Reverse graft suturing to avoid Descemet’s membrane detachment of glycerol-preserved donor cornea used for therapeutic penetrating keratoplasty during COVID-19 to overcome the tissue shortage – A novel surgical technique

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To overcome tissue shortage during pandemic, we switched to 100% glycerol preservation of the donor cornea, which is economical and provides longer duration of storage than the short and intermediate storage mediums we normally use like McCAREY Kaufman (MK) or cornisol. During our initial few cases of therapeutic penetrating keratoplasty using glycerol preserved donor cornea, we faced spontaneous Descemet’s detachments resistant to air tamponade. We tried reverse graft suturing and successfully reinforced Descemet’s attachment along with air tamponade, in one of the cases after multiple failed air injections. In the subsequent two cases of infective keratitis needing therapeutic penetrating Keratoplasty, we took eight reverse sutures in between the eight cardinals, to anchor the Descemet’s membrane of the graft. Both the grafts showed attached Descemet’s and maintained good graft clarity. The reverse corneal suturing technique has not been described to the best of our knowledge and hope this helps our corneal fraternity.

Key words: Covid-19, Descemet’s membrane detachment, glycerol-preserved donor cornea, reverse suturing, therapeutic penetrating keratoplasty

Case Reports

Case 1
A 66-year-old man came with total corneal ulcer that occurred after injury at construction site 15 days back. Corneal scraping grew aerobic Gram-negative bacillus Burholderia Cepacia; he underwent TPK with GPC and the corneal button grew Fusarium species. On the second week post TPK, he suddenly developed a gradually increasing DMD, for which he underwent air tamponade twice with no improvement [Fig. 1a]. Desperate to find a solution on the third attempt of air injection at 1 month post TPK, reverse corneal suturing in between radial sutures was taken randomly, to anchor the DM.

Intraoperative examination with handheld spectral domain ophthalmic imaging system (Bioptigen Inc.) confirmed the attachment which was also maintained in the postoperative period [Fig. 1b-d].

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Case 2

A 55-year-old female with corneal scar, from an ulcer treated locally 8 years back, came to our hospital for visual rehabilitation, and had optical PK and extracapsular cataract surgery with IOL implantation in 2014. She underwent complete graft suture removal a year later and maintained 6/15 vision for 6 years.

Figure 1: (a) Case 1: slit view of DMD. (b) Case 1: Intra-op Bioptigen of DMD. (c) Case 1: Bioptigen post attached. (d) Case 1: failing graft at 1 month. (e) Case 2: IOL explantation. (f) Case 2: 1 month ASOCT of attached DM. (g) Case 2: clear graft at 1 month. (h) Case 3: infected graft. (i) Case 3: arrow at iris incarceration. (j) Case 3: clear graft at 40 days. (k) Partial thickness host bite at inferior quadrant. (l) Full thickness graft bite the same site. (m) Partial thickness host bite at superior quadrant. (n) Full thickness bite at the same site
She stopped topical steroids during pandemic, due to local nonavailability of drugs and reported to us 1 month later, with graft rejection. Though the initial intensive topical and systemic steroids arrested the graft rejection, lack of proper follow-up during the lockdown period caused the situation to escalate to a total graft melt with extruding IOL. When she underwent IOL explantation with TPK using GPC, we did primary reverse suturing [Fig. 1e-g], by applying our experience learned from the previous case. Both the explanted IOL and corneal button showed negative microbiological results.

Case 3
A 74-year-old man came to us for visual rehabilitation with a total corneal opacity that he developed after endophthalmitis postcataract surgery done 1.5 years back elsewhere. He underwent optical PK and his vision improved up to 6/9 that he maintained with regular follow-up for 5 years. During pandemic, he could not maintain regular follow-up, and due to nonavailability of medication in the lock down period, he stopped topical treatment. He reported to us with total graft failure of approximately 2 months duration, then further developed persistent epithelial defect, for which, he underwent amniotic membrane transplantation. With continued lockdown, his follow-ups became more irregular, and he later reported with multiple graft infiltrates due to methicillin-resistant Staphylococcus epidermidis. With persistent ulcer in spite of appropriate medical management, we did TPK using GPC along with primary reverse suturing [Fig. 1h-j].

Surgical Technique
We use 100% glycerol prepared by adding 3 g of aluminosilicate molecular sieves (0.2-0.8 mm, Sorbade India) to 5 mL of glycerol (100%) (Molecular biology grade, HiMedia, India) for donor corneal preservation and store it between 2 and 8°C for up to 1 year. All the GPC used in this case series were initially stored in MK medium for 4 days, later in cornisol for 10 days, then transferred to 100% glycerol, and were utilized within 10 days. The age of the corneal donor were 78, 82, and 70 years with specular counts of 2590, 2353, and 2610 cells/mm² for the cases 1–3, respectively. Intraoperatively, GPC was immersed in normal saline to thaw and wash away residual glycerol for 30 min prior to usage. In the case 1 with spontaneous DMD after two failed attempts of air tamponade [Fig. 1a], eight reverse sutures were taken wherever possible, in-between the radial sutures along with air tamponade to reattach DM. Intraoperatively, a handheld spectral domain ophthalmic imaging system (Bioptigen Inc.) was used to confirm the proper DM attachment [Fig. 1b and c]. In the subsequent two cases, where primary reverse suturing was done, the usual steps for TPK was followed up to the stage of eight cardinal sutures. These were briefly as follows: general anesthesia for case 2 and local for case 3, fleringa ring fixation in both, host corneal trephination of 10 mm in case 2 and 8 in case 3, punching oversized donor corneas by 1.0 mm in case 2 and 0.5 in case 3, additional intraocular manipulations as needed, including clearing of exudative debris, cautery of possible bleeder, debridement of nonviable iris tissue and two surgical peripheral iridectomies in both, while IOL explantation with limited anterior vitrectomy in case 2 [Fig. 1e], and retention of IOL in case 3 and finally donor graft anchoring with eight radial sutures using 9-0 nylon in case 2 and 10-0 in case 3. Then, the anterior chamber was reasonably formed with saline and further suturing depended on the size of the graft. For case 2 with 11 mm graft, two sutures were placed in between each cardinal suture, and one out of the two was a reverse suture. For the case 3 with 8.5 mm graft, the next eight were reverse sutures, taken one in between each cardinal suture. The reverse suture bite starts from the host cornea as a partial thickness bite of 90% depth [Fig. 1k and m] and then continues as a full thickness bite of donor GPC to anchor the DM [Fig. 1l and n]. Care should be taken to avoid iris looping into the host corneal bite by avoiding a full thickness bite and while suturing the GPC by lifting the cornea rather than pressing to anchor DM for the full thickness bites [Fig. 2 and Video 1]. The reverse bite usually ends up being longer than the routine ones. Intracameral voriconazole 50 μgm in 0.1 mL of normal saline for case 1, and vancomycin 1 mg in 0.1 mL of normal saline for cases 2 and 3 were injected. At the end of suturing, all the three cases were stained with moistened strips impregnated with 0.6 mg of fluorescein sodium to check for leaks, identified by dye dilution upon gentle pressure around the suture tract. Detailed postoperative medical management is beyond the scope of this article, but all were maintained on appropriate antimicrobials and adjuvants and none had a recurrent infection up to 3 months postsurgery now.

Results
Case 1 showed attached DM after the reverse suturing combined with the third attempt of air tamponade. Topical steroids were not initiated in this case due to the fungal etiology of the infective keratitis and the graft eventually failed [Fig. 1d]. This patient reported a month later with perforated graft, underwent tectonic PK using a donor cornea stored in MK medium, as it was available, and the removed GPC button showed negative microbiological results. For cases 2 and 3, after epithelial healing, topical prednisolone acetate 1% (Pred Forte; Allergan, Inc., Irvine, California, USA) was started at 8 times per day dosage initially and then tapered weekly. Case 2 was aphakic, maintained good graft clarity, responded well to systemic treatment. Case 3 showed negative microbiological results. For cases 2 and 3, after epithelial healing, topical prednisolone acetate 1% (Pred Forte; Allergan, Inc., Irvine, California, USA) was started at 8 times per day dosage initially and then tapered weekly. Case 2 was aphakic, maintained good graft clarity, responded well to systemic treatment. Case 3 showed negative microbiological results.

Figure 2: The diagram shows partial thickness bite of host cornea, thread passing through the GHJ, and full thickness bite of the gentry lifted edge of the graft.

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steroids started for choroidal detachment, and her vision pre
TPK of PR accurate improved to CFCF [Fig. 1g] at 3 months.
Case 3 was pseudophakic, had a better graft clarity, attached
retina, and improved from CFCF preop to 6/18 postop [Fig. 1j].
None of the patients had postoperative suture leaks at the site
of reverse suture bites or at the graft host junction; checked
under slit-lamp bio microscopy, observed with cobalt blue
filter, after surface staining with moistened strips impregnated
with 0.6 mg of fluorescein sodium. Absence of the leak using
GPC could be attributed to the biomechanical stiffness and the
varying parallelism of collagen as noted by Wei Chen study.[4]
We considered therapeutic success as anatomical restoration
and eradication of infection, and therapeutic failure as loss of
globe integrity and recurrence of infection post TPK using GPC.
Our first patient achieved partial therapeutic success due to
graft perforation though no recurrent infection occurred, but
the other two cases achieved therapeutic success, maintained
up to 3 months post TPK. Only one out of the three patients
achieved good visual improvement, while the first two patients
failed to benefit visually.

Discussion
In order to optimize the corneal tissue utilization, a longer
duration of corneal preservation is crucial, since it avoids
wastage due to nonusage. Among the longer duration of corneal
preservation, like the cryopreservation, lyophilization, organ
culture, or the gamma radiation, the glycerol preservation
has many good attributes. It is relatively inexpensive,
easily adaptable by eye banks, does not require specialized
instruments, and gives a wide option of storing from room
temperature to sub-zero up to ~80°C.[5] It is well documented
from the skin and the bone banking that glycerol preservation
provides good antimicrobial property.[6–9] GPC will not induce
graft rejection as it is devoid of cells including the epithelium,
keratocytes, and the bone marrow-derived cells, which form
the major histocompatibility complex antigens and minor H
antigens.[10] The introduction of glycerol corneal preservation
started as an experiment in feline eyes by J H Singh, who
later started human corneal preservation using 95% glycerol
under vacuum to ensure its anhydrous state. In 1961, he
achieved glycerol dehydration by adding sodium and calcium
alumina silicates as a physical adsorptive agents, obviating the
cumbrous vacuum system.[11] These molecular sieves are
synthetic crystals absorbing and trapping water molecules in
their pores, thereby dehydrating glycerol. J H Singh performed
the first LK after achieving reasonable dehydration of the
glycerol preserved corneas.[3]

When using GPC for PK, poor graft clarity needing
subsequent optical PK and persistent epithelial defect are
the most commonly known complications. However, when
we started using GPC for emergency TPK during pandemic,
we encountered spontaneous DMD around a week in the
postoperative period, though well attached DM was
documented in the early postop days. GPC is thicker and
acellular, specifically with the absence of endothelium, which
can make it prone for DMD which calls for careful tissue
handling. Additionally, the repeated failure to reattach the
DM, after attempts of air tamponade could be attributed to
the absence of healthy endothelial pump function in GPC.
With the acute shortage in donor corneal tissue and increasing
emergency cases needing TPK, we resorted to the desperate
attempt of reverse graft suturing along with the third attempt
of reverse suture bites in one of the initial cases. When we found
success with this approach, our preventive reverse suturing
in the subsequent two cases was better executed with proper
radial placement of sutures in between the cardinal sutures
and both had attached DM with good graft clarity up to the
last follow-up of 3 months. The mechanical anchoring by the
reverse sutures might prevent an easy separation of the DM
in GPC lacking endothelium, especially in the initial postop
period, when major inflammatory changes occur in the anterior
chamber after TPK.

Specific indications for GPC can be lamellar surgery not
needing viable endothelium,[11] corneal melt or perforation
needing tectonic support using stiffer cornea with low
immunogenicity to neutralize host’s persistent inflammation,[12]
therapeutic PK utilizing antimicrobial property of GPC for
medically uncontrolled infective keratitis, and PK in poor
prognosis eyes with multiple rejections. There are other
indications that need to be explored like, as a K-pro bearer
reducing graft melts with biomechanically stiffer and less
immunogenic GPC. Another area that can be explored is,
as a patch graft to cover tube exposure in glaucoma implants,
since stiffer GPC especially stored in room temperature,[5]
can be easily trimmed to the desired thickness, and its translucency
can be cosmically more appealing than the white scleral patch
grafts visible sub-conjunctivally. In nonseeing eyes with corneal
leucoma, which are unsuitable for cosmetic contact lenses, LK
using GPC with lamellar corneal tattoo can optimize cosmesis
with lesser chances of rejection. Further, varying properties
like transmittance, translucence, stiffness, decellularization,
anti-immunogenicity, and varying collagen parallelism of
GPC with different storage techniques as proven by Wei Chen
et al. can also be optimized to the needs.[4] This will help us
form proper prevention protocols to optimize GPC usage for
the intended surgical goal. Though our novel surgical technique
can open up opportunities of GPC usage in certain situations,
successful outcome of more number of cases is preferred. The
present increase in level of corneal retrieval prevents us from
including more cases, hence forming our study limitation.
However, by sharing our limited experience, it could prove
to be handy, in view of future uncertainties created by the
pandemic.

Conclusion
This method of reverse corneal suturing will be useful to avoid
DMD when TPK is done using GPC. Though the resultant graft
clarity is not good enough to annul the need for optical PK, it
can still enhance the chamber visibility, to better titrate postop
antimicrobial treatment. Further studies with a bigger sample
size and longer duration of follow-up can further shed light
on the other aspects of this technique.

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Conflicts of interest
There are no conflicts of interest.

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