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Chapter 5

Updates in Anterior Lamellar Keratoplasty: The State of the Debates

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ABSTRACT

**Introduction:** Deep and Superficial anterior lamellar keratoplasty (DALK and SALK, respectively) are rapidly replacing penetrating keratoplasty (PK) as the treatments of choice for anterior corneal disorders worldwide. Nevertheless, significant disagreements remain which encompass nearly every aspect of both operations and whether there are better alternatives.

**Areas Covered:** Here, we perform a comprehensive literature review of all articles published in the English language, indexed on Pubmed, and within the past 5 years on the subject of “anterior lamellar keratoplasty.” From these articles, the most salient disputes are enumerated and presented.

**Expert Commentary:** Presently, there is no consensus in the areas of graft preparation, instrumentation, or operative technique for DALK/ SALK. As new evidence emerges, these debates may be clarified, or – instead – merely forgotten, as alternative surgical techniques arise to supplant ALK entirely.

**KEYWORDS:** anterior lamellar keratoplasty, Bowman layer transplantation, DALK, SALK, review
1.0 INTRODUCTION

The past decade has seen a blossoming of anterior lamellar keratoplasty (ALK) as a surgical strategy and a proliferation of studies scrutinizing its various applications. From these myriad investigations, a consensus has emerged that ALK (properly performed) confers the same visual benefits as its predecessor (penetrating keratoplasty, PK), but with fewer potential complications.1,2 In this article, we put that general agreement aside and focus instead on the remaining controversies. After “staging the debate” by describing the evolving trends in corneal transplantation, we shift to enumerating the five largest contemporary disputes surrounding ALK. As a collection, these span the gamut: from graft selection and preparation, to operative technique, to instrumentation, to surgical anatomy, to potential alternatives. Overall, the intention is not to resolve any of these points of contention, but rather, to provide an overview of the landscape of competing claims. By identifying the various opinions, and by displaying their rationales, we hope to apprise readers of these ongoing disputes and enable them to interpret new research vis-a-vis existing debates.

1.1 Modern Trends

Globally, the number of PKs performed each year has been increasing: in 2011, the total number of such procedures (domestically and internationally) using tissue prepared by American eye banks was 36,998.3 That number has grown steadily and stands in the Eye Bank Association of America’s most recent report at a modestly greater 38,919.4 Nevertheless, as a percentage of all corneal transplants, PK is becoming less preferred, both in the United States and abroad, largely secondary to the introduction of posterior lamellar keratoplasty, particularly Descemet Stripping (Automated) Endothelial Keratoplasty (DSAEK) and Descemet Membrane Endothelial Keratoplasty (DMEK), which have almost totally displaced PK from the treatment of corneal endothelial disorders.3,4

The emergence of ALK has also undercut the number of PKs performed, although to a lesser extent. Particularly in the United States, PK still remains the more popular option for anterior corneal pathologies, accounting for 90% of those transplantations.3,4 This fraction has been tilting steadily in ALK’s direction for the past decade, however, and has reached parity in much of Europe and swung decisively into ALK’s favor within regions of the Middle East.5-10 ALK’s most common indication - representing >70% of all cases - is advanced keratoconus (KC), followed by stromal dystrophies and postinfectious scarring.3,4 Overall, the number of transplantations performed for KC appears to be decreasing, potentially owing to modern disease arresting therapies including ultra-violet corneal crosslinking (UV-CXL) and intra-corneal ring segments (ICRS).10,11
2.0 CONTROVERSY ONE: GRAFT PREPARATION

The debates here are multifaceted, and center upon the use of fresh vs. preserved human corneas vs. xenografts (deriving primarily from pigs), and upon the proper instrumentation for graft creation.

2.1 Fresh (human) corneal tissue (FCT)

The case for the use of FCT is based on tradition and simplicity. It is also the graft type with the largest literature base, and recently, Russo et al. have reported that with Descemet membrane baring deep anterior lamellar keratoplasty (D-DALK), FCTs provide better visual results and longer longevity than do decellularized grafts.12 Prolonged death-to-preservation and storage times conduce to epithelial defects in the immediate postoperative phase, but do not impact ultimate visual outcome.13 Conversely, Borderie et al. published that the use of tissue from donors >80 years old yields significantly worse visual results (with average corrected acuities of 20/55, compared to 20/30 with younger donors).14 FCT may be commonly stored in Optisol-GS, as in the United States, or in Organ Culture, as in Europe. After a week of storage in organ culture, ALK tissue may be transplanted with good results.15,16 However, during storage, the donor grafts swell (up to 1200μm) and become opaque. Although these features reverse after surgery, preventing this storage-related transformation may permit a technically easier operation and less postoperative surface change. For this purpose, Lie et al. proposed a new dehydrating solution, buffered with PEG, for soaking donor lenticules stored in organ culture, starting 24-48 hours before surgery.17 These recent discoveries aside, for those using FCT for DALK, the biggest controversy is whether to leave the donor endothelium on, or strip it off, prior to transplantation.

2.1.A. DM-on FCT tissue: Leaving the donor-DM intact may result in less trauma to the graft’s posterior surface during stripping (and also, less epithelial disruption). Meanwhile, it shortens the surgery, has no measurable impact on Snellen acuity or higher order aberrations, and has never been proved responsible for a heightened occurrence of graft rejection.18,19

2.1.B. DM-off FCT tissue: Opponents counter that DM-on transplants may undermine healing at the lamellar interface, precipitating a double anterior chamber and interface haze which - while not diminishing Snellen acuity (and the stromal haze may tend to resolve over time) - does appear to compromise contrast sensitivity.19-22 There is also the theoretical risk of an increased antigenic load, which may incite additional allograft reaction.
2.2 Preserved (human) corneal tissue (PCT)

PCTs are decellularized grafts stored in sterile solutions. Compared to FCTs, they carry a significantly longer shelf-life, a lower probability of harboring infection, and a diminished risk of inciting allograft reaction (since they have been purified of antigen presenting cells). For the same reason, these grafts possess a lower density of keratocytes (even years after surgery), and - when combined with D-DALK - may thin and opacify over time. The competing strategies for producing PCT include:

2.2.a. Cryopreservation and/or dehydration: “Lyophilizing” is among the oldest methods for PCT production; it consists of freezing followed by dehydration under high vacuum. Cryopreservation remains popular (and may be performed alone, without a subsequent lyophilizing step, yielding results equivalent to the use of FCT, per Javadi et al.) although today dehydration is more commonly accomplished chemically by osmotic agents (such as glycerol/glycerin). Stored at -78°C, donor corneas may remain viable for years. In a recent study of DALK in high-risk patients, Li et al. reported that 0/31 (0%) eyes experienced an episode of allograft reaction during the first two years when operated with glycerol cryopreserved corneas compared to 10/33 (21.2%) operated with FCT. Excepting Russo et al.’s previously mentioned report, no study has found worsened visual outcomes with cryopreserved or dehydrated tissue compared to FCT (and, in fact, Farias et al. described improved contrast sensitivity using lyophilized vs. Optisol stored grafts in patients receiving DALK for KC). The chief reported problem with glycerol storage is swelling and opacification of the donor tissue prior to surgery, which though resolving spontaneously after surgery - may make the operation itself more technically challenging.

2.2.b. Gamma irradiation: Gamma-irradiated corneas are available commercially under the product name “VisionGraft Sterile Cornea” (VisionGraft, Tissue Banks International Inc., Baltimore, MD, USA). Following irradiation, the tissues are stored in an Albumin solution where they can remain viable at room temperature for one year. Most of the literature describes their use as patch-grafts for corneal perforations. However, the several studies evaluating their role as stand-alone corneal transplants have found equivalent Snellen acuities compared to FCT and reduced risk of allograft reaction, but more stromal haze (and, therefore, the heightened possibility of diminished contrast sensitivity).

2.3 Xenotransplantation

Particularly in the population-dense third world, the donor cornea shortage has rendered xenotransplantation and “xenobridging” positions of extreme interest. Porcine corneas perhaps have the greatest potential, since they have approximately the same size, shape, and refractive properties as human corneas, and since porcine-to-human transplantations are already commonplace in other areas of medicine. To date, most
studies have been animal based, evaluating various protocols for decellularizing porcine corneas and transplanting them into rabbits and non-human primates. However, Zhang et al. recently reported the results of 47 eyes receiving decellularized, porcine DALK grafts for intractable fungal ulcers: 6 months after surgery, all patients were apparently free of infection, and 41/47 (87%) had clear, epithelialized grafts.

2.4 Instrumentation
For DALK, graft preparation consists only of removing the DM and endothelium from a full-thickness donor cornea (and for surgeons who prefer DM-on FCT, even this represents an optional step). But for superficial ALK (intending only partial-thickness stromal replacement), the donor graft must be specially crafted. Formerly, this was accomplished by hand: after mounting a corneoscleral button within an artificial anterior chamber (or some facsimile) a manual lamellar dissection was performed. Now, however, three automated alternatives exist: the microkeratome and femtosecond and excimer lasers.

2.4.A. Microkeratome facilitated graft preparation: This is the older strategy, having been originally conceived by Barraquer in 1972. Different cut depths (and thereby graft thicknesses) can be achieved by varying the cutting speed, blade size, and pressure within the artificial chamber holding the donor tissue in position. In general, ALK grafts prepared by microkeratome tend to approximate their intended depth better when the cut is shallower, so thinner grafts are more likely to be accurately prepared than thicker ones. For each individual graft, the thickness may also be uneven, with the center thinner than the periphery (by, on average, 25μm). Perhaps the greatest liability for microkeratome graft preparation, however, is that it may oblige the intraoperative use of the microkeratome to likewise fashion the recipient bed, and - of all the strategies for recipient stromal dissection - the microkeratome may yield the worst results.

2.4.B. Femtosecond and excimer laser facilitated graft preparation: Compared to the microkeratome, the femtosecond laser is more accurate and more precise. It also permits the edges of the donor lenticules to be shaped into one of several configurations, theoretically enabling better tissue apposition. However, the femtosecond laser is considerably more expensive to use and, with deeper cuts (>250μm), produces ridges in the graft’s posterior stroma that compromise its optical performance.

The excimer laser is an older, alternative technique for graft preparation: it ablates, rather than incises, the donor tissue to the desired depth. Its advantages include precisely perpendicular graft edges, which may reduce horizontal and vertical tilting (and correspondingly, astigmatism) compared to donor grafts trephined by hand. However, unlike the femtosecond laser, the excimer laser does not permit the graft edges to be “shaped” into various configurations, making it potentially less suitable for SALK applications. In addition, the excimer laser has conventionally been an expensive
instrument to use, and not widely available. As a result, there is correspondingly little published about its use.

3.0 CONTROVERSY TWO: PRE DESCEMET STROMA AND DALK DISSECTION DEPTH

Pre Descemet Stroma layer (the so-called “Dua layer”) remains a hotly contested subject. The referenced tissue no doubt exists, although perhaps not as a discrete structure. Specifically, whereas some stroma does indeed typically remain adherent to the underlying DM during pneumo/visco/manual dissection (an old, if not widely appreciated finding), the amount is not constant, nor is it otherwise endowed with any special features. Regardless, Pre Descemet Stroma layer(s) may still be useful as a reference plane: stromal dissections reaching this level might be characterized as “deep” and distinguished from those terminating superficially. On this issue, the modern debate centers around what dissection depth is ideal.

3.1 Maximal

The totality of evidence suggests that deep dissection (to the level of Pre Descemet Stroma/DM) provides visual results that are equivalent to PK and better than those obtained with mid-stromal dissection. Compared to sub-maximal dissection (close to, but not quite to the level of Pre Descemet Stroma/DM), the visual recovery is faster, interface reflectivity is lower, and keratocyte activation is lesser, at least through the first 6 postoperative months. Thereafter, these disparities diminish (as stromal haze decreases), but may not totally disappear.

3.2 Sub-maximal

Most studies of sub-maximal dissection are the result of failed “big-bubble” attempts, after which, layer-by-layer manual stromal removal is performed. (This might bias the results against submaximal dissection, since those eyes receiving it may be somehow architecturally /structurally different from those in which big-bubble dissection succeeded). As a result, the stromal layer tends to be not only thicker, but also, more irregular. Although this irregularity may be optically limiting, there may not be a correlation between residual stromal thickness and visual outcome. Grafts transplanted onto a thicker stromal bed also have a higher keratocyte density postoperatively, and - if using decellularized donor material - may be less likely to develop progressive thinning / anterior stromal haze, which tends to worsen over time (whereas, with FCT tissue, stromal haze tends to gradually improve over time). According to Borderie et al., corneas undergoing manual stromal (vs. big-bubble) dissection additionally pos-
sess higher postoperative endothelial densities, presumably secondary to their more anterior cleavage planes. Therefore, a submaximal dissection depth may be desirable in patients with concomitant endothelial dysfunction. Additional theoretical reasons to prefer a thicker recipient bed include: a technically easier surgery, protection against inadvertent perforation, and added tectonic stability.

4.0 CONTROVERSY THREE: BIG-BUBBLE UPDATES AND DEBATES

DALK with pneumatic dissection was introduced by Anwar in 2002 via his “big-bubble” technique. (Hydrodissection and visco-dissection strategies were previously introduced, but neither achieved a popular following.) Since then, a litany of modifications to the original procedure have been described, mostly geared toward increasing the success rate of big-bubble production (varying widely in the literature between 35-95%). The most debated factors influencing this success rate include:

4.1 Patient demographics

Feizi et al. reported that female sex predicts against successful big bubble creation (odds ratio of 0.4), but that patient age, personal history of vernal keratoconjunctivitis, and family history of keratoconus do not. Conversely, Goweida published that advanced patient age predisposes toward the formation of type-2 bubbles following intrastromal air injection (with a cleavage plane formed between Pre Descemet stroma and DM), which have a higher-rate of intraoperative rupture compared to type-1 bubbles (formed between posterior stroma and Pre Descemet Stroma.)

4.2 Patient disease severity

KC severity is partially reflected in measured corneal steepness, thickness, and the presence of stromal scarring. Studies analyzing the effects of these features on big-bubble creation have returned conflicting results. Fontana et al. and Huang et al. reported that milder KC results in more frustrated attempts (Fontana: 73% success rate in corneas with central mean keratometry > 62 diopters (D) vs. 55% of patients <62D; and Huang: 80.6% success in corneas with advanced KC vs. 36.4% with moderate KC). In contrast, Michieletto et al. published that corneas thinner than 250µm, particularly if accompanied by significant stromal scarring, are more likely to suffer DM perforations during air injection, necessitating conversion to PK. Goweida likewise found thinner corneas more susceptible to inadvertent type-2 bubble formation and intraoperative perforation. Meanwhile, in what may be the largest dedicated study of the subject, Feizi et al. uncovered no association between corneal steepness, thickness, or anterior stromal scarring (not involving DM) and big-bubble creation.
4.3 Surgeon learning curve
Some learning curve certainly exists - Caporossi et al. (in two separate investigations) and Smadja et al. both reported a significant decrease in all complications after their first 10 cases. However, because most studies report a success rate of ≤ 80%, it is probable that an inescapable risk of DM perforation is intrinsic the procedure, regardless of the level of surgical experience.

4.4 Location of intrastromal air-injection (central vs. peripheral)
The originally described technique calls for air injection in the corneal center. However, Busin et al. published that peripheral injections (1-2mm inside the corneal trephination) are equally efficacious in big-bubble generation. Moreover, Feizi et al. reported that peripheral air injections (outside the original trephination, into the corneal periphery) achieve the same effect, while avoiding obscuring/whitening the central cornea, thereby preserving intraoperative visibility. Although, a potential downside of this latter procedure is enhanced risk of type-2 bubble creation with subsequent perforation.

4.5 Depth of intrastromal air-injection (superficial vs. deep)
Overwhelmingly, big-bubble formation seems to be a function of the stromal depth at which air is injected, with deeper injections more likely to succeed than shallower ones. This consensus finding has generated a litany of competing, ancillary techniques for facilitating deep injection. These include:

4.5.A. Enhanced visualization techniques: Melles et al. reported that inflating the anterior chamber with air prior to injection generated an "air-endothelial" light reflex, providing a guide for advancing a needle into the deep stroma with minimal risk of inadvertent perforation. Recently, Scorcia et al. described a similar visual cue demonstrated by retro-illumination (therefore, requiring pupillary dilation of the operative eye).

4.5.B. Facilitated visualization techniques: Several have described the intraoperative use of optical coherence tomography (OCT) to guide air injection, but none have demonstrated an improved rate of big-bubble creation.

4.5.C. Facilitated injection techniques: Principally, these employ intraoperative pachymetry to guide deep incisions inside the trephination area, into which the intrastromal injection is delivered. In a large trial of the technique, Ghanem et al. reported a 90.5% success rate of big-bubble formation, rising to 95.5% if - after an initial attempt failed - a second injection was delivered using visco-elastic, rather than air.
5.0 CONTROVERSY FOUR: MANUAL, MICROKERATOME, EXCIMER, AND FEMTOSECOND ASSISTED ALK

For both SALK and DALK, recipient lamellar dissection may proceed manually, by microkeratome, and by femtosecond or excimer laser. In general, SALK is limited to patients with stromal scarring in the anterior 200μm of the cornea, and as a result, is performed more commonly for post-infectious or traumatic scarring, and less frequently for ectatic disorders such as keratoconus. Compared to DALK for the same indications, SALK may offer equivalent visual results and, theoretically, enhanced tectonic stability.

5.1 Manual dissection

Manual dissection has been virtually abandoned as a strategy for SALK, since microkeratome and femtosecond cuts have proven faster and smoother. For DALK, however, manual dissection remains a popular strategy. First introduced by Melles in 1998, a controlled manual dissection down to the level of DM is possible using curved spatulas, guided by the air-endothelial light reflex (previously discussed). Visual outcomes approximate those achieved by big-bubble dissection, and the chances of inadvertent perforation may be reduced by 50%, although the interface may be less regular, compromising contrast sensitivity.

5.2 Microkeratome:

Microkeratomes are rarely used to facilitate DALK: their cut depth is too variable/unreliable to consistently achieve a deep dissection, particularly in severely irregular (especially KC) corneas, where the risk of various complications is also increased. Despite Busin et al.’s positive report describing their own results, Borderie et al. found that - compared to femtosecond and manual dissection - microkeratome cuts resulted in the worst visual outcomes among the three strategies. As a result, the microkeratome is more commonly used to facilitate SALK, since its cut accuracy and precision are better with shallower passes. An advantage of using the microkeratome for SALK is that the recipient beds tend to be smoother than when the femtosecond laser is used, instead. The microkeratome may also be preferred in cases of dense corneal opacities, below which the femtosecond laser may have difficulty focusing.

5.3 Femtosecond and excimer laser:

Like the microkeratome, the femtosecond laser is rarely used to perform deep lamellar dissection with DALK, since - the deeper its application - the larger and the more visually significant are the interface ridges produced. (Higher frequency laser application and excimer laser ablation of the femtosecond dissected bed somewhat diminish these ridges, but only to a limited extent.) However, the laser nevertheless finds frequent us-
age in DALK surgeries to shape the edges of the recipient and donor tissues: by cutting interlocking profiles in the two tissues, their fit may be enhanced, resulting in better tissue apposition, a stronger wound, and the possibility of earlier suture removal.\textsuperscript{93,97,99} Wetlab studies have indeed confirmed an increased resistance to wound gape/leak using femtosecond cut edges compared to simple, mechanical trephination methods.\textsuperscript{49,100} However, there have been no astigmatic improvements noted (which disputes the notion that better tissue apposition is achieved.) Moreover, in the only study to directly assess whether earlier suture removal is possible following femtosecond vs. conventional trephination, Shehadeh-Mashor et al. found that - on the contrary - suture removal was significantly delayed in the femtosecond group compared to the mechanical method.\textsuperscript{101} Additional disadvantages to incorporating the femtosecond laser into DALK procedures including substantially increased surgical time and cost. Femtosecond assisted SALK, however, is an operation growing in popularity, and several studies have shown that - with specially cut donor and recipient profiles - the graft may be secured without sutures, thereby alleviating one of the largest potential sources of postoperative complications.\textsuperscript{90,91}

As mentioned previously, the excimer laser has likewise been used to shape the recipient and donor surfaces, achieving visual and astigmatic results that compare favorably to the above mentioned alternative modalities.\textsuperscript{52-54,102-104} However, their expense entailed, relative scarcity, and the inability to shape the donor edge profile with excimer laser has somewhat undermined their popularity.

**6.0 CONTROVERSY FIVE: ALTERNATIVES TO DALK/SALK - BOWMAN LAYER (BL) TRANSPLANTATION**

BL transplantation was introduced by van Dijk et al. in 2013 as a procedure for patients with advanced, progressive KC.\textsuperscript{105} The operation consists of transplanting an isolated, donor BL into the midstroma of a keratoconic cornea. As the recipient cornea heals around the transplanted tissue, it flattens (with maximum keratometry values decreasing by, on average, approximately 9D).\textsuperscript{105,106} The ocular surface likewise experiences a significant reduction of higher order visual aberrations, especially spherical aberration.\textsuperscript{107} The effect is to improve best spectacle corrected visual acuity and patient subjective visual satisfaction. Best contact lens acuity frequently remains unchanged, but rigid contact lens tolerance may be increased. Like DALK/SALK, the operation itself is largely “extraocular,” taking place entirely within the recipient cornea, but it entails no surface incisions or corneal sutures - only a manual mid-stromal dissection (facilitated by manual DALK dissection spatulas.) Because the graft is acellular, the risk of allograft reaction and graft rejection may be diminished. As a result, some of the most significant complications of PK
and DALK may be avoided, including wound healing, ocular surface, and suture related problems. As with DALK, the operation’s most common complication is intraoperative DM perforation, which may occur in approximately 10% of eyes. Thereafter, the surgery may be converted to PK or aborted: since no surface incisions have been made, the perforation site may be allowed to heal and the operation reattempted at a later date.

Presently, BL grafts are prepared by hand: donor corneas are mounted in artificial anterior chambers, debrided of their epithelium, stained with trypan blue, and then stripped of their BL using fine forceps. Groeneveld-van Beek et al. reported a success rate for BL graft preparation of 70%, indicative of the current technical difficulty of the procedure. Before stripping BL, the donor cornea’s endothelium may harvested for Descemet Membrane Endothelium Keratoplasty (DMEK). Thereby, a single donor cornea may be sectioned for use in two separate patients. 108,109

To date, BL transplantation has been reserved exclusively for patients with extremely advanced KC (maximum keratometry values >70D). Its application to less severely ectatic corneas has not yet been investigated. However, considering that many KC disease arresting therapies are not-yet available in the United States (including ultraviolet corneal cross-linking and a variety of intracorneal ring subtypes), BL transplantation may see an expanded role in the future.

7.0 EXPERT COMMENTARY

In each of the five areas listed above, legitimate controversies exist. None are likely to be resolved soon, although – as new technologies emerge – the list of “most important” controversies is likely to change. It is possible that none of them will be resolved, so much as they will be “forgotten,” as have many of the disputes lingering with regard to PK.

8.0 5-YEAR VIEW

Despite continual advances in PK, DALK, and SALK, the future may involve fewer penetrating surgeries of all sorts. With the spread of disease arresting therapies (including ultraviolet crosslinking, intracorneal ring segments, and Bowman layer transplantation), the trend will be toward improved visual outcomes and fewer postoperative complications.
9.0 **KEY ISSUES:**

- Deep and Superficial anterior lamellar keratoplasty (DALK and SALK, respectively) offer equivalent visual results with fewer complications compared to PK.
- Both operations are increasing in popularity, although significant disagreement remains concerning their application.
- For both, donor corneal tissue may be prepared fresh, from decellularized stores, and increasingly from animal sources. Microkeratomes and femtosecond lasers may be useful instruments for sculpting grafts.
- The surgeries themselves may be facilitated by “big bubble” techniques, by manual dissection, or by recourse to microkeratome and femtosecond technology. The propriety of each method may depend on the particular features of the individual patient.
- For superficial stromal scars, SALK may offer an additional advantage over DALK by providing a tectonically stronger eye, and by eliminating the need for sutures.
- Meanwhile, Bowman Layer transplantation is a new operation for patients with advanced keratoconus: it may eventually supersede PK and DALK/SALK as the treatment of choice for patients with corneal ectasias.
Chapter 5

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SPECIAL / ANNOTATED REFERENCES

   This article represents a recent, comprehensive overview of the existing surgical treatments for keratoconus, and compiles nearly all the published data regarding the surgical outcomes of DALK.

   This report provides the best data regarding recent trends in corneal transplantation

   The first large-scale, successful study of the use of acellular porcine corneal tissue for human use.

   The original big-bubble technique, described

   The original Melles manual dissection technique, described

   This article reports the first results with BL transplantation.