Early endothelialization of ab interno stromal tectonic patch in the management of corneal perforation secondary to bacterial keratitis.

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Abstract

Purpose – To describe a novel surgical technique using an ab interno stromal patch to treat corneal perforation, and to present the histological findings of the patch following its removal during definitive mushroom keratoplasty.

Methods – A 22-year-old female presented with a large paracentral corneal perforation affecting the visual axis, secondary to a large pseudomonas corneal abscess.

Results – The patient was managed successfully with a sutureless ab interno stromal tectonic patch. 2 months later, definitive mushroom keratoplasty was performed and the patch was sent for histological examination. Immunohistochemistry revealed a reactive endothelium covering the posterior surface of the graft. Nine months later her best corrected visual acuity was 6/9.

Conclusions – This is the first case to our knowledge demonstrating that stromal tectonic grafts without endothelium can successfully attach to host tissue and seal a perforation. Stroma may undergo re-endothelialization and begin to restore vision, even prior to PK.

Introduction

Corneal perforation is a sight-threatening complication of corneal pathology, trauma or ocular surgery. Emergency treatment options for small perforations have historically included bandage contact lens, cyanoacrylate glue, amniotic membrane graft and pedicle conjunctival flap. Tectonic keratoplasty for larger perforations has conventionally been approached anteriorly using either full-thickness or lamellar donor tissue.[1] However, sutures placed during an anterior approach can induce significant astigmatism, and other suture related ocular surface complications. For this reason, various technical permutations of Descemet stripping (automated) endothelial keratoplasty (DS(A)EK) have recently been proposed in sealing the perforation from within the anterior chamber.[2–6] Unlike emergency gluing with cyanoacrylate, which is often the first-line treatment for such patients, tectonic DSAEK may be a definitive rather than an often temporizing measure, and the stromal substrate of the DSAEK may promote re-epithelialization across the perforation site sooner than glue which is cytotoxic.[4]

In conventional DSAEK, it is presumed that adherence of the lenticule requires the donor endothelium to create a negative pressure across the graft-host interface.[7,8] Conversely, in tectonic DSAEK we have previously hypothesized that the perforation itself acts as a large ‘venting incision’ (until re-epithelialization is complete) which promotes DSAEK adherence due to the pressure gradient across the perforation site.[5] We recently had the opportunity to test this hypothesis via a case in which stromal material without endothelium was used as an ab interno patch to seal a corneal perforation. We herein present the clinical outcomes of this case, along with the

histological findings from this stromal button following its subsequent explantation during rehabilitative surgery.

Materials & methods

A 22-year-old female contact lens user presented with a 2-day history of painful red right eye and loss of vision. Initial examination revealed a 7mm central corneal abscess and a 3mm paracentral corneal perforation with a flat anterior chamber (AC). Visual acuity was perception of light at presentation. Corneal scrapes were taken, the perforation was sealed with cyanoacrylate glue and a 4mm drape patch and bandage contact lens were applied. Broad-spectrum topical and oral antimicrobial therapy was initiated, and treatment was tailored after cultures grew *Pseudomonas aeruginosa* sensitive to quinolones.

Application of cyanoacrylate glue was successful in reforming the anterior chamber for 24 days, after which the glue became dislodged and re-gluing was attempted. However, the size of the perforation had extended to become greater than 4mm, therefore a tectonic graft was required to maintain the integrity of the globe. A corneal button which had already been used to harvest a Descemet's Membrane Endothelial Keratoplasty (DMEK) on the same day was available for the ab interno tectonic graft. The endothelium had already been removed via the SCUBA technique and a 2mm port had been trephined in the mid-periphery for marking the DMEK.[9] It was not possible to prepare a tectonic DSAEK as the endothelium had been removed and microkeratome of the graft was not possible due to the 2mm trephination. Thus, the epithelium of the donor cornea was removed and a 6mm corneal trephine (SmithKline Beecham Ltd, Berks, UK) was placed eccentrically to the center to harvest a 6mm diameter graft of full-thickness stroma.

Surgery was performed with a sub-tenons anesthetic. The iris was displaced posteriorly from the perforation by filling the AC with sodium hyaluronate through the perforation. Following this, a 6 mm 3-step clear incision was made in the peripheral temporal cornea. A supero-nasal paracentesis was made with a 23G MVR blade. The bevel of a 23G needle was bent to 90 degrees and passed through the paracentesis across the AC, out of the temporal section and embedded in the graft which was being held with notched forceps. The needle was then withdrawn back into the AC, dragging the graft with it. The reason a needle was used to pull through the graft is that the graft was too thick to fit in the Busin glide or be grasped by DSAEK forceps[10]. The graft was positioned under the perforation and the needle was disengaged and withdrawn. Viscoelastic was exchanged for balanced saline solution, which was subsequently exchanged for 100% air. The patient was instructed to lie on her back for 2 hours to aid attachment.

Methods for analysis of histological specimen

The tissue was fixed in 10% neutral buffered formalin and then processed and embedded in paraffin wax. Microtomy was performed to yield 4 micron sections. These were initially stained with hematoxylin and eosin, periodic acid-Schiff (PAS), Gram and Grocott. Subsequent immunohistochemistry was performed using antibodies from Dako (vimentin) and Leica (AE1/AE3, CD56) on a Leica BondMax immunostainer.

Results

Clinical Outcome

The patient was examined 2 hours after surgery, the following day and at 1, 2 and 4 weeks when she was subsequently listed for definitive elective surgery. Throughout follow-up, the stromal graft remained in situ and AC was deep with no evidence of leak (Fig. 1A). The anterior surface of the graft was fully re-epithelialized by the 1 month review. At 2 months, best corrected visual acuity (BCVA) was 6/24 in a quiet stable eye with no evidence of infection and an intact epithelium. At this point the patient underwent rehabilitative microkeratome-assisted two-piece mushroom keratoplasty (8.5mm anterior lamella on 6mm posterior lamella).[11] The explanted initial stromal graft was sent for histological analysis. When reviewed at 9 months her BCVA had improved to 6/9 and she was listed for suture removal (Fig. 1B).

Histological findings

The specimen was received in two parts. There was an anterior portion of scarred and mildly inflamed host tissue showing re-epithelialized and thinned stroma adjacent to the presumed perforation site. The stromal graft also showed epithelialization along its anterior surface and edge.

Additionally, the posterior surface of the graft was partly lined with a monolayer of flattened cells. On immunohistochemistry, these co-expressed CD56 and vimentin as well as AE1/AE3 (typically expressed in epithelium). The presence of all three markers strongly suggested that the cells were reactive endothelial cells. By comparison, the endothelium on the host portion of tissue expressed CD56 and vimentin, but not AE1/AE3. Figure 2 is a composite display of these findings.

Discussion

We have previously demonstrated that tectonic mini-DSAEK can be used in the management of corneal perforation as an effective temporizing measure prior to visually rehabilitative keratoplasty, but also as a definitive intervention in itself.[4,5] This case demonstrates successful closure of a perforation using donor stroma without endothelium. It is likely that the perforation itself acted as a venting incision large enough to create a pressure gradient to draw the stromal graft into sealing the perforation. The OCT image in Fig. 3A shows that the graft is only adherent over the perforation and detaches away from this site. The lack of endothelium and therefore negative pressure pump may explain why the graft detaches peripheral to the venting incision site, suggesting that in tectonic DSAEK for corneal perforation, the most important factor in graft adherence is the pressure gradient across the venting incision, rather than graft endothelial function.

The histological findings from this case indicate that following ab-interno stromal patching (no donor endothelium or epithelium present), a reactive endothelium began to line the posterior stromal surface within two months. These cells are likely to have migrated from healthy peripheral host endothelium; possibly recruited due to inflammation and cell signaling induced at the time of perforation. Theoretically, the endothelialization may alternatively result from metaplastic processes originating in donor stroma. This mechanism however is unlikely having only been observed *in vitro* when stromal keratocytes, grafted into an embryonic environment, lost their phenotypic traits and differentiated into endothelium.[12]

One advantage to using stromal patches without endothelium is that this tissue can be harvested from leftover material from same-day elective DMEK surgery, reducing

pressure on tissue availability. A large series on split grafts reported good outcomes with no intraocular infections.[13] The current recommendation however from tissue authorities in the UK is not to split corneal grafts for two elective recipients in the UK, although it is ultimately left to the responsible consultant's discretion. In this particular case of a deeply infected cornea with a large perforation already too large to glue, the risk of sight loss from endophthalmitis was significant and therefore leftover corneal tissue to close the perforation was utilized.

One theoretical advantage of transplanting stromal tissue only (without endothelium) in such cases is that it may avoid sensitizing a 'hot' eye to donor endothelium, reducing the risk of rejection of subsequent keratoplasty. Nonetheless it still must be accounted for as a first corneal graft and therefore the risk of rejection of subsequent keratoplasty is still likely to be higher than compared to a first corneal graft.

Finally, the case provides proof-of-concept that in centers without microkeratome availability, a full thickness corneal button can be used safely via an internal approach.

This is the first case to our knowledge to report the use of a 6mm full thickness ab interno stromal patch graft to successfully treat a large corneal perforation, and provides some histological evidence that new endothelium may repopulate onto stroma without the requirement for donor endothelium. This study was limited to one case and further experience with this technique is mandated.

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Figures

Fig. 1A. Anterior segment photograph of the right eye after stromal ab interno keratoplasty for corneal perforation. **Fig. 1B.** Anterior segment photograph of the same eye after subsequent definitive mushroom keratoplasty. Note the deliberately decentred posterior stromal component to match the site of perforation.

Fig. 2. Top left: Hematoxylin and eosin staining of explanted host and donor tissue at low power to demonstrate architecture. **Bottom left:** AE1/AE3 immunohistochemistry (x10 objective) with surface epithelium indicated with green arrow and endothelial cell marked with red arrow. **Top right:** AE1/AE3 immunohistochemistry (x20 objective). Brown staining demonstrates epithelial-type proteins within endothelial cell cytoplasm. **Bottom right:** CD56 immunohistochemistry (x20 objective) with black arrow marking brown-staining CD56, a protein expressed in corneal endothelium.

Fig. 3A. Anterior segment optical coherence tomography (OCT) showing the tectonic stromal patch closing corneal perforation. **Fig. 3B.** Anterior segment OCT 9 months after definitive two-piece lamellar mushroom keratoplasty.