). Although the graft edges were protected, these steep grafts could be considered more likely to be extruded due to trauma to the projecting optical apex of the graft by the eyelid. We did not use tarsorrhaphies in the postoperative period to aid graft retention, as did Rosenthal and colleagues,[3] but fitted the animals with specially made bicurved bandage soft contact lenses. These were generally retained until epithelialization was complete and may have helped to stabilize the graft. However, they were not consistently retained, viz, animal 6.

Buschmann[7] found it necessary, when using Tisseel for gluing conjunctival wounds, to apply topical aprotinin to inhibit premature fibrinolysis, and we also used topical aprotinin in the hope of prolonging the adhesive action. Although reepithelialization of the lenticule is typically complete by around four days postoperatively when using sutures,[10] with the first two animals in group 1 treated with aprotinin, there was hardly any reepithelialization by five days. This was attributed to an inhibitory effect of the aprotinin drops; consequently, we reduced the treatment period in the rest of the group to 24 hours only. The remaining animals all reepithelialized more quickly. It is unclear from these preliminary experiments to judge to what extent the use of aprotinin contributed to the success of the technique.

Of those animals that lost grafts, animal 6 was followed up for a further four days, during which time the graft bed reepithelialized and returned to a normal state with a clear central cornea. If an epikeratophakia graft is lost, the possibility remains of repeating the procedure at a later data, but we did not attempt any repeated grafts in this series. The remaining animals that lost grafts were not followed up for long enough to allow reepithelialization.

All the grafts that successfully epithelialized did, however, undergo later complications with secondary epithelial breakdown. Since this complication occurred in both the glued graft and control groups, it could not have been caused by the presence of the adhesive. In our previous work using sutured grafts in this animal model, we had not encountered similar problems of epithelial breakdown.[10] In this previous study, we maintained bandage contact lens wear until a few days after the sutures were removed at ten days postoperatively. However, in the current series, the contact lenses were removed on the day that epithelial cover of the graft was established. It seems probable that this early removal of the contact lens accounted for the subsequent epithelial breakdown since the epithelium had not had time to stabilize on the graft.

If an adhesive technique could be successfully applied to human epikeratophakia, the operative time would be significantly reduced with benefit to both patient and surgeon. By expediting the procedure, the use of local anesthesia could be more readily recommended; there would be a reduction of both surgical trauma and the risk of retinal damage from the light of the operating microscope. In addition, postoperative management could be simplified, and further anesthesia for suture removal in children could be avoided. It has been reported that incorrect suture technique can have a significant effect on refractive outcome of the graft,[14] and it is possible that by removing this source of error in the procedure, the accuracy of refractive correction could be further enhanced.

This initial study has shown the effectiveness of Tisseel adhesive as a bonding agent for epikeratophakia grafts. The success of the technique seems also dependent on our modification of lenticule design and operative technique.

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D. C. Bouch, MRCPath, performed the histopathological studies, and J. R. Rostron prepared the manuscript.

References

1. Katzin HM: Aqueous fibrin fixation of corneal transplants in the rabbit. *Arch Ophthalmol* 1945;35:415-420.

2. Tassman IS: The use of fibrin coagulum fixation in ocular surgery: Keratoplasty. *Trans Am Acad Ophthalmol* 1949;54:134-139.

3. Rosenthal AR, Harbury C, Egbert PR, et al: Use of a platelet-fibrinogen-thrombin mixture as a corneal adhesive: Experiments with sutureless lamellar keratoplasty in the rabbit. *Invest Ophthalmol Vis Sci* 1975;14:872-875.

4. Klemen UM, Freyler H, Prskavec FH, et al: Die Einheilung lamellarer Hornhauttransplantate beim Kaninchen nach Klebung mit Fibrinogen. *Graefes Arch Clin Exp Ophthalmol* 1979; 210:261-267.

5. Puchkovskay NA, Gorgiladze TU: Results of keratoplasty. *Cornea* 1987;6:70.

6. Robin JB, Salazar J: The use of mussel adhesive protein in epikeratophakia. *Invest Ophthalmol Vis Sci* 1987;28(suppl):225.

7. Buschmann W: Fibrogen concentrate and topical antifibrinolytic treatment for conjunctival wounds and fistulas in Schlag G, Redl H (eds): *Fibrin Sealant in Operative Medicine.* New York, Springer-Verlag NY Inc, 1986, vol 2, pp 68-69.

8. Buschmann W: Microsurgical treatment of lens capsule perforations: II. Clinical applications and results. *Ophthalmic Surg* 1987;18:276-282.

9. Henrick A, Gaster RN, Silverstone PJ: Organic tissue glue in the closure of cataract incisions. *J Cataract Refract Surg* 1987;13:551-553.

10. Rostron CK, Sandford-Smith JH, Morton DB: Experimental epikeratophakia using tissue lathed at room temperature. *Br J Ophthalmol* 1988;72:354-360.

11. Hilfenhaus J, Herrmann A, Mauler R, et al: Inactivation of the AIDS-causing retrovirus and other human viruses in antihemophilic plasma protein preparations by pasteurization. *Vox Sang* 1986;50:208-211.

12. Eder G, Numann M, Cerwenka R, et al: Preliminary results of a randomised controlled study on the risk of hepatitis transmission of a two-component fibrin sealant (Tissucol/Tisseel), Schlag G, Redl H (eds): *Fibrin Sealant in Operative Medicine.* New York, Springer-Verlag NY Inc, 1986, vol 3, pp 51-59.

13. Felding P, Nilsson IM, Hansson BG, et al: Absence of antibodies to LAV/HTLV-III in haemophiliacs treated with heat-treated factor VIII concentrate of American origin. *Lancet* 1985; 1:832-833.

14. Rostron CK: Epikeratophakia: Clinical results and experimental development. *Eye* 1988;2:56-62.

15. Cardarelli J, Basu PK: Lamellar corneal transplantation in rabbits using isobutyl cyanoacylate. *Can J Ophthalmol* 1969;4:179-182.