Deep Anterior Lamellar Keratoplasty (DALK)

with vacuum-dried corneal tissue

– a versatile approach.

Mr Chad K Rostron MS BS DO FRCS FRCOphth

Consultant Ophthalmologist

Moorfields Eye Department

St George's Hospital

London SW17 0QT, England

Tel: +44 20 8725 2325

Fax: +44 20 8725 3026

Email: rostron@sghms.ac.uk

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There is now a profusion of different techniques with which to accomplish anterior lamellar keratoplasty. Where is the trade-off between the difficulty and complexity of learning several techniques, against the benefit of having extensive experience with a single approach? One answer to this question may well be the femto-second intra-stromal laser[1], used to cut both the donor button and recipient bed, but for many ophthalmologists performance of the procedure using manual surgical skills is likely to remain a necessity for some time to come.

Historical development

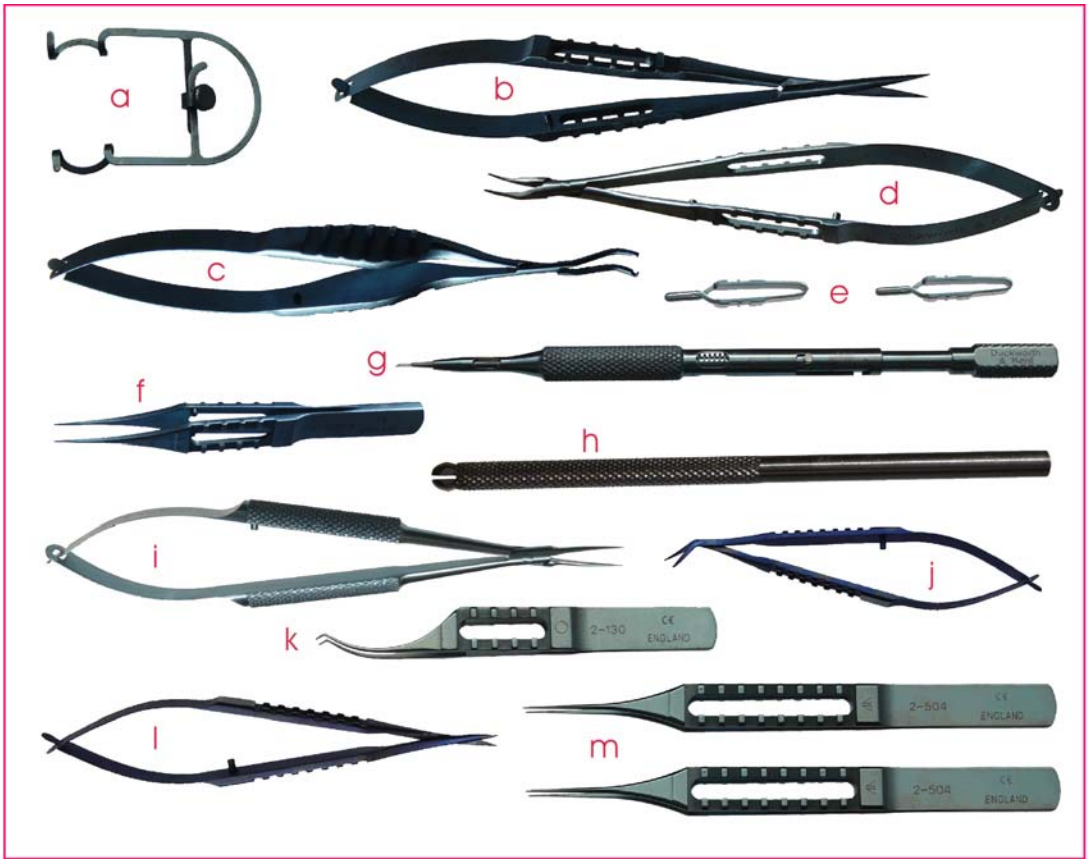
Anterior lamellar keratoplasty performed by simple manual dissection has been carried out throughout the past century, but visual results were often sub-optimal due to interface irregularity and scarring. The introduction of the microkeratome by Barraquer[2] enabled a much better quality interface to be achieved, and this style of mechanised superficial lamellar keratoplasty remains a useful technique for many cases, but is less suitable where there is significant irregularity in corneal thickness, or where there is deep stromal scarring.

In the past twenty years there have been a number of further advances which have transformed lamellar keratoplasty from being a poor second choice to being the treatment of choice for corneal disease where endothelial function is normal. In 1985, Archila[3] described air dissection of the cornea, and this method was subsequently taken up by Price *et al.*[4]. Air dissection has the advantages of doubling the corneal thickness, cleaving the inter-lamellar planes, and making the transparent corneal tissue opaque and so more easily visualised. In Japan, Sugita and Kondo[5] described hydro-dissection, and these two techniques were then brought together by us and combined with visco-dissection (Chau *et al.*[6], Coombes *et al.*[7]). More recently Anwar and Teichmann[8] described the 'big bubble' variation of the air dissection technique. An altogether different inter-lamellar dissection method has been described by Melles[9,10]. In addition Krumeich[11] has obtained good results using his guided trephine system.

A versatile approach

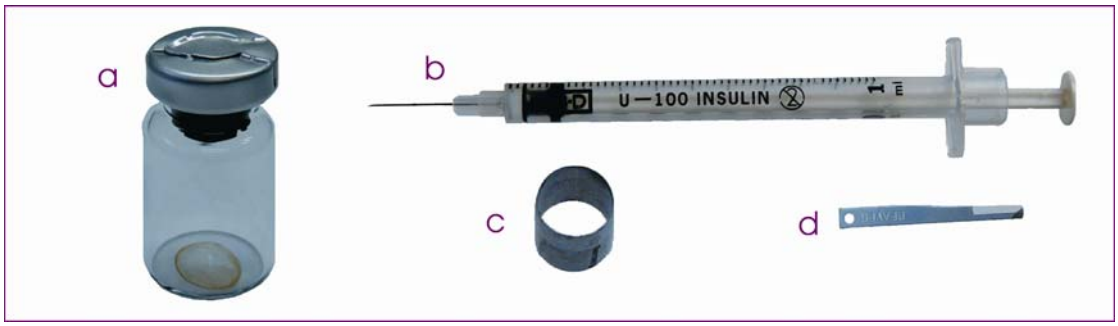
The approach to lamellar keratoplasty outlined in this chapter is one that has been developed particularly to maximise the benefits of lamellar surgery, whilst at the same time avoiding the use of any complex or expensive equipment (Figure 1, Table 1). It is designed to be used in virtually any scenario where an operating microscope is available, can treat a wide variety of corneal pathologies that are suitable for lamellar keratoplasty, and as such can be considered a versatile approach.

Figure 1a. Instruments.



a	Barrett adjustable speculum	D&K	9-590	Retract eye lids.
b	Westcott style stitch scissors	D&K	1-500	Cut Steridrape.
c	Barrett rectus fixation forceps	D&K	2-183	Rectus muscle fixation, and tarsorrhaphy.
d	DK needle holder, curved	D&K	3-503	Needle holding.
e	DK Serrefine clamp x 2	D&K	6-805	Rectus suture fixation.
f	DK Pierse notched forceps	D&K	2-100	Tissue holding during down cut.
g	DK 45º single edge diamond knife	D&K	4-100	Vertical cutting through stroma.
h	Beaver blade handle	Weiss	601338	Inter-lamellar cleavage.
i	Anis Corneal Forceps	D&K	2-197	Strong traction on tissue during dissection.
j	Castroviejo Corneal scissors, curved	D&K	1-401	Cutting posterior stromal layers.
k	DK Pierse notched colibri forceps	D&K	2-130	Tissue holding during suturing.
l	Vannas scissors	D&K	1-110	Suture cutting.
m	Harms Tubigen tying forceps x 2	D&K	2-504	Suture tying.

Figure 1b. Special consumables.



a	Vacuum-dried corneal lenticule	Keratec		Corneal transplant.
b	1ml insulin syringe	B D	U100	Air- and hydro-dissection.
c	Short trephine blade	Dixey	D1125b/*	Mark graft position. * = trephine diam.
d	Beaver blade No:64 round tip	Weiss	601169	Lamellar dissection.
	6/0 Prolene suture	Ethicon	W8597	Rectus fixation, and tarsorrhaphy.
	10/0 Ethilon suture	Ethicon	W1770	Graft fixation.
	11/0 Mersilene suture	Ethicon	W1758	Non-biodegradable graft fixation.

Table 1. Suppliers.

Duckworth & Kent Terence House 7 Marquis Business Centre Royston Road, Baldock Herts. SG7 6XL, England Tel: + 44 1462 893 254 Fax: +44 1462 896 288 www.duckworth-and-kent.com	John Weiss 89 Alston Drive Bradwell Abbey Milton Keynes MK13 9HF, England Tel: + 44 1908 318 017 Fax: + 44 1908 318 708 www.johnweiss.com	Keratec Eye Bank St George's Hospital Medical School Cranmer Terrace,London SW17 0RE, England Tel: +44 20 8672 1238 Fax: +44 20 8682 0718 www.kerateceyebank.co.uk
Becton, Dickinson & Co. 1 Becton Drive Franklin Lanes NJ, USA 07417 Tel: 1 800 237 2174 Fax: 1 800 804 7489 www.bd.com	Dixey Instruments 5 High Street Brixworth NN6 9DD, England Tel: +44 1604 882480 Fax: +44 1604 882488 www.dixeyinstruments.com	Ethicon Inc. Johnson & Johnson Health Care Systems 425 Hoes Lane, PO Box 6800 Piscataway, NJ, USA 08855 Tel: 800 255 2500 ecatalog.ethicon.com

Indications for DALK

Indications for deep anterior lamellar keratoplasty are all corneal stromal pathological conditions where corneal endothelial and epithelial function is adequate. This includes keratoconus, keratoglobus, granular, lattice, and macular dystrophy, and corneal scarring from previous infection including herpes simplex and herpes zoster.

Tissue Preparation

Vacuum-dried lenticules for DALK are prepared in the Keratec Eye Bank, St George's Hospital Medical School, London, England. Corneal donors are screened according to the same criteria as for those providing tissue to be used for penetrating keratoplasty. After an initial period of organ culture at 34ºC, and microbiological screening, the tissue undergoes further processing. The donor epithelium, endothelium, and Descemet's membrane are stripped mechanically, and the cornea trephined from the epithelial side whilst held on an artificial anterior chamber. After drying, the lenticule is sealed under vacuum in a vial, and has a nominal shelf life of three months at ambient temperature.

It has been shown in experimental animal models that when transplanted, freeze-dried corneas are free from problems of rejection[12]. Although there may be antigens on the stromal keratocytes in the dried tissue[13], it is thought that the cell remnants are present too transiently in the recipient to elicit any immune response.

Pre-operative

Because the vacuum-dried corneal lenticules are pre-cut to selected diameters, it is necessary to decide on the graft size at the time when the patient is listed for surgery. For most cases of corneal scarring with otherwise normal architecture, an 8mm or 8.5mm diameter lenticule is generally appropriate. In keratoconus, careful slit-lamp examination to determine the location and extent of the ectasia is important, and corneal topographic examination can also be helpful. For many keratoconus patients, a 9mm or 9.5mm graft will be adequate to ensure complete resection of the cone. In extensive ectasia or keratoglobus, larger grafts may be desirable. For graft sizes greater than 10mm, modification of the surgical technique to an epikeratoplasty type procedure can be beneficial in helping to maintain a sufficient reserve of host epithelium to ensure rapid re-epithelialisation of the graft post-operatively.

Pre-operative specular microscopy is rarely necessary. The corneal endothelial density in keratoconus is not abnormal, and whilst there may be endothelial depletion in corneas that have had severe keratitis, it is frequently impossible to visualise the endothelium in such cases due to the resultant corneal scarring. Provided that there is no clinical evidence of epithelial oedema, it can be assumed that there is still enough residual endothelial function to maintain corneal transparency postoperatively, as the endothelial cell loss incurred by DALK is generally minimal[14].

Anaesthesia

General anaesthesia is preferred, but if local anaesthesia is employed, the anaesthetic block must achieve full akinesia of the globe and eye lids, and a soft eye be obtained by oculo-pressure. A poor local anaesthetic will create a high risk of perforation if Descemet's membrane is exposed, so if local anaesthesia is necessary, then a less-than-full-thickness approach to stromal resection may be more appropriate.

Surgical technique

The lid speculum should achieve good corneal exposure without pressure on the globe, so a manually adjustable and lockable speculum is preferred to an uncontrolled spring-loaded one. Inferior and superior rectus stay sutures may be placed to ensure that the corneal surface can be observed perpendicularly through the operating microscope.

Air dissection

A one millilitre insulin syringe with an integral 29 gauge needle is filled with air and advanced obliquely into the patient's stroma with the needle bevel side down. In corneas with normal architecture the location of the needle tip can be central, but in keratoconus the mid-peripheral cornea is generally safer. The needle is advanced as deep into the stroma as possible without perforating the anterior chamber, and air is injected. Initially, as the plunger of the syringe is advanced, nothing happens as the air pressure builds up. Once a critical pressure is reached, the air will rapidly dissect the stromal inter-lamellar planes (Figure 2).

Watch for the possibility of a large pre-Descemet's bubble. Typically the air will dissect all around the corneal periphery, and subsequently extend into the sub-conjunctival tissues, and / or reflux into the anterior chamber through the trabecular meshwork. Incomplete air dissection of the stroma can be topped up by additional air injection starting elsewhere in virgin cornea. It is not mandatory to achieve full central corneal air dissection, which may be difficult in cases of central keratoconus or scarring, but it is generally advisable to try and achieve air dissection all around the periphery where the trephination is to be made.

When the air dissection has been done, the globe will often be found to have a high intra-ocular pressure due to air in the anterior chamber, which may not be visible due to complete corneal opacification. This high pressure must be addressed urgently to avoid retinal, or iris sphincter, ischaemia. A paracentesis is made at the limbus, typically at three o'clock. A Rycroft cannula is gently introduced to allow air / aqueous to drain. Reduce the intra-ocular pressure to a normal level, but no further, as the globe needs to maintain its shape during the trephination.

When making the paracentesis and draining fluid from the anterior chamber, one must bear in mind the possibility that a large pre-Descemet's air bubble may be present. When the intra-ocular pressure is lowered this bubble will expand, and any instrument introduced into the anterior chamber can then potentially come into contact with and rupture Descemet's membrane.

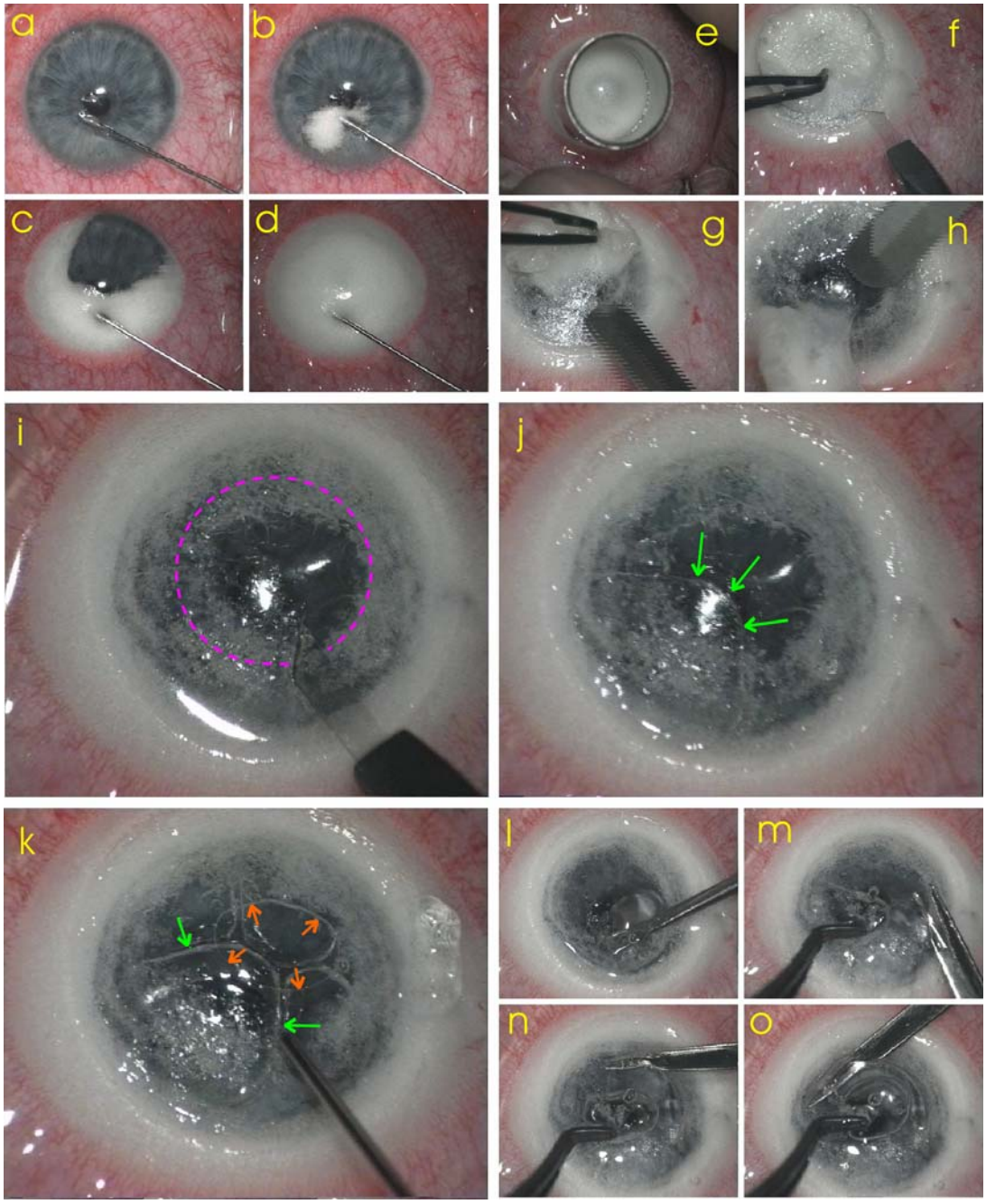


Figure 2 *a) air injection of the cornea of a patient with keratoconus. b), c), d) progressive, and ultimately complete, air dissection of the stroma. e) the trephine blade is balanced on the cornea. f) cutting down through the opaque stroma. g) deep lamellar resection. h) the stroma is pulled free leaving a smooth central bed. i) in this case there is a large central 'big bubble' in the pre-Descemet's plane extending as far as the purple dotted line. j) after puncture of the posterior stroma, the pre-Descemet's bubble has been expelled, allowing bubbles in the anterior chamber to move forward and become more visible – green arrows. k) re-entering the puncture wound with a Rycroft cannula, the pre-Descemet's space is filled with visco-elastic, a small pre-Descemet's air bubble has also been introduced (orange arrows) which cannot fuse with the bubbles in the anterior chamber (green arrows). l) as scissors commence cutting from the puncture point, the visco-elastic starts to come out. m), n), o) as the wound is extended, more visco-elastic is expelled allowing an air bubble in the anterior chamber to take up a progressively more axial position.*

Trephination

A short trephine blade (e.g. Gibbs punch blade) is placed on the corneal surface. If the globe is positioned with the visual axis vertical, a short trephine blade will balance on the cornea without being held, and this gives a good view to ensure that the positioning is ideal before any cut is made. In keratoconus, the Fleischer ring will have been highlighted against the white of the air dissection, and the trephine should be positioned to excise all of the area of the cone, even if this means going right to the limbus in one quadrant, and the graft being eccentric. Once the position of the trephine is verified, a gentle quarter turn back and forth is all that is required to mark the epithelium, without attempting to achieve any depth of stromal cut. Beware of using ultra-sharp trephines since these will cut through air dissected corneas with surprising ease, and there is a real risk of making a perforating cut.

Before the epithelial mark is lost, go over it quickly with a diamond knife to ensure that the site for the graft is clearly marked out. Now lower the intra-ocular pressure by draining aqueous from the paracentesis again so that the eye is soft. This facilitates opening the trephine wound to see the depth of the cut. Hold the inner tissue edge with notched Colibri forceps, open the wound, and use a diamond knife to deepen the cut. So long as the stroma is white, there is still considerable tissue to cut, but as Descemet's membrane is approached, the dark colouration of the iris is seen in the depth of the wound. Aim to achieve around a 90% depth cut.

Hydro-dissection

If a large air bubble is present in the pre-Descemet's plane, then hydro-dissection is not necessary, but if not, or if one is unsure, then hydro-dissection of the central stroma is done next. Fill the insulin syringe with balanced salt solution and introduce the needle tangentially into the edge of the central stromal button. The hydro-dissection is slower and more controlled than the air dissection, and almost inevitably requires two or three puncture points around the central stromal pie in order to achieve a uniform degree of hydro-dissection. Typically this hydro-dissection will again double the stromal thickness as well as create additional inter-lamellar cleavage.

Lamellar dissection

For the lamellar dissection, a single-use curved ended Beaver blade (blade 376400) has the advantage of being predictable without being too sharp. The lamellar resection is more of a peel[15] than a cut, and the blade is used as a fulcrum point over which the stromal fibres separate, rather than as a true cutting blade. Toothed forceps are required to exert sufficient traction on the central stromal tissue to effect the cleavage, and a round-and-round centripetal approach to the dissection ensures that the residual stromal bed achieved is as smooth and uniform as possible in the central optical area.

Visco-dissection

Once the bulk of the stroma has been excised, a better view of the deep residual stroma and anterior chamber will be apparent. If there is a large pre-Descemet's air bubble, then complete stromal resection is readily possible, and generally desirable to achieve. In which case, puncture the posterior stromal fibres with a rapid in-and-out oblique movement with a diamond blade. The air will egress, and Descemet's membrane will move forward to once again lie in touch with the posterior stroma. Visco-elastic such as Healonid GV can then be injected through the

puncture point to reform the pre-Descemet's space. This allows one blade of a pair of scissors to be introduced into the puncture point, and a circular window to be cut in the posterior stroma. This window should be at least 6mm in diameter. Before allowing a pre-Descemet's bubble to escape, make a mental note of its extent, as once the air is replaced with visco-elastic, the extent of the cleavage cannot readily be seen. If scissors are used to cut beyond the extent of the Descemet's cleavage, then perforation of the membrane will occur.

Before the posterior stromal excision is attempted, make sure that the donor tissue is ready to be used, and also that the intra-ocular pressure is minimised by repeat paracentesis drainage. Leave one or two small air bubbles in the anterior chamber as these will be helpful as indirect markers of the location of Descemet's membrane.

Variations on the deep dissection

When there has not been spontaneous pre-Descemet's dissection by a large air bubble, a decision has to be made as to whether or not to take further measures to achieve a deeper dissection. For the majority of cases where air and hydro-dissection of the central stroma has been carried out, the central residual stromal bed will be extremely thin and smooth centrally, and an adequate visual result will be obtained without further dissection being necessary. If there is still significant residual stromal tissue and / or the bed is irregular, a further layer of lamellar resection is indicated.

To do this, further hydro-dissection is done by applying a 5ml syringe with balanced salt solution and a Rycroft cannula to the stromal bed. As soon as a part of the stroma starts taking up the fluid, a greater purchase can be achieved to press the cannula tip against the tissue to maximise the hydrostatic force, or alternatively one can revert to a fine gauge needle in the tissue.

Occasionally, hydro-dissection can produce a pre-Descemet's cleavage and produce a large lake of fluid separating Descemet's from the posterior stroma. Such an effect can be difficult to see through the operating microscope, but the key is to have a few small air bubbles in the anterior chamber, and as the pre-Descemet's dissection takes place, Descemet's membrane bows backwards into the anterior chamber, and the bubbles will be seen to be progressively displaced into the periphery of the anterior chamber. Occasionally a free Descemet's membrane can be visualised in the anterior chamber by the subtle light reflexes from its undulating surface, but generally its location has to be inferred from the movement of bubbles and fluids in the anterior chamber and pre-Descemet's plane.

If a pre-Descemet's fluid lake is achieved, then it can be treated in the same way as a pre-Descemet's air bubble, by entering and filling the space with visco-elastic. It is however inadvisable to attempt to enlarge a pre-Descemet's cleavage by direct injection of visco-elastic, as this will almost certainly lead to rupture of the membrane.

Graft

Since the vacuum-dried lenticules from Keratec are trephined from the epithelial side in the eye bank, the same diameter donor tissue and recipient bed are used. All that is required is to re-hydrate the tissue with saline a few minutes before it

is needed. The graft is placed on the bed and four interrupted cardinal sutures tied. Only at this point should attempts be made to remove the visco-elastic from the pre-Descemet's plane. A little air can be injected into the anterior chamber, and the unsutured quadrants of the graft lifted a little to allow the visco-elastic to be expelled from the interface. 10/0 polyamide-6 sutures (Ethilon W1770, Ethicon) are suitable for most patients, particularly when it is anticipated that complete suture removal will be carried out at some stage, with a view to possible laser surgery for any remaining refractive defect. However, for patients where follow-up may be unreliable, 11/0 polyester (Mersilene W1758, Ethicon) has the advantage of being non-biodegradable, although it is a more difficult suture to obtain an even wound tension with, due to its more limited elastic range.

Once the graft is fully sutured, and the knots buried, any large air bubbles should be removed from the anterior chamber, and sub-conjunctival antibiotic / steroid given. To enhance re-epithelialisation of a vacuum-dried graft, a central temporary tarsorrhaphy suture of 6/0 polypropylene (W8597 Prolene, Ethicon), tied over bolsters, is the most reliable means of ensuring rapid re-epithelialisation. In patients unlikely to be compliant with examination in the presence of a tarsorrhaphy suture (e.g. Downs' syndrome) the alternatives to consider are bandage contact lens wear, or botulinum toxin induced temporary ptosis[16].

Post-operative management

Patients often stay as an in-patient overnight following surgery, but if they have made a good recovery from the anaesthesia, they can go home on the same day.

On the first post-operative day, the eye is examined on the slit-lamp. Although the view may be difficult due to lid swelling and tenderness, the lids can be manually opened medial to the tarsorrhaphy suture. With the patient looking medially, the graft can be inspected, and the extent of re-epithelialisation assessed by fluorescein staining.

Treatment is commenced with topical *G.* cyclopentolate 1% *qid, Oc.* chloramphenicol *bd*, and *Oc.* Betnesol N (betamethasone / neomycin) *bd*. Full re-epithelialisation of the graft is usually achieved by the fourth or fifth post-operative day, and once there is complete epithelial cover, the tarsorrhaphy suture can be removed. If there is failure or significant delay in re-epithelialisation, if there is significant pain, or if the view of the graft is inadequate, the tarsorrhaphy suture should be released. Otherwise inadvertent problems such as broken sutures, or lashes trapped by the tarsorrhaphy and abrading the cornea, will be missed. Once the tarsorrhaphy has been opened, the topical treatment is changed to *Oc.* Betnesol N *tds*, which is tapered off over three months. If the tarsorrhaphy is opened before epithelialisation is complete, alternative strategies to enhance re-epithelialisation such as padding, or bandage contact lenses etc. should be employed.

Discussion

How do the different approaches to anterior lamellar keratoplasty compare? Anwar's 'big bubble' technique requires ultrasonic pachymetry and a vacuum trephine system. However, a big bubble can often be obtained with the method described in this chapter, although this method is not necessarily reliant on achieving a big bubble, and requires no special equipment.

The prime advantage of lamellar keratoplasty over penetrating keratoplasty is the avoidance of graft failure from endothelial rejection or endothelial cell depletion. A secondary advantage is the possibility of performing large grafts, a factor which is important to minimise suture- and wound-induced astigmatism and to ensure that the edge of the graft extends beyond the limits of the ectasia in keratoconus. It has been shown that large penetrating grafts have a higher risk of rejection and failure than smaller grafts[17], and for this reason conversion from a planned lamellar to a penetrating graft is particularly undesirable if a large diameter graft bed has been prepared. In Melles' technique of inter-lamellar dissection, the most risky part of the surgery is completed before the size of the graft needs to be decided, and this is beneficial if one was planning a large lamellar graft but decide to convert to a smaller penetrating graft because of an inadvertent corneal perforation.

In lamellar keratoplasty it is of course possible to inadvertently perforate at any stage of the procedure, but the maximal risk of perforation is probably when working in the immediate pre-Descemet's plane. If one is going to attempt maximal-depth anterior lamellar keratoplasty (i.e. full stromal resection) then in a way it is irrelevant by what means one reaches a preliminary deep-but-partial-thickness lamellar resection, as with all the current techniques, including Melles', the graft size has had to be decided and cut before attempting the final residual stromal resection.

Obtaining a complete separation of Descemet's membrane from the posterior stroma is ideal since it facilitates complete stromal resection, and gives optimal visual results. Air dissection or hydro-dissection are the easiest methods of achieving this aim. Sugita's method of piecemeal dissection of posterior stromal fibres is technically challenging and is particularly risky when Descemet's membrane is stretched and thin as in young patients with keratoconus. Melles' technique of inter-lamellar dissection is also difficult where there is significant corneal ectasia, and anyway only achieves partial thickness lamellar resection.

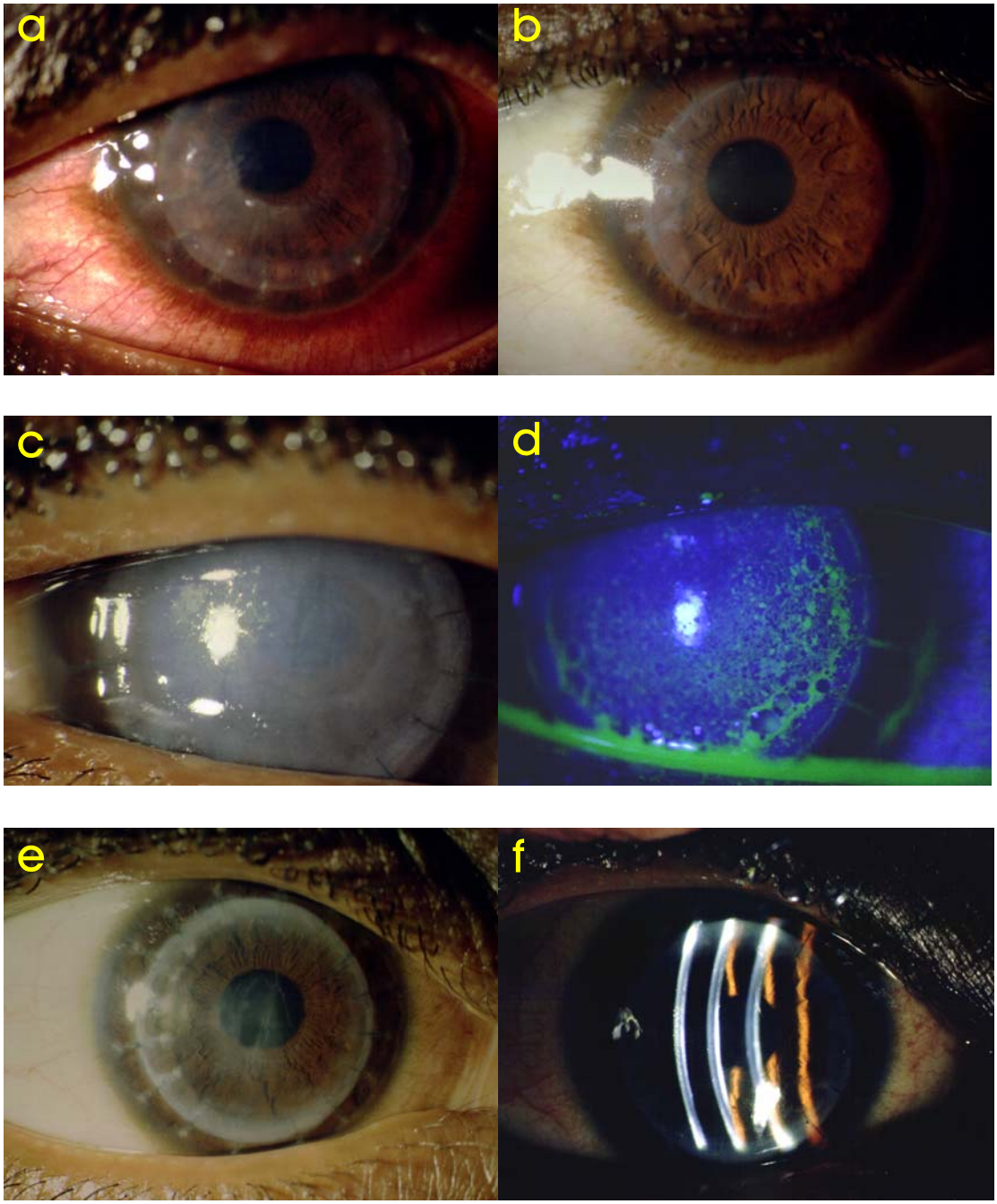


Figure3. *a) At one week post-operatively the central area of full-thickness stromal resection is outlined by some haze in the residual deep host stroma. b) At four months the graft / host interface is clear. c+d) This case was complicated by tearing of Descemet's membrane. At one week, the graft is oedematous with bullous keratopathy and punctuate epitheliopathy. e+f) The same eye at four months post-operatively with spontaneous re-attachment of Descemet's membrane, and clearing of the graft oedema. Final best corrected acuity was 20/40.*

Management of perforation

The issue of management of corneal perforation during lamellar surgery is of importance since perforation quite commonly occurs. Given that patients selected for lamellar keratoplasty are likely to have a normal or near normal endothelium before surgery, almost inevitably conversion to a penetrating graft will result in a major decrease in endothelial density post-operatively, particularly when a large graft is done.

For some patients such as those with herpes simplex keratitis, or where post-operative compliance is a problem such as in mental retardation, the risk / benefit ratio of performing a lamellar graft might be considered acceptable, whereas that of a penetrating graft might be unacceptable.

For these reasons, conversion to a penetrating graft is undesirable, as well as the fact that it requires the surgeon to have donor tissue available that has an endothelium adequate for use in a penetrating graft. If, however, conversion to penetrating keratoplasty is not considered to be an option, then vacuum-dried donor tissue can routinely be used, with the additional benefit that the patient is not sensitized to any donor antigens. If this strategy is adopted, some patients in whom corneal perforation occurs will need secondary procedures to treat double anterior chambers etc., but by far the majority of perforations heal spontaneously in patients with normal endothelial function (Figure 3). Conversion to penetrating keratoplasty can be seen as trading the possible short-term complications of double anterior chamber for the possible longer-term risks of endothelial rejection and failure, and this may be a particularly pertinent issue in younger patients.

Conclusion

Resection of a deep lamella of anterior stromal tissue can be achieved by a number of different approaches including controlled depth trephination, inter-lamellar dissection techniques, or an open direct approach as described in this paper. However if maximal-depth complete stromal resection is desired, then some combination of air, hydro- and visco-dissection facilitates definition of the pre-Descemet's plane.

This chapter was based on the paper: Rostron CK. Deep Anterior Lamellar Keratoplasty (DALK) – a versatile approach. Asoc Am Oftalmol Optom 2004, and Figure 3 was reproduced from Coombes AGA, Kirwan JF, Rostron CK. Deep lamellar keratoplasty with lyophilized tissue in the management of keratoconus. Br J Ophthalmol 2001;85:788–791, with the permission of the publishers.

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