

Novel adaptation of a running suture technique in a mouse model of corneal transplantation

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ABSTRACT

Several murine models of corneal transplantation have been developed over the years to study the immunopathological processes that lead to the failure of grafted corneas. In all of them, the classic eight interrupted sutures technique is utilized for transplanting the donor cornea on the host bed. However, in clinical practice, a single continuous suture with a single knot is generally performed for corneal transplantation. Here, we describe the adaptation of the single continuous suture technique in a mouse model of corneal transplantation.

Keywords: animal model, corneal transplantation, running suture technique

INTRODUCTION

Over the past 115 years, keratoplasty has progressed to become the most widely performed solid tissue transplantation procedure [1]. In 2019, the Eye Bank Association of America reported 51336 corneal transplantations performed in the United States [2]. The development of experimental murine models of corneal transplantation beginning three decades ago has contributed greatly to our understanding of the pathological processes that lead to graft failure [3-8]. These models have been used to evaluate the host alloimmune response to the graft, to investigate the role of angiogenesis in the immunological response [9,10], and to assess the therapeutic effect of various immunomodulatory strategies [11-13]. In murine models of corneal transplantation, the most commonly used technique is the interrupted suturing method, in which a 2 mm donor corneal graft is secured to a 1.5 mm diameter recipient bed with eight interrupted (individual) 11-0 nylon sutures which are left un-buried [14]. Seven days post-transplant, the sutures are removed from the grafted cornea [15]. In clinical practice, surgeons performing corneal transplantation traditionally utilize a similar interrupted suturing technique, which allows for individual suture removal; however, the “running” suture technique, which consists of a single continuous suture with one knot, is also commonly performed. Potential benefits of the running suture technique include less irritation, suture loosening, and neovascularization [16], as well as even distribution of suture tension to reduce postoperative astigmatism [17,18].

Here, we report the adaptation of a continuous suturing method, commonly used in the clinical setting, to a murine model of corneal

transplantation. Although a comparison between running and interrupted suture techniques has been described in the literature [19], here we provide a step-by-step description of the single continuous suture technique which will be of potential use for future investigators interested in using this model. Follow-up with slit-lamp photographs confirming the feasibility of this technique is shown.

MATERIALS

Mice

- ✓ 6 to 8-week-old male/female* C57BL/6** mice (donor)
- ✓ 6 to 8-week-old male/female BALB/c mice (allogeneic recipient)
 - *Age- and sex-matched mice have fewer variable outcomes.
 - **Depending on the aim of the study, the technique can be applied to different strains, genders, ages, and animal models for donor and recipient animals

Reagents

- ✓ Ocular viscoelastic device (OVD) (Bausch & Lomb, Rochester, NY, USA, Cat. # 59081L)
- ✓ Propacaine hydrochloride ophthalmic solution 0.5% (wt/vol), (Bausch & Lomb Incorporated, Tampa, FL, USA, NDC 24208-730-06)
- ✓ Phenylephrine ophthalmic solution 5% (ALTAIRE Pharma-

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- ✓ ceuticals, Inc., Aquebogue, NY, USA, NDC 59390-193-13)
- ✓ Tropicamide ophthalmic solution 1% (Bausch & Lomb Incorporated, Tampa, FL, USA, NDC 24208-590-64)
- ✓ Triple antibiotic eye ointment (Bacitracin Zinc, Neomycin, polymyxin B Sulfates), (Bausch & Lomb Incorporated, Tampa, FL, USA, NDC 24208-780-55)
- ✓ Ketamine (20 mg/kg)
- ✓ Xylazine (100–200 mg/kg)
- ✓ Buprenorphine (0.05–0.1 mg/kg)
- ✓ Sterile Phosphate Buffer Saline (PBS), (Life Technologies Corporation, Grand Island, NY, USA, Cat. # 10010-031)
- ✓ 11-0 nylon suture for corneal suturing (MANI, Inc., Tochigi, Japan, Cat. # 0550S)
- ✓ 8-0 nylon suture for eyelid tarsorrhaphy (MANI, Inc., Tochigi, Japan, Cat. # 2056)
- ✓ Jeweler’s forceps
- ✓ Scissors (Vannas and curved)
- ✓ Sterile 1.5 mm and 2 mm diameter trephines. (HealthLink, Jacksonville, FL, USA, Cat. # BP15, BP20)
- ✓ Sterile 25G and 30G needles
- ✓ Weck-cel sponges (Beaver Visitec International, Inc., Waltham, MA, USA, Cat. # 008680)
- ✓ Binocular surgical microscope
- ✓ Slit lamp

Equipment

PROCEDURE

Mice were housed in the Schepens Eye Research Institute animal vivarium and treated according to the guidelines set forth by the Association for Research in Vision and Ophthalmology (ARVO). All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee.

Allogeneic full-thickness corneal transplantation

1. Obtaining the donor cornea
 - 1.1. Euthanize the donor C57BL/6 mouse *via* CO₂ inhalation.
 - 1.2. Mark the central cornea with a 2 mm diameter trephine. Apply enough pressure to penetrate the deep stroma without perforating the cornea.
 - 1.3. With a 30 G needle, enter the anterior chamber: proper tunneling leads to leakage of aqueous humor and partial collapse of the anterior chamber.
 - 1.4. Inject OVD to restore the anterior chamber.
 - 1.5. Carefully dry the corneal surface with a Weck-cel sponge.
 - 1.6. Along the marked, trephined edge, excise the central cornea using Vannas scissors.
2. Anesthesia
 - 2.1. Induce general anesthesia by intraperitoneal injection of ketamine (120 mg/kg) and xylazine (20 mg/kg) to the recipient BALB/c mouse.
 - 2.2. Apply one drop of 0.5% procainamide to the corneal surface as topical anesthesia.
3. Preparing the graft bed
 - 3.1. Once the animal is fully anesthetized, achieve mydriasis using one drop of 2.5% phenylephrine hydrochloride and 1% tropicamide.
 - 3.2. Place the mouse in a recumbent lateral position, placing the head so the eye can be visualized under the surgical microscope.
 - 3.3. Wash the ocular surface with PBS and dry it using Weck-cel sponges.
 - 3.4. Mark the central cornea with a 1.5 mm diameter trephine. Apply enough pressure to penetrate the deep stroma without perforating the cornea. (**Fig. 1A**)
 - 3.5. Using a 30 G needle, enter the anterior chamber.
 - 3.6. Inject OVD to restore the anterior chamber.
 - 3.7. Carefully dry the surface with Weck-cel sponges.
 - 3.8. Along the marked, trephined edge, completely excise the central cornea using Vannas scissors and discard.
4. Suturing the graft (**Movie S1**)

- 4.1. Anchor the 2 mm donor cornea on the 1.5 mm recipient bed using a single interrupted 11-0 nylon suture at 12 o'clock, penetrating the donor graft first, and biting through 90% of the recipient corneal depth.
- 4.2. Place three additional interrupted cardinal sutures at 3, 6, and 9 o'clock (**Fig. 1B** and **Fig. 2A**).
- 4.3. During and after suture placement, inject OVD to maintain anterior chamber depth.
- 4.4. Start the continuous suture using an 11-0 nylon between 12 and 1 o'clock, running clockwise, placing two evenly-spaced suture bites within each quadrant (**Fig. 1C** and **Fig. 2B**).
- 4.5. Adjust suture tension along each suture segment.
- 4.6. Wash the anterior chamber with PBS to remove OVD, maintaining the anterior chamber and achieving a physiologic intraocular pressure (**Fig. 1D**).
- 4.7. Adjust suture tension along each suture segment and permanently secure the suture knot once the desired tension is achieved (**Fig. 1E** and **Fig. 2C**).
- 4.8. Remove the four cardinal interrupted sutures (**Fig. 1F**).

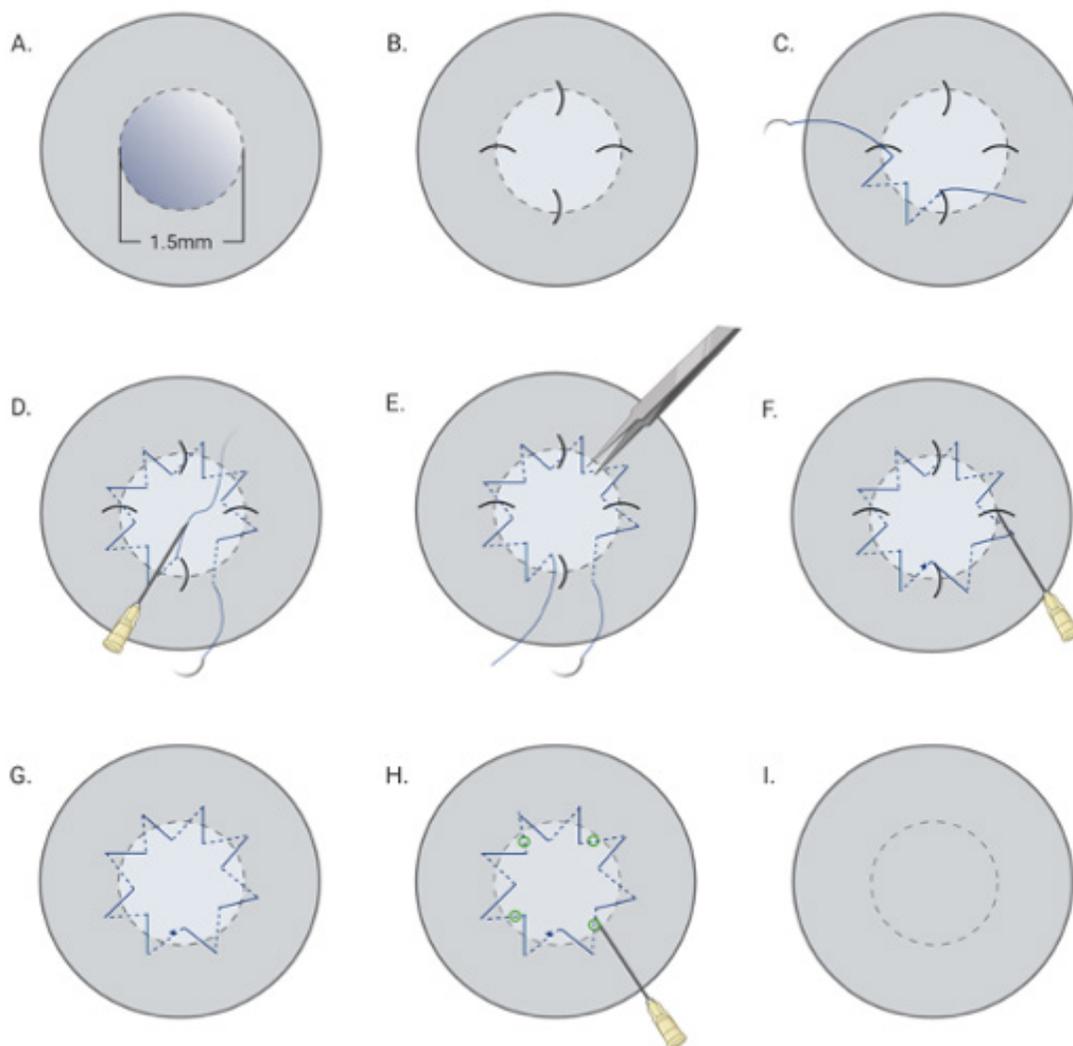


Figure 1. Schematic drawing of running suture placement. **A.** Mark the recipient central cornea with a 1.5 mm trephine and excise cornea. **B.** Place 4 intrastromal interrupted 11-0 nylon sutures to secure the donor cornea, creating 4 quadrants. **C.** Place running suture by taking 2 suture bites per quadrant. **D.** Rinse the anterior chamber with PBS. **E.** Adjust suture tension. **F.** Cut the 4 interrupted cardinal sutures using a 30 Gauge needle. **G.** Schematic representation of the cornea post-transplantation. **H.** At day 7, cut the running suture within each quadrant (green circles) and remove suture. **I.** Schematic representation of healed, transplanted cornea.

5. Final evaluation
 - 5.1. Observe the eye to make sure the pupil is round and the anterior chamber depth is normal (**Fig. 1G** and **Fig. 2D**).
 - 5.2. Apply antibiotic ointment to the operated eye.
 - 5.3. Perform tarsorrhaphy with an 8-0 nylon suture to close the eyelids
 - 5.4. Administer buprenorphine (0.05–0.1 mg/kg) by subcutaneous injection immediately after surgery to minimize post-operative pain.
6. Suture removal
 - 6.1. At 24 hours post-transplantation, anesthetize mice as described above and remove tarsorrhaphy.
 - 6.2. At seven days post-transplantation, anesthetize mice as described above and cut the suture at one location in each quadrant and remove the suture (**Fig. 1H**).
7. Clinical assessment
 - 7.1. Examine eyes by slit-lamp microscopy weekly for eight weeks to assess corneal opacity and neovascularization using a standard scoring system [14].

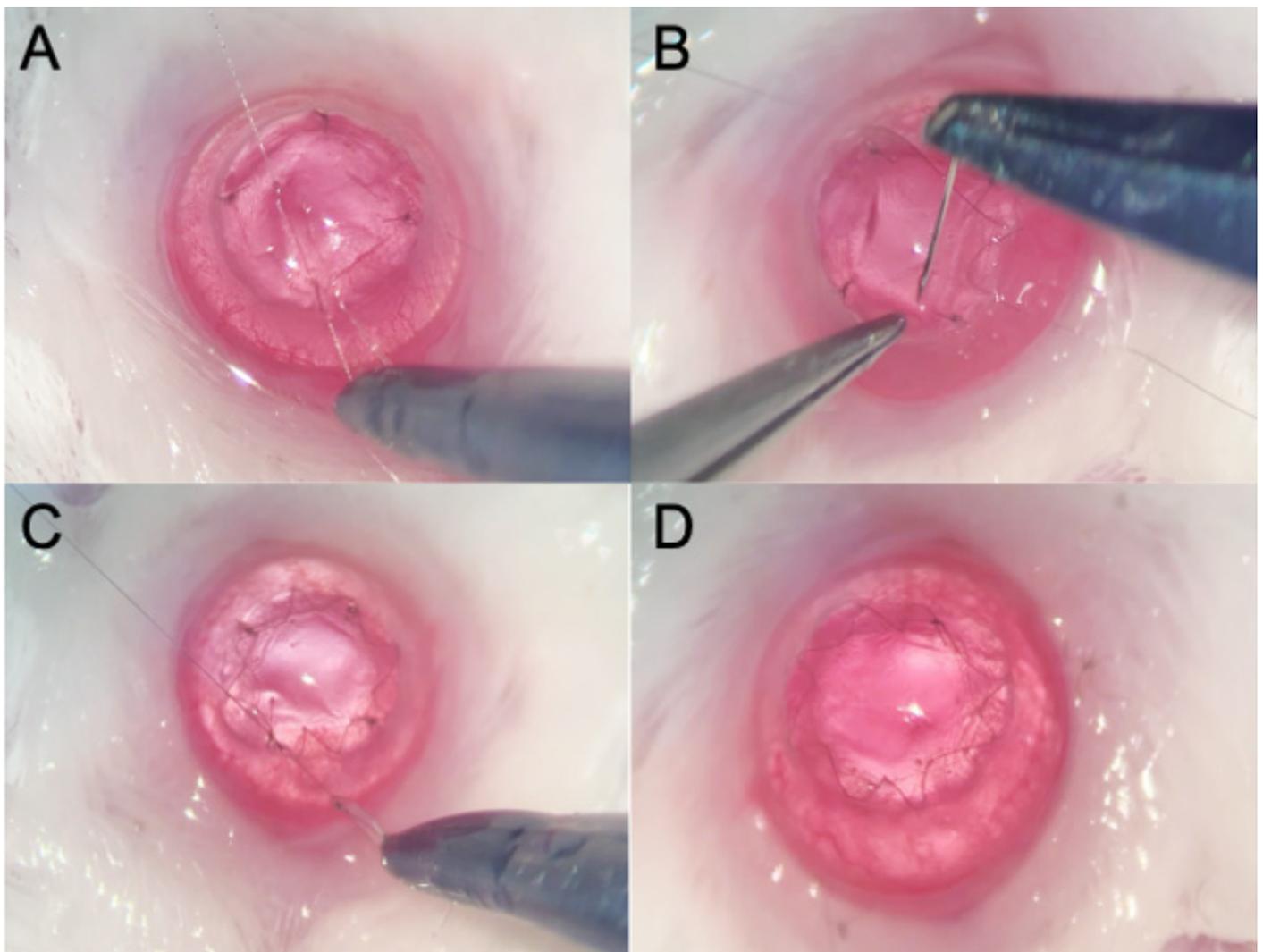


Figure 2. Running suture surgical procedure. **A.** Place 4 intrastromal interrupted 11-0 nylon sutures to secure the donor cornea to the recipient bed. **B.** Place continuous suture by placing 2 suture bites within each quadrant. **C.** Tie-off the running suture knot and remove the 4 cardinal interrupted sutures. **D.** Post-operative appearance immediately after surgery.

RESULTS

The continuous suture technique was successfully developed in a mouse model of corneal transplantation, and no complications were observed through 8-weeks of follow-up (Fig. 3).

All sutures remained in place and no suture breakage or changes in tension were observed. In contrast to corneal transplants with interrupted sutures, where neovascularization is sometimes observed due to un-buried suture knots, no neovascularization was observed in corneas with the continuous technique. During suture removal, we did not observe any complications such as aqueous humor leakage, anterior chamber decompensation, or graft dehiscence. Regarding the operative time for transplantation and suture removal, we found that the running suture technique requires more time for suturing the graft and less time for suture removal compared to the interrupted suture technique, but there was no difference in overall surgical time (Table 1). Incidentally,

we observed fewer anterior synechiae in the continuous suture group (4/26 mice, 15%) as compared to the interrupted suture group (8/24 mice, 33%) (see Fig. 4 for representative images). Anterior synechiae formation is a common complication in corneal transplantation which may increase the rate of graft rejection and future studies may be warranted to investigate the possible contribution of anterior synechiae to graft rejection and failure [20].

In conclusion, the continuous running suturing technique is feasible in a mouse model of corneal transplantation and represents an adaptation of a very well-established clinical technique to an animal model. The current report provides detailed step-by-step instructions for investigators to put this model into practice, and in future studies it will be important to compare the running and interrupted suture techniques in terms of clinical characteristics (neovascularization and graft opacity) and graft survival.

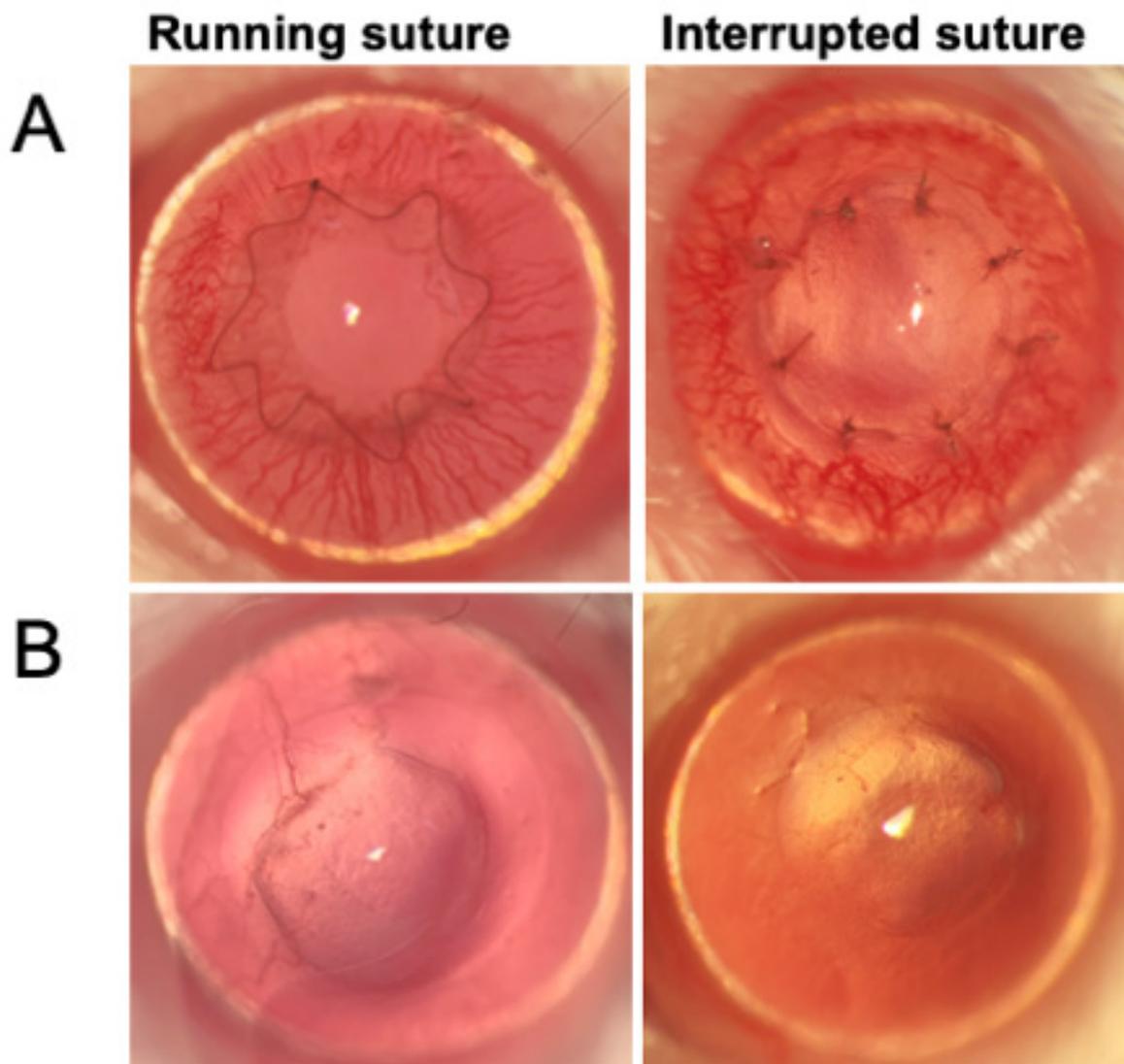


Figure 3. Representative post-operative images after corneal transplantation. A. Postoperative slit-lamp appearance 1 week after running (left) and interrupted (right) suture techniques. **B.** Postoperative slit-lamp appearance 8 weeks after running (left) and interrupted (right) suture techniques.

Table 1. Surgical time using the running and interrupted suture techniques*.

	Transplantation (min)	Suture removal (min)	Total time (min)
Running	23.2 ± 2.8	4.7 ± 1.4	27.9 ± 3.0
Interrupted	18.3 ± 2.6	9 ± 1.9	28 ± 2.7
<i>P</i> value	0.001	0.001	0.920

*Min, minutes; data given as mean ± SD.

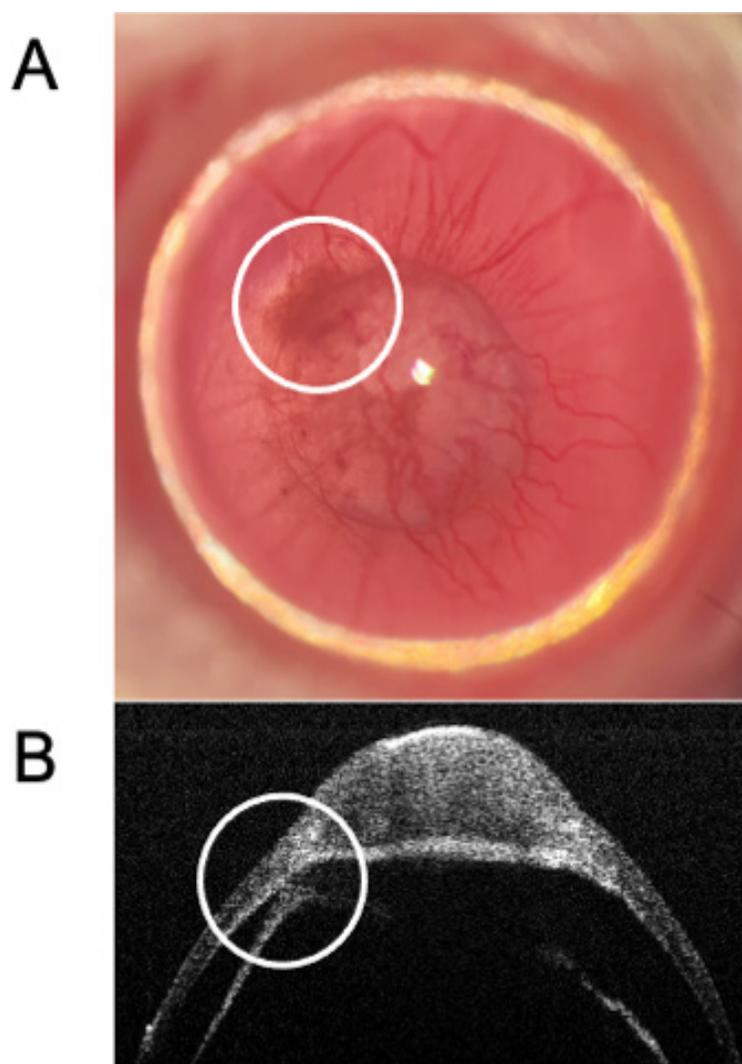


Figure 4. Anterior synechiae: a severe complication of corneal transplantation. **A.** Slit-lamp image showing anterior synechiae and corneal neovascularization. **B.** Anterior synechiae in the anterior chamber visualized on Optical Coherence Tomography.

POTENTIAL COMPLICATIONS

Potential issues that may be encountered with corneal transplantation using the continuous suture technique are outlined below.

1. A cataract is a major complication that results from physical contact with the lens capsule while preparing the recipient bed.
2. Hemorrhage in the anterior chamber and subsequent hyphema
3. It is essential to keep the surgical field clear as a foreign body can become trapped within the suture and potentially result in suture breakage.
4. Anterior synechiae between the iris and cornea may form

formation may occur due to surgery-related trauma, typically due to accidental injury to the iris during the procedure. Anterior chamber washout with PBS may be performed to clear the anterior chamber.

and may be identified by slit lamp exam and/or anterior segment OCT.

NOTES: These are typical complications observed with the classical interrupted technique, and although we cannot rule out other specific complications related to this new technique, it likely that encountered complications are related more to the surgical learning curve rather than the technique itself.

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Supplementary information

Movie S1. The video included how to place the interrupted suture, how to start the continuous suture, how to adjust suture tension at the end.

Supplementary information of this article can be found online at <https://jbm.wistia.com/medias/xkkdlg2v01>.



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