stromal opacity and a distinct increase in best spectacle-corrected visual acuity (BSCVA).

METHODS

Three eyes of three patients with progressive keratoconus were included in this interventional case series. Progression was defined as an increase in K_{max}>1.0 D within 12 months.[3] Pre- and postoperative examinations included corneal topography (Keratograph C, Oculus, Wetzlar, Germany), Scheimpflug imaging (Pentacam, Oculus, Wetzlar, Germany) and slit-lamp examination. CXL was performed as published previously.[4]

REPRESENTATIVE CASE

For an overview of corneal characteristics, please refer to table 1.

A 27-year-old man (case 1) was referred to us for bilateral keratoconus in April 2007. Previous topographies demonstrated progression in the right cornea, whereas the left cornea was stable.

We performed CXL in the right eye using hypo-osmolar riboflavin[4]: after abrasion, the minimal stromal thickness was 376 µm. Isoosmolar riboflavin solution was applied for 30 min, and ultrasound pachymetry showed a minimal stromal thickness of 382 µm. Hypo-osmolar riboflavin was applied every 20 s for a further 5 min, and corneal thickness increased to 417 µm. In cases 2 and 3, minimal corneal thickness was more than 400 µm after abrasio, and the standard protocol with isoosmolar riboflavin solution was used.

A trace haze was noted at 4 weeks postoperatively.[5] At 6 months, the haze had disappeared, and Scheimpflug analysis showed the (commonly observed) regression of K_{max} values of up to 2.8 D (data not shown).

At this point, the patient reported a decrease in VA in his left eye. Scheimpflug analysis showed keratoconus progression, and we performed CXL in November 2008. The early postoperative period was uneventful with a 1.0 haze at 4 weeks. At 6 months postoperatively, a 2.0 haze was noted, and regression was 6.8 D. At 12 months after surgery, the haze had consolidated to a deep stromal opacity (figure 1B, C, E), and the maximal regression was 9.5 D (figure 1A). BSCVA had increased to 20/20 from 20/50 preoperatively, and confocal microscopy (HRT Cornea module, Heidelberg Engineering, Germany) showed fibrosis of the stroma at a depth of 160–250 µm (figure 1D).

DISCUSSION

Regression of K_{max} is commonly observed following CXL.[2 6] Recently, a K_{max} regression by a mean of 2.68 D in 62% of patients at 1 year after CXL was reported.[2] Regression occurs between 6 and 36 months postoperatively and is often accompanied by an increase in BSCVA.

In contrast, our cases showed massive corneal remodelling with K_{max} regression of up to 9.5 D. This remodelling might be due to a reorganisation of the corneal stroma: subsequent to CXL, cellular processes such as keratocyte apoptosis and necrosis, and transformation of surrounding keratocytes to myofibroblasts take place in the anterior stroma.[7 8] Other morphological changes include a significant increase in the keratocyte proliferation in cross-linked corneas and an increase in collagen fibre diameter.[9]

Remodelling was accompanied by formation of a central stromal haze-like opacity, and BSCVA improved distinctly. The underlying mechanisms of this flattening effect are unknown and may include unintentional increases in riboflavin concentration, prolonged UV irradiation but also an individual predisposition.

Little is known about the similarities in the nature of haze after PRK and CXL. Subepithelial haze following PRK extends to a depth of approximately 60 µm below the epithelium and is transient in nature. In the case of the transient post-CXL haze commonly observed, the changes in reflectivity extend to the depth of the demarcation line (270–330 µm),[5] possibly due to the migration of inflammatory cells. The stromal opacities observed here also extend to a depth of approximately 300 µm (figure 1D). We propose the term 'deep stromal haze' for this phenomenon. The current follow-up in these patients is up to 12 months, and only a minor diminution was observed in the extent of 'deep stromal haze,' suggesting that the changes observed here might not be transient but rather permanent. The occurrence of this flattening effect is rare. We have performed more than 1000 CXL procedures in previous years and have observed this effect in three eyes only.

We report a massive corneal remodelling following CXL, concomitant with a distinct increase in BSCVA. Understanding the underlying mechanism would allow this effect to be induced reproducibly in every CXL procedure for the benefit of the patient.

Farhad Hafezi,[1,2] Tobias Koller,[3] Paolo Vinciguerra,[4] Theo Seiler[2]

[1]Department of Ophthalmology, Geneva University Hospitals, Geneva, Switzerland; [2]IROC, Institute for Refractive and Ophthalmic Surgery, Zurich, Switzerland; [3]Private practice, Dietikon, Switzerland; [4]Department of Ophthalmology, Istituto Clinico Humanitas, Milano, Italy

Correspondence to Professor F Hafezi, Department of Ophthalmology, Geneva University Hospitals, Rue Alcide-Jentzer 22, 1211, Geneva 14, Switzerland; farhad@hafezi.ch

Competing interests None.

Patient consent Obtained.

Ethics approval Ethics approval was provided by the cantonal ethics committee of the Canton of Zurich.

Provenance and peer review Not commissioned; externally peer reviewed.

Accepted 14 August 2010
Published Online First 8 October 2010

Br J Ophthalmol 2011;**95**:1171–1172.
doi:10.1136/bjo.2010.184978

REFERENCES

1. **Hafezi F,** Kanellopoulos J, Wiltfang R, *et al*. Corneal collagen crosslinking with riboflavin and ultraviolet A to treat induced keratectasia after laser in situ keratomileusis. *J Cataract Refract Surg* 2007;**33**:2035–40.
2. **Raiskup-Wolf F,** Hoyer A, Spoerl E, *et al*. Collagen crosslinking with riboflavin and ultraviolet-A light in keratoconus: long-term results. *J Cataract Refract Surg* 2008;**34**:796–801.
3. **Koller T,** Iseli HP, Hafezi F, *et al*. Scheimpflug imaging of corneas after collagen cross-linking. *Cornea* 2009;**28**:510–15.
4. **Hafezi F,** Mrochen M, Iseli HP, *et al*. Collagen crosslinking with ultraviolet-A and hypoosmolar riboflavin solution in thin corneas. *J Cataract Refract Surg* 2009;**35**:621–4.
5. **Seiler T,** Hafezi F. Corneal cross-linking-induced stromal demarcation line. *Cornea* 2006;**25**:1057–9.
6. **Coskunseven E,** Jankov MR 2nd, Hafezi F. Contralateral eye study of corneal collagen cross-linking with riboflavin and UVA irradiation in patients with keratoconus. *J Refract Surg* 2009;**25**:371–6.
7. **Esquenazi S,** He J, Li N, *et al*. Immunofluoresence of rabbit corneas after collagen cross-linking treatment with riboflavin and ultraviolet A. *Cornea* 2010.
8. **Wollensak G,** Iomdina E, Dittert DD, *et al*. Wound healing in the rabbit cornea after corneal collagen cross-linking with riboflavin and UVA. *Cornea* 2007;**26**:600–5.
9. **Mencucci R,** Marini M, Paladini I, *et al*. Effects of riboflavin/UVA corneal cross-linking on keratocytes and collagen fibres in human cornea. *Clin Exp Ophthalmol* 2010;**38**:49–56.

Donor tissue preparation for Descemet membrane endothelial keratoplasty

We are pleased to note that Dr Zarei-Ghanavati and his team have replicated our technique (originally presented at the American Academy of Ophthalmology meeting in Atlanta 2008 and subsequently published)[1] and agree that it is a viable, quicker and easier alternative to conventional donor preparation for Descemet membrane endothelial keratoplasty (DMEK) surgery.[2] The process of pneumatic dissection described is essentially similar to ours; however, they have overlooked some key adaptations which further maximise the potential of this novel technique.

We performed a superficial keratectomy using a 300 µm microkeratome head prior to performing the pneumatic dissection. In the event of failure to separate Descemet and endothelium (5% in our series), this additional step ensures that the tissue can still be used for Descemet stripping automated endothelial keratoplasty surgery and thus eliminates wastage. Furthermore, the anterior lamella obtained may be utilised for other cases.

Advancing the needle to a distance of approximately 2 mm from the limbus is sufficient to obtain the correct depth for separating Descemet and endothelium. The authors advocate advancing the needle into the central cornea. In our experience, this increases the chances of penetrating stroma or perforating through the endothelium. This results in either failure to separate Descemet and endothelium or tissue wastage.

The dissected tissue can be prepared and stored for up to 7 days in tissue culture medium without significant endothelial cell loss. We performed cell counts prior to preparation and 7 days after storage. Average endothelial cell loss was 4.44±4.3% which is lower than that reported using other techniques.

Staining the area of dissected Descemet and endothelium by injecting trypan blue into the bubble allows delineation of the dissected tissue. Although the authors have stained the endothelium directly, they advise against its use due to endothelial toxicity. While there is lack of convincing evidence that a low concentration of trypan blue and short exposure is toxic, injecting it into the bubble offers the advantage of (a) preventing direct contact with endothelium and (b) allowing eccentric punching to obtain a rim of stromal support.[3]

A key advantage of this technique is that the Descemet membrane (DM) is prevented from coiling into a roll as the surface tension between it and the underlying stroma keeps it flat. This facilitates correct orientation and eases delivery. Lifting the DM off the underlying stroma or filling the punching block with fluid would result in loss of surface tension, allowing the DM to form a roll. In our series, the DM tissue remained flat in all cases. Although the authors do not elaborate on how the roll of DM was formed at the end of their preparation, we advocate avoiding excessive fluid within the punch block and lifting of the graft from the underlying stromal bed.

We encourage readers to adopt the above variations which enhance the advantages that this technique offers and allow eye banks and surgeons greater flexibility and ease.

Massimo Busin,[1,2,3] Vincenzo Scorcia,[1,2] Amit K Patel,[1,3] Gianni Salvalaio,[3] Diego Ponzin[3]

[1]Villa Serena Hospital, Department of Ophthalmology, Forlì, Italy; [2]University of Magna Graecia, Department of Ophthalmology, Catanzaro, Italy; [3]Fondazione Banca degli Occhi del Veneto, Venice, Italy

Correspondence to Professor Massimo Busin, Villa Serena Hospital, Via Camaldolino 8, Forlì 47100, Italy; mbusin@yahoo.com

Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

Accepted 20 November 2010
Published Online First 16 December 2010

Br J Ophthalmol 2011;**95**:1172–1173.
doi:10.1136/bjo.2010.195651

REFERENCES

1. **Busin M,** Scorcia V, Patel AK, *et al*. Pneumatic dissection and storage of donor endothelial tissue for Descemet's membrane endothelial keratoplasty: a novel technique. *Ophthalmology* 2010;**117**:1517–20.
2. **Zarei-Ghanavati S,** Khakshoor H, Zarei-Ghanavati M. Reverse big bubble: a new technique for preparing donor tissue of Descemet membrane endothelial keratoplasty. *Br J Ophthalmol* 2010;**94**:1110–11.
3. **Busin M,** Patel AK, Scorcia V, *et al*. Stromal support for Descemet's membrane endothelial keratoplasty. *Ophthalmology* 2010;**117**:2273–7.

Authors' response

We thank Busin *et al*[1] for their letter regarding our article 'Reverse big bubble: a new technique for preparing donor tissue of Descemet membrane endothelial keratoplasty'[2] and would like to answer briefly the main questions raised.

Regarding their claim of novelty, we recently read their study regarding air-assisted donor preparation for Descemet membrane endothelial keratoplasty (DMEK) published in August 2010,[3] but we were not aware of their presentation at the American Academy of Ophthalmology meeting in Atlanta. We also used an air-assisted technique (reverse big bubble)[2] to prepare the Descemet membrane (DM) for DMEK. We presented the videograph of our technique at the 19th Iranian Congress of Ophthalmology, 2009.

We think their technique needs some modifications. They use 'peripheral cornea approximately 1 mm from the limbus' for insertion of the needle. Puncture of the DM at peripheral cornea might cause an air leak or DM rupture during the injection and bubble formation. We think the preferred entry point for the needle should be outside the Schwalbe's line (a pigmented trabecular meshwork is used as a marker) which makes this technique more atraumatic.

Busin *et al*[1] reported a 5% failure with their technique. In our experience, we found that air-assisted DM dissection is more difficult in young patients (<40 years old) and sometimes the attempt to make a complete detachment may lead to DM rupture. In these cases, we prefer to use a 2 cc air-filled syringe and restrict DM dissection up to 8–9 mm diameter, to reduce the possibility of DM rupture. In our first 10 cases, with an average age of 32 years, we had two patients with incomplete detachment (<8 mm diameter) and one case of DM rupture. Therefore, the high rate of success in the Busin *et al*[3] study might be related to the older age of donors (average=63 years).

We would like to add that we intentionally make a DM roll at the end of the procedure as it allows us to inject the DM through a small corneal incision, which is one of the obvious advantages of DMEK over Descemet stripping automated endothelial keratoplasty.

Siamak Zarei-Ghanavati,[1] Mehran Zarei-Ghanavati,[1] Arturo Ramirez-Miranda[2]

[1]Cornea Department and Eye Research Center, Khatam-al-Anbia Eye Hospital, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran; [2]Department of Cornea and Refractive Surgery, Institute of Ophthalmology "Conde de Valenciana", National Autonomous University of Mexico, Mexico DF, Mexico

Correspondence to Dr Siamak Zarei-Ghanavati, Mashhad University of Medical Sciences (MUMS), Khatam-al-Anbia Eye Hospital, Cornea Department and Eye Research Center, Abootaleb Blvd, Mashhad, Iran; zareis@mums.ac.ir

Competing interests None.

Patient consent Obtained.

Provenance and peer review Not commissioned; not externally peer reviewed.

Accepted 6 February 2011
Published Online First 8 April 2011

Br J Ophthalmol 2011;**95**:1173.
doi:10.1136/bjo.2011.203943

REFERENCES

1. **Busin M,** Scorcia V, Patel AK, *et al*. Donor tissue preparation for Descemet membrane endothelial keratoplasty. *Br J Ophthalmol* 2011;**95**:1172–3.
2. **Zarei-Ghanavati S,** Khakshoor H, Zarei-Ghanavati M. Reverse big bubble: a new technique for preparing donor tissue of Descemet membrane endothelial keratoplasty. *Br J Ophthalmol* 2010;**94**:1110–11.
3. **Busin M,** Scorcia V, Patel AK, *et al*. Pneumatic dissection and storage of donor endothelial tissue for Descemet's membrane endothelial keratoplasty: a novel technique. *Ophthalmology* 2010;**117**:1517–20.

Incidence and regression of Charles Bonnet syndrome in vascular age-related macular degeneration

It appears that the incidence as well as the regression of Charles Bonnet syndrome (CBS) depends on the presence of several retinal circumstances. There is general agreement that symptoms of CBS are frequently reported in eyes with presumably long duration of age-related macular degeneration (AMD), consecutively large subretinal (SR) lesions and poor visual acuity (VA). It is our personal experience that CBS rarely occurs in acute retinal pathologies such as acute retinal pigment epithelial (RPE) tears,[1] SR haemorrhages[2] or recent onset of a geographic atrophy (GA) with a small lesion area.[3] However, clinical signs of CBS may become apparent after 9–12 months, when the final VA remains poor and an enlarged atrophy is observed due to a denuded RPE bed in RPE tears,[1] RPE atrophy after rtPa injections in SR haemorrhages[2] or a large GA.[3] Treating acute choroidal neovascularisation (CNV) with anti-VEGF can reduce inner and SR fluid, increasing the final VA.

In two recent publications, Singh and Sørensen studied patients with vascular

Donor tissue preparation for Descemet membrane endothelial keratoplasty

Massimo Busin, Vincenzo Scorcia, Amit K Patel, et al.

Br J Ophthalmol 2011 95: 1172-1173 originally published online December 16, 2010
doi: 10.1136/bjo.2010.195651