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# Factors Associated with Successful Tissue Harvest for Descemet's Membrane Endothelial Keratoplasty

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**FACTORS ASSOCIATED WITH SUCCESSFUL TISSUE HARVEST FOR  
DESCEMET'S MEMBRANE ENDOTHELIAL KERATOPLASTY**

**A Thesis Submitted to the  
Yale University School of Medicine  
in Partial Fulfillment of the Requirements for the  
Degree of Doctor of Medicine**

**by**

**David Charles Shield**

**2010**

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## ABSTRACT

### FACTORS ASSOCIATED WITH SUCCESSFUL TISSUE HARVEST FOR DESCemet'S MEMBRANE ENDOTHELIAL KERATOPLASTY

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Several challenges prohibit Descemet's Membrane Endothelial Keratoplasty (DMEK) from becoming widely adopted as the next generation of corneal transplant for corneal endothelial dysfunction. To date, there have been no evaluations of donor cornea attributes associated with tissue harvest for DMEK. The aim of this study was to describe a method for harvesting the Descemet's membrane and endothelial graft and to evaluate a series of dissections of human corneas for factors associated with successful harvest. Forty human donor corneas were obtained from tissue and eye banks. Endothelial surfaces were stained with trypan blue dye and then scored with 8.5 mm disposable trephines. Harvesting of Descemet's membrane-endothelial graft was attempted using smooth forceps and hydrodissection with application of balanced salt solution (BSS) to the interface between tissue graft and adherent stroma. Donor cornea variables (sex, age, time from death to preservation, time from death to dissection) and factors such as warming donor tissue to room temperature were recorded for each harvest attempt. Twenty-eight of forty (70%) Descemet's membranes were peeled successfully with this technique. After adjusting for donor cornea age, sex, and time from death to preservation, soaking the donor tissue for 60 minutes in ambient temperature BSS was found to be the only factor significantly associated with successful membrane harvest ( $p=0.044$ ). Twenty-six of 32 corneas (81.3%) that had been warmed were successfully peeled, whereas on 2 of 8 (25.0%) of unwarmed corneal peels were successful. These results highlight the importance of warming and rehydrating the donor cornea for an hour or more in BSS prior to dissection. However, additional challenges trouble the remaining steps of Descemet's membrane endothelial keratoplasty; future directions for the field are presented, including possibilities for refinement of the DMEK technique and hybrid DSAEK/DMEK techniques. We have developed one such hybrid technique using thin-cut grafts (40-70 microns) to combine the advantages of DMEK (topical anesthesia, clear corneal incision, less induced hyperopia) with the ease of graft manipulation allowed by thicker endothelial grafts used in traditional DSAEK.

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## I. INTRODUCTION

### **Overview of corneal function, anatomy, and histology**

The cornea is the anterior transparent wall of the globe of the eye. A well-functioning cornea is crucial to the function of the eye and to enabling vision. The cornea, along with the tear film, is the major refractive component of the eye, providing approximately two-thirds of the optical power of the eye (about 43 diopters), with the rest of the optical power provided by the aqueous humor, vitreous humor, and the lens. The cornea also provides structural integrity to the anterior portion of the eye and protects the eye from infection. Its transparency and near total lack of vascularity are critical to transmitting light to the posterior components of the visual system. When the cornea is damaged or becomes diseased, its normal function is disrupted and it can become translucent or opaque, resulting in varying levels of decreased vision. Corneal

transplantation seeks to replace the diseased cornea with a healthy cadaveric cornea.

The cornea is made up of discrete layers or *lamellae* (Figure 1). The outermost layer is a non-keratinized stratified squamous epithelium approximately 5-6 cell layers in thickness. The epithelial layer serves to protect the eye and provides refractive surface that is kept smooth by the overlying tear film. It is this interface between air and tear film and cornea that provides the bulk of the eye's refractive power. The exposed layer of epithelium is continuously shed and replaced by division of the basal layer of columnar epithelium, with the entire epithelium replacing itself every 7-10 days. Epithelial stem cells are present in the basal layer of the limbus, at the circumference of the cornea. The stem cells undergo differentiation into basal cells and epithelial cells as they migrate towards the center of the cornea; their complex role is responsible for the maintenance of a constant epithelial cell density (1).

Under the basement membrane of the epithelial cell layer lies Bowman's layer of acellular condensed collagen fibers, which measures approximately 14 microns in thickness. Beyond being a structural component of the cornea, it is richly innervated by the terminal branches of the nasociliary nerve, providing sensation to the cornea (2). Beneath Bowman's layer begins the thickest layer of the cornea, the transparent stroma (histologically the *lamina propria*), which takes up over three-quarters of the thickness of the cornea. The stroma consists of approximately 200 layers of type I collagen fibers organized in parallel along with a sparsely distributed network of keratocyte. The stroma provides important structural integrity to the globe of the eye and the precise organization of the collagen fibers is important for transmitting light with minimal scattering. It is theorized that transparency of the stroma is maintained by the tight and regular

arrangement of these collagen fibers, with one layer of parallel fibers at an angular offset from the layers above and below it (3). The major cell type of the stroma is the keratocyte, which are interspersed within the stroma and normally quiescent. However, they become activated when repair or maintenance is needed, as in the case of a corneal wound or trauma, enlarging and transforming into fibroblasts to assist in repair of the injury. They are further responsible for maintenance of the stromal extracellular matrix (4).

Descemet's membrane (also called the posterior limiting membrane) is the basement membrane that underlies the stroma; it extends to the periphery of the anterior globe wall to fuse with the trabecular meshwork at Schwalbe's line. It is secreted by the endothelial cell layer that lies just below it, and thickens over a lifetime from approximately 3 microns at birth to 10 microns in adulthood.

The corneal endothelium is a monolayer of simple squamous cells that take on a hexagonal honeycomb appearance due to their tight arrangement. The endothelium is bathed in the aqueous humor of the anterior chamber of the eye and its cells are responsible for regulating fluid and solute transport between the aqueous humor and the rest of the corneal tissue. Through multiple mechanisms it produces a net flow of fluid from the cornea into the aqueous humor and maintains the normal cornea at 78% water content (5). This relatively dry state of the cornea is instrumental to maintaining corneal transparency.

Unlike the corneal epithelium, endothelial cells do not regenerate and their numbers decline throughout the lifespan, beginning at about 3000-4000 cells/mm<sup>2</sup> in childhood and declining to approximately 2600 cells/mm<sup>2</sup> in the elderly at a rate of cell



loss of 0.6% per year(6). Endothelial cells may be lost as part of the normal aging process, or through a variety of other mechanisms, including trauma (both direct trauma to the endothelium and the downstream effects of indirect trauma to the anterior cornea), surgical manipulation, intracameral pharmacological agents, increased intraocular pressure, and several endothelium-specific disease processes (7). When cells are lost, the remaining endothelial cells stretch and become larger in size (polymegathism) to compensate for the reduced cell density. The regular hexagonal honeycomb pattern of the endothelial cells becomes less regular as cells take on a variety of sizes and shapes (pleomorphism). Research suggests that both polymegathism and pleomorphism are signs of impaired endothelial function (8).

The impairment of endothelial function may begin gradually as endothelial cells are lost, but as the cell density drops lower (below 1000 cells/mm<sup>2</sup> and especially below 500 cells/mm<sup>2</sup>), the endothelium can no longer adequately extract fluid from the cornea, resulting in corneal edema and loss of transparency. Endothelial dysfunction from any etiology is thus an important cause of decreased vision and blindness and one of the major indications for corneal transplantation.

### **Indications for corneal transplantation**

Corneal transplantation (*keratoplasty*) aims to replace the injured or diseased portions of the cornea with tissue from a deceased donor cornea when other medical and surgical modalities of treatment have failed. There are several major indications for keratoplasty including Fuchs' endothelial dystrophy, pseudophakic and aphakic bullous

keratopathies, keratoconus, keratitis of various kinds, trauma, stromal dystrophies, and to correct a previous failed graft. In a retrospective chart review of over 1,500 corneal transplants at the Wills Eye Hospital from 1996 to 2000, Cosar et al. found the leading indications to be pseudophakic bullous keratopathy (27.2%), regrant (18.1%), keratoconus (15.4%), and Fuchs' endothelial dystrophy (15.2%) (9). The following review of these diseases and their respective indications for keratoplasty, while not intended to be exhaustive, serves as a foundation for understanding the research into endothelial keratoplasty that was performed in the present study.

### *Fuchs' Endothelial Dystrophy*

Fuchs' endothelial dystrophy is a bilateral degenerative disorder of the corneal endothelium characterized by thickening of Descemet's membrane, the accumulation of discrete excrescences (called guttae or corneal guttata) in the central area of the cornea, folds of Descemet's membrane, loss and decreased function of endothelial cells, and resulting decreased vision from corneal edema (See Figure 2). Fuchs' is more common among women and has been linked to an autosomal dominant inheritance pattern (10,11).

As the corneal endothelium degenerates, there is a decrease in the concentration of endothelial cells and increased polymegathism and pleomorphism, resulting in corneal edema, as previously mentioned. Edema of the stroma first occurs upon wakening with resolution throughout the day; patients report blurred vision primarily in the morning. Later in the disease process, the edema does not resolve over the course of the day and patients have no daily improvement in vision. As the disease progresses to its end-stage, the endothelium becomes progressively more dysfunctional and edema affects both the

stroma and eventually the epithelium as well. Epithelial involvement can severely limit vision, both through loss of the smooth interface with the tear film and the air and from the increased risk of corneal erosions. Stromal edema can cause blisters and bullae to form on the epithelial surface, with resulting pain, photophobia, epiphoria, and sloughing of the epithelium as bullae rupture. Treatment is limited to surgical options at this late stage of Fuchs', which traditionally have focused on full-thickness penetrating keratoplasty. However, in recent years endothelial keratoplasties such as DLEK (Deep Lamellar Endothelial Keratoplasty), DSEK (Descemet's Stripping Endothelial Keratoplasty), and DMEK (Descemet's Membrane Endothelial Keratoplasty) have started to supplant penetrating keratoplasty for this disease entity, with post-surgical visual acuity of 20/40 or better in about 60 to 70 percent of patients (12-15).

#### *Pseudophakic and Aphakic Bullous Keratopathies*

Pseudophakic and aphakic bullous keratopathy are also diseases of endothelial dysfunction that occur as a result of cataract extraction with or without intraocular lens placement. Routine cataract extraction can reduce the endothelial cell count by around 10 percent, which may result in endothelial dysfunction in a patient with low cell density or other predispositions (e.g., mild unrecognized endothelial dystrophy).

Damage to the endothelium can occur during cataract surgery through several mechanisms. Direct mechanical trauma to the endothelium can result from surgical instrumentation or from the clear corneal incision. Intraocular lenses (especially those made of polymethylmethacrylate) can severely damage the endothelial surface if contact occurs between the two surfaces on insertion of the lens. Iris-fixated lenses and anterior

chamber lenses with a closed loop design have been shown to come into intermittent contact with the endothelium, thus contributing to endothelial cell loss. Further, the preservatives in some intraocular medications, such as some preparations of lidocaine, as well as high doses of intracameral vancomycin have been shown to result in endothelial cell loss.

Surgical techniques, such as the use of viscoelastics (which can protect the endothelium to varying degrees, depending on the type of viscoelastic used), use of preservative-free intracameral anesthesia, and deepening of the anterior chamber prior to IOL insertion, are used to reduce the risk of contact between the intraocular lens and the endothelial surface (16).

Patients with pseudophakic and aphakic bullous keratopathy may be candidates for either penetrating keratoplasty or endothelial keratoplasty, depending on the severity of the disease. At the time of surgery, the intraocular lens may be exchanged.

### *Keratoconus*

Keratoconus is a sporadically occurring disease that affects the structure and curvature of the cornea, resulting in progressive corneal steepening, corneal thinning, induced myopia, and astigmatism. Although the cause of keratoconus is unknown and in fact it may be caused by a variety of underlying etiologies, evidence suggests that the disorder is generally associated with increased activity of epithelial proteolytic enzymes and matrix metalloproteases, which degrade the integrity of the stroma (17). Stromal degradation results in irregular steepening of the cornea, which is more pronounced inferiorly, and thinning of the central cornea. The distorted curvature of the cornea can

be observed clinically, just as the ocular pulse can be appreciated as it is transmitted through the thinned corneal tissue. As the cornea bulges out, stress lines can be seen in the stroma on examination and breaks can occur in Bowman's layer. Likewise, Descemet's membrane can rupture acutely causing rapid increase in corneal edema and loss of vision (18).

Treatment for keratoconus begins with corrective lenses for myopia and astigmatism, followed by rigid contact lenses. Surgical treatment is indicated once contact lenses fail to provide adequate visual acuity. Penetrating keratoplasty is the preferred surgical treatment for this disease, although deep anterior lamellar keratoplasty (DALK) is also possible and may have some advantages such as reduced graft rejection despite being a technically more challenging procedure (19). Keratoconus rarely recurs after keratoplasty.

Other surgeries, such as placement of intracorneal ring segments, may reduce myopia and astigmatism and improve visual acuity. There is also promising research into less-invasive combined treatment with riboflavin and ultraviolet-A (UVA) radiation. This treatment induces collagen cross-linking in the stroma and thus increases the structural integrity of the cornea, limiting progression of the disease (20).

### *Keratitis*

Keratitis may be infectious or non-infectious. Infectious keratitis may be caused by bacteria, fungi, parasites, or viruses and is one of the leading causes of blindness in the world (21). Once the diagnosis has been made via laboratory analysis of scrapings of a corneal ulcer and appropriate antibiotic therapy has been initiated, the extent of necrotic

cornea must be determined. If the infection is limited to the anterior portion of the cornea, the surgeon may choose to perform only an anterior lamellar keratoplasty (22). However, if the depth of the infection is unclear or deeper than an anterior resection can successfully remove, a penetrating keratoplasty should be performed to ensure complete removal of the necrotic and infected tissue. If the infection has resulted in perforation of the cornea, then penetrating keratoplasty may be used to replace the perforated cornea. In cases of small perforations, cyanoacrylate glue may be applied to seal the perforation, at the surgeon's discretion, instead of keratoplasty. Likewise, in the case of a large, non-perforating ulcer, a surgeon may choose to perform a penetrating keratoplasty (23).

In the case of fungal keratitis, which can be particularly insensitive to antifungal therapies, surgical intervention may be considered early-on in instances of presumed therapeutic failure (24). In viral keratitis due to herpes simplex (HSV), penetrating keratoplasty is performed for scarring due to recurrent infections or when the infection is leading to perforation, but rates of graft survival are low (25, 26). Lamellar keratoplasty is typically not effective in these cases. Penetrating keratoplasty may also be used in cases of peripheral ulcerative keratitis (PUK), but results are generally poor (27). A variety of systemic diseases have ocular manifestations that include corneal inflammation to the extent that they may require penetrating keratoplasty (28).

### *Trauma and Burns*

Trauma to the eye can result in corneal perforation or scarring, both of which may be indications for penetrating keratoplasty. Exposure to the cornea of either alkali or acid chemicals can have a large range of effects on the cornea, from mild irritation to severe

damage and vision loss, although alkali burns tend to be more severe than those caused by acidic agents. After a chemical exposure is acutely managed, the extent of damage to the ocular surface must be assessed. The chemical exposure may damage only the epithelium or it may progress deeper into the eye, damaging the stroma (destroying stromal keratocytes and limiting the capacity of the cornea to heal itself), endothelium, or deeper structures of the eye. Lamellar keratoplasty generally does not have a role in chemical burns. In mild cases of chemical burn, penetrating keratoplasty may be performed to good effect, but the results of keratoplasty worsen with more extensive damage. For example, burns to the conjunctiva and the limbus can destroy the limbal stem cells. If enough stem cells are destroyed, the epithelium will not be able to replenish itself and a penetrating keratoplasty will fail. Therefore, limbal stem cell transplantation must be performed prior to replacing the damaged cornea, in order to provide a new source of epithelial cells. Other complications of chemical burns, such as glaucoma and hypotony, may further complicate the prognosis for surgical interventions (29).

### *Corneal Dystrophies*

Corneal dystrophies are progressive, inherited, bilateral diseases, usually of a discrete layer of the cornea. There are a variety of dystrophies that affect the anterior layers of the cornea including Bowman's layer and the anterior stroma (such as honeycomb dystrophy, superficial granular dystrophy, and Reis-Bücklers' dystrophy). These diseases are candidates for surgical treatment with penetrating keratoplasty or anterior lamellar keratoplasty for recurring cases after other surgical modalities (e.g.,

superficial keratectomy) have failed.

Stromal dystrophies such as the three types of lattice dystrophy, macular corneal dystrophy, and congenital hereditary stromal dystrophy may be candidates for penetrating keratoplasty or deep anterior lamellar keratoplasty (DALK) (30). Phototherapeutic keratectomy (PTK) with excimer laser is also an option for anterior and stromal dystrophies, although patients may later need DALK or penetrating keratoplasty.

### *Regraft*

Regrafts may be necessary for any failed keratoplasty. Graft failure may occur if the corneal edema fails to resolve following surgery. This may be due to an inadequate endothelial cell concentration of the donor tissue or damage to the tissue from manipulation during surgery.

Graft rejection is the most common cause for graft failure and occurs in approximately 20% of corneal transplants (31). Rejection of the donor cornea may occur at three levels: epithelial, subepithelial, or endothelial, the last of which is the most severe. In general, anterior lamellar keratoplasties in which the endothelium is not replaced are more successful, inducing less host reaction. Endothelial keratoplasties have similar rates of rejection as penetrating keratoplasty (32).

### **History of corneal transplantation**

Corneal transplantation has been performed since the 19<sup>th</sup> century (32). Since that time, improvements in antiseptic technique, instruments, operating microscopes, suture



material, and tissue banking protocols have made the procedure the most frequently performed organ transplant in modern times. Currently, approximately 40,000 corneal transplants are performed each year in the United States (33). Visual outcomes of keratoplasty have improved as surgical techniques and post-operative management have evolved. For example, as the role of the endothelium was elucidated, more care was taken to preserve those cells during surgical manipulations. Knowledge of limbal stem cells resulted in advances in treating patients with alkali burns of the cornea and limbal stem cell transfer. Improvements in tissue preservation and tissue banking procedures increased the availability of high-quality tissue for transplantation. Advances in immunomodulatory therapies decreased the incidence of graft rejection. With each advance in knowledge, technology, and technique came further challenges and opportunities for improvement.

Although partial thickness transplantation of the cornea, or *lamellar keratoplasty*, has been performed as far back as 1886, it was not until the past decade that this technique has gained popularity as a treatment for diseases restricted to either the anterior cornea or the endothelium. This situation can be attributed in part to the success of full-thickness transplantation of the cornea, or *penetrating keratoplasty* (PK or PKP), throughout the latter half of the 20<sup>th</sup> century.

PK has been the gold standard keratoplasty technique for many years but bears with it some significant limitations. When the full thickness of the cornea is replaced with a donor cornea, the replacement corneal button changes the refraction of the eye and induces astigmatism, due to differences in curvature between the native and donor corneas as well as the manipulation and suturing of the graft into place. This induced

refractive error and astigmatism is managed either through the use of spectacles or contact lenses or through a variety of surgical techniques to reduce the astigmatism (e.g., relaxing incisions, suture adjustment or removal, wedge resections, photorefractive keratectomy, laser in situ keratomileusis or LASIK). It can take up to several years for the post-operative refraction to stabilize in a transplanted eye (34).

PK inherently reduces the structural integrity of the globe, increasing the risk of globe rupture with traumatic injury. In 2004, Elder and Stack reported on a case-control study comparing the rates of globe rupture for 139 patients who underwent penetrating keratoplasty with the rates for patients undergoing cataract extraction. They found a 5.8% incidence of globe rupture following PK versus 0.45% for extracapsular cataract extraction and 0% for phacoemulsification. The highest risk of rupture was found to be in the first month following surgery; however, the structural integrity of the eye following PK never returns to normal, and patients are always at increased risk of globe rupture (35).

Penetrating keratoplasty also results in absent corneal sensation due to lack of innervation of the graft tissue, increasing the risk of post-operative complications and graft failure. The presence of corneal sutures can exacerbate this issue, providing a nidus for infection or causing eye-rubbing. In one study of 1819 eyes undergoing PK, although 27% of failures were due to graft rejection, another 25% of failures were due to ocular surface disease (36). In recent years these limitations have given rise to novel methods of transplanting either the anterior cornea (*deep anterior lamellar keratoplasty*, DALK) or the endothelium with varying amounts of attached stroma (*endothelial keratoplasty*), depending on the specific disease process. It is thus possible to avoid or reduce some of

the major limitations of penetrating keratoplasty.

DALK has gained favor in recent years and may be a treatment option for patients with corneal disease that does not affect the posterior stroma or endothelium. Clearly, the risk of graft rejection is lessened in DALK when compared with either PK or selective endothelial keratoplasty, as patients retain their native endothelium (18). It may be an appropriate treatment for patients with early-stage keratoconus instead of penetrating keratoplasty (37). Similar visual outcomes have been observed using DALK and penetrating keratoplasty for the treatment of lattice dystrophy and macular corneal dystrophy, although endothelial cell counts declined more in patients with macular corneal dystrophy who underwent DALK (38). DALK remains a technically challenging surgery, but progress has been made to increase its success. In 2002, Anwar and Teichmann proposed a method for separate the anterior cornea from Descemet's membrane using a "big-bubble technique," in which air is injected deep into the central cornea (39). This creates a bubble between stroma and Descemet's membrane that can then be used to facilitate the excision of the remaining stroma. However, there remains a danger of perforating Descemet's membrane while using the big-bubble technique. A recent study reported that 23% of attempted DALK procedures had to be converted to penetrating keratoplasty due to this complication (40).

Endothelial keratoplasty, on the other hand, specifically aims to remove a diseased endothelium from the patient's eye and replace it with a healthy and functional donor endothelium. This method has clear advantages over full-thickness corneal grafting. In its current form, endothelial keratoplasty is performed through a small (5 mm) clear corneal incision, which reduces the surgery's impact on the structural integrity

of the cornea. Likewise, post-operative astigmatism and refractive errors are minimal using this technique, since the patient's own stroma and epithelium are preserved and only a small amount of donor stroma is introduced. Lastly, visual recovery is faster with a smaller incision. Post-operative complications, such as those related to long-term suture placement and lack of corneal sensation, are also significantly reduced (32).

José Barraquer first attempted a method for endothelial keratoplasty in the 1960s, but technical challenges in transplanting only a portion of the cornea limited its use vis-à-vis penetrating keratoplasty. Refinements in technique were necessary to make it a realistic alternative to PK. Those advances began in the late 1990s when Gerrit Melles reported on his technique for *posterior lamellar keratoplasty* (PLK) (later renamed *deep lamellar endothelial keratoplasty* or DLEK), which replaced the endothelium in a sutureless procedure (41). In these initial innovations, the patient's native endothelium and stroma had to be painstakingly dissected out prior to replacement with the donor stroma and endothelium, all through a 9 mm incision. Over the next four years, Melles refined his technique first through use of an air bubble in the anterior chamber to aid visualization (42) and later by folding the donor tissue in half and inserting it through a 5 mm incision (43), thus reducing post-operative astigmatism and refractive errors (44).

The next major innovation came in 2004 when Melles reported that it was not necessary to dissect out the patient's native stroma and endothelium (45). Rather, he showed that a *descemetorhexis* procedure could be used to score and strip the dysfunctional endothelium and Descemet's membrane from the recipient's eye. This new technique was termed *Descemet's stripping with endothelial keratoplasty* (DSEK). The use of a microkeratome to automate the harvesting of the graft tissue became known as

*Descemet's stripping automated endothelial keratoplasty* (DSAEK) and was first reported by Gorovoy in 2006 (44, 46).

### **Current state of endothelial keratoplasty**

DSEK/DSAEK has now been widely adopted as the preferred method for selective transplantation of the corneal endothelium. DSAEK has its own limitations, however, despite the advances that have been made and the availability of pre-cut donor tissue from eye banks. Endothelial keratoplasty in general requires more tissue manipulation (resulting in endothelial cell death) than does penetrating keratoplasty. Techniques for inserting the donor tissue into the anterior chamber of the recipient eye involve either pushing the graft through a scleral tunnel with forceps or pulling it with either forceps or by a suture attached to one edge, both of which have the potential to damage endothelial cells (44). More recently, efforts have been made to insert the DSAEK graft with injectors.

The donor graft in DSAEK is approximately 150 microns in thickness, representing about 10 microns of endothelium and Descemet's membrane attached to 140 microns of stroma. While the presence of the stroma does not seem to induce post-operative astigmatism or refractive changes, nevertheless it can limit visual outcomes. The main difficulty has been the accumulation of fluid at the interface between the host and donor stroma leading to haziness and, if allowed to progress, dislocation of the graft. Price et al. reported a donor tissue dislocation rate of 14% in a series of his first 200 DSEK cases (47). Methods have been proposed to drain the interface fluid when it

occurs, either by massaging the eye and introducing a series of mid-peripheral corneal incisions to drain the fluid, as Price proposed, or by draining it with a syringe at the slit-lamp (48).

Other refinements have been made to the DSEK technique to address high rates of graft dislocation. Terry et al. used electron microscopy to analyze graft failures in DSEK. They found that roughening the recipient stromal surface was important in promoting adherence of the graft, through a “Velcro-like” effect between the two stromal surfaces (49).

In 2002, Melles et al. described the next frontier for selectively transplanting only the Descemet’s membrane and endothelial cell layer, a graft only 10 microns in thickness, which he termed *Descemet’s membrane endothelial keratoplasty* (DMEK) (50, 51). In DMEK, the Descemet’s membrane-endothelial graft is harvested from the donor corneo-scleral button, leaving behind all of the donor stroma. The resulting membrane spontaneously scrolls up with the endothelium on the outside. This cigar-shaped graft can then be injected into the anterior chamber through a clear corneal incision, carefully unscrolled in the anterior chamber, and positioned on the prepared surface of the host stroma. Though technically challenging, this procedure addresses some of the disadvantages of DSEK/DSAEK, the primary being the potential for superior visual outcomes and fewer graft dislocations. Ham et al. have reported the results of 50 consecutive cases of DMEK for Fuchs’ endothelial dystrophy, showing visual acuity of 20/25 or better within 6 months for 75% of patients who underwent successful DMEK (52). Price et al. have reported similarly encouraging results in a prospective series of 60 (53). An additional benefit is that DMEK eliminates the need for expensive equipment

for the dissection of the donor tissue, such as the femtosecond laser or microkeratome. In DMEK, the surgeon harvests the membrane immediately prior to transplant using simple surgical instruments. Alternatively, the Descemet's membrane-endothelial graft may be harvested by a highly trained technician and preserved for later transplant. Ham et al. demonstrated that this may be done with minimal decline in endothelial cell density (54).

Alternatives to corneal transplantation exist currently in the form of an artificial cornea or keratoprosthesis. This approach may be taken after multiple failed keratoplasties or as a temporizing measure before keratoplasty (32). The artificial cornea also has the potential to provide a permanent synthetic replacement to diseased corneas. Similarly, there are exciting developments for the future treatment of endothelial dysfunction. Endothelial cell sheets have been successfully cultured from donor corneal endothelium. This raises the possibility of autologous transplantation of corneal endothelium that has been raised from the patient's own cells, eliminating the risk of graft rejection (55).

For the time being, DMEK appears to be the next step in corneal transplantation for endothelial dysfunction. However, as previously mentioned, significant barriers remain to the widespread adoption of DMEK, including difficulty with reliable graft harvesting, problems with unscrolling the graft, and proper positioning once inserted into the recipient's anterior chamber. In a report by Ham et al. of the first 50 cases of DMEK, 20% required a second transplant (DSEK) for graft failure (52, 54, 56). In terms of difficulties with graft preparation, the Descemet's membrane-endothelial graft is extraordinarily thin and prone to damage during the harvesting procedure (57). Estimates and anecdotal reports of harvest failure range widely, from 5% to over 30%. A recent

case series demonstrated harvest failure in 12 out of 72 attempted dissections (16.7%)

(53). If DMEK is to be widely adopted, reliable techniques for harvesting and preparation of donor tissue must be further demonstrated and refined. Furthermore, factors associated with harvest outcomes must be elucidated. This is the purpose of the present study.



## II. STATEMENT OF PURPOSE AND HYPOTHESIS

We describe here a case series of 40 consecutive human donor corneas that were dissected in order to harvest Descemet's membrane-endothelial grafts for DMEK. The primary objective of this study was to describe and refine a saline dissection technique for harvesting the Descemet's membrane-endothelial graft for DMEK in a manner suitable for the surgical setting. While others have used the submerged dissection technique ("SCUBA") and described a "big-bubble" technique of injecting saline or air under Descemet's membrane to separate it from the stroma (58), we chose the method described below based on our past experience examining a variety of other techniques (59).

Our secondary purpose was to assess the characteristics of donor corneas for factors associated with successful harvesting of the membrane. Although several case series of DMEK have been published (53, 57), no study has attempted to quantify those factors that may be associated with successful graft harvest for DMEK. We hypothesized that donor tissue that had been brought to room temperature and rinsed of its preservative solution would be easier to dissect and result in more grafts suitable for transplantation. The null hypothesis is that warming and rehydrating the donor corneas would have no impact on harvesting the graft tissue.

We further hypothesized that the age of the donor tissue was an important factor that could be associated with successful harvest of graft tissue, with donors of younger age and tissue from more recently deceased donors would result in easier dissections.

The null hypothesis is that donor age and amount of time from death to preservation and from death to dissection would have no impact on successful harvest.

Our final purpose in undertaking this project was to gain experience in these novel modalities for endothelial keratoplasty and to theorize on modifications that could have the potential to either improve upon the visual outcomes of patients or simplify the surgical techniques required for these procedures. Our resulting hybrid surgical technique, *Injector-assisted Endothelial Keratoplasty* or InjEK, is described in the Discussion section.

### III. METHODS

#### **Donor Tissue Acquisition**

A research waiver was obtained from the Yale Human Investigation Committee prior to beginning research. Expired tissue suitable for transplantation was requested from over 50 US-based eye banks. No compensation was offered to the eye banks for providing tissue. A total of 40 corneoscleral buttons were received by and stored at the Yale New Haven Hospital Blood Bank prior to dissection with temperature-controlled refrigeration (between 2 and 8°C). All dissections were performed under the operating microscope of an ambulatory surgical center.

Of note, one initial human donor cornea was obtained for experimentation and basic refinement of technique. Guided by our knowledge and experience with this cornea, we decided upon the following dissection methods to be used for rest of the graft harvests.

#### **Membrane Harvesting Procedure (See Figure 3)**

##### *Donor tissue preparation*

Donor tissue, in the form of corneoscleral buttons, was removed from refrigeration at least 60 minutes prior to harvesting procedure for 32 of the 40 corneas. For an additional 8 trials, due to limitations of operating room scheduling and staffing, corneas were dissected within 30 minutes of removal from refrigeration. Optisol GS

preservation medium (Chiron Ophthalmics, Irvine, California, USA) was removed and the donor tissue was rinsed several times with balanced salt solution (BSS) Plus (Alcon Laboratories, Fort Worth, Texas, USA). The rinsing procedure involves aspiration of the Optisol GS medium from the graft container with a 10 or 20 cc blunt-tipped syringe. A new syringe is then used to introduce enough BSS Plus to cover the graft, after which the saline is immediately aspirated out. This procedure is repeated two to three more times with free BSS Plus each time. After the final introduction of saline into the container, the graft is left to soak in the ambient temperature BSS Plus until dissection. Attempts to harvest immediately after refrigeration had a statistically significant high failure rate (see Results section).

#### *Staining and Trephination*

Once the tissue had been soaked for the required time, it was removed from the BSS Plus and placed endothelial side up on a dry trephination block. The endothelium was stained with trypan blue dye for one minute for improved visibility during dissection and, once in the recipient anterior chamber, for unscrolling of the tissue. Once stained, trypan blue dye was carefully removed with Weck-Cel sponges (Medtronic ENT, Jacksonville, Florida, USA) and adequate staining was confirmed.

A plastic trephination guide was placed over the tissue and an 8.5 mm disposable trephination blade was used to score the endothelium and Descemet's membrane. A combination of firm downward pressure, rocking, and rotation of the blade was used to ensure complete scoring. Care was taken to completely score the endothelium and Descemet's membrane, without scoring into or through the stroma.

### *Membrane Peeling*

Smooth-tipped forceps were used to lift the scored edge of the graft for a suitable dissection plane. The edge of the scleral rim was grasped with 0.12 mm toothed forceps to provide counter-traction. Areas with Descemet's folds were avoided as they tended to tear easily. Gentle manipulation of the graft edge was used to obtain a suitable dissection plane; too firm a grip was seen to tear the membrane. However, the graft could still be salvaged even when small tears developed at the graft edge. In this case, a new starting point was sought along the scored edge of the graft or gentle dissection around the area of the tear was used to minimize its effect.

Once a peeling graft edge had been established, BSS Plus was applied in a dropwise fashion from a 25-gauge cannula to assist in peeling the leading edge of the graft. The force of the falling drops seemed to minimize the tractional force needed to peel the graft. If drops of BSS Plus did not adequately aid the membrane peeling (i.e., if the graft membrane appeared to be strongly adherent to the underlying stroma, requiring excessive tractional force on the membrane), hydrodissection with BSS Plus on a cannula was employed as an additional tool.

Once the majority of the membrane had been peeled, the corneal well was further hydrated in order to facilitate the graft scrolling. Prior to completely separating the graft from the stroma, it was important to lay it flat again to confirm its orientation and ensure that the endothelium remained on the outside of the scrolled graft.

## **Data Collection**

Data were obtained from the tissue information sheets prepared by the eye bank, including age and sex of donor, cause of death, time and date of death, and time and date of tissue preservation. Other data, such as endothelial cell count and morphology was not uniformly reported by the various eye banks that provided tissue for research. Specular microscopy was not available to assess the endothelial cell counts before or after the harvesting procedure.

Further data was recorded at the time of each dissection trial, including the time and date of dissection, whether or not the tissue had been soaked in ambient temperature saline solution for an hour or greater, and the outcome of each peeling attempt.

Successful peeling of the Descemet's membrane-endothelial graft was defined as a procedure that produced a contiguous piece of graft tissue large enough for a typical transplantation (8 mm in diameter). Small tears in the periphery of the tissue were deemed acceptable if the bulk of the tissue was of sufficient size and had not torn into separate pieces. Small defects at the periphery of the graft would be unlikely to result in visually-significant stromal edema, due to their small size and their location outside of the central visual axis.

## **Statistical Analysis**

All statistical analyses were implemented in Stata/SE v10.1 (StataCorp LP, College Station, Texas, USA). All inferences of significance were made based on a type

I error rate equal to 0.05. Logistic regression analysis was employed with successful peeling as the dependent variable while controlling for donor age, sex, time from death to preservation (in minutes), time from donor death to dissection (in days), and pre-soaking the tissue for more than 60 minutes with ambient temperature BSS Plus as independent variables. We also included the trial number as an independent variable to account for our own learning curve in the harvesting technique. Stepwise backward elimination of variables was used to confirm the best-fit logistic regression model.

### **Statement of student contribution**

With the oversight and input from my advisor, I performed an initial literature review. Based on the results of that review, I wrote an initial hypothesis and surgical harvesting procedure. I applied for and received exemption from review from the Yale Human Investigations Committee (HIC) prior to obtaining human donor tissue for experimentation. I solicited human donor corneas from 66 eye banks throughout the United States and was eventually able to secure donor tissue from six of those eye banks. I assisted in the arrangement for storage of the corneal tissue at the Yale-New Haven Hospital Blood Bank and ensured timely retrieval of the tissue on the days when we attempted dissection.

In the operating room, I assisted my advisor on all 40 attempts to harvest the Descemet's membrane-endothelial graft from donor tissue, working alongside him at the operating microscope. I recorded and maintained the data and results from each of these

sessions. With the oversight of and input from my advisor, I decided on the statistical methods to be used in analysis of the acquired data, programmed the analytical code, performed the data analyses, interpreted the results, synthesized the conclusions, and prepared this written report including accompanying tables and figures.



## IV. RESULTS

### **Characteristics of donor tissue**

Forty consecutive donor corneoscleral buttons were dissected in this case series, using the methods described above. The average age of donors was  $61 \pm 16$  years, with the youngest donor being 19 and the oldest 77 years of age. Seventeen (42.5%) of the corneas came from female donors. The average time from death to tissue preservation was 639 minutes (10.65 hours), with a range from 92 minutes to 2490 minutes (1.5 to 41.5 hours). The average time from donor death to corneal dissection was 19.76 days, with a range from 3.68 days to 47.37 days (Table 1).

As previously mentioned, we were able to presoak 32 of the corneoscleral buttons in ambient temperature BSS Plus for at least one hour prior to dissection. Due to limitations of operating room scheduling and staffing, the remaining 8 corneas were soaked in ambient temperature BSS Plus for less than a half hour prior to dissection. These 8 corneal dissections occurred in the second quarter of the series.

### **Results of harvesting procedure**

Overall, 28 of 40 trials resulted in a tissue graft deemed suitable for transplantation, giving a success rate of 70.0% (Table 2). The success rate of harvesting a Descemet's membrane-endothelial graft from a cornea that had been soaked in BSS

Plus for more than 60 minutes was 26 of 32 (81.3%), while the success rate of harvesting from a cornea that been soaked in BSS Plus for less than a half hour was 2 of 8 (25.0%).

### **Logistic regression analysis**

After adjusting for age, sex, time from death to preservation, and time from death to dissection, warming the corneas for 60 minutes to room temperature was found to be the only significant factor associated with successful membrane peel (adjusted odds ratio [OR] 17.22, 95% confidence interval [CI] 1.08-273.89,  $p = 0.043$ ) (Table 3). Age, sex, time from death to preservation, and time from death to dissection were not associated with successful graft harvest. Furthermore, individual trial sequence number was not significantly associated with the success of graft harvest. That is to say, earlier attempts were no more or less likely to result in successful tissue harvest than later attempts.

## V. DISCUSSION

### **Summary and implications of current research**

Ideally, diseases of endothelial dysfunction should be treated by selective replacement of the diseased endothelium with a healthy layer of new cells. DMEK comes closer to this goal than any previous method of endothelial keratoplasty, using Descemet's membrane as a natural carrier for an endothelial layer. The division between corneal stroma and Descemet's membrane is a natural tissue plane, which is theoretically less prone to the complications of interface fluid accumulation seen in DSEK. Furthermore, DMEK offers some significant practical advantages over DSEK. DMEK may be performed using a small clear corneal incision and topical anesthesia, thus speeding visual recovery time and further limiting any induced refractive error and astigmatism.

The success of DMEK as the next paradigm in endothelial keratoplasty relies on four critical steps:

- 1) reliable harvesting of Descemet's membrane-endothelial grafts;
- 2) an acceptable method of insertion of the Descemet's membrane-endothelial graft into the anterior chamber with minimal damage to the endothelial cell layer;
- 3) a consistent means of unscrolling and properly positioning the graft once in the anterior chamber; and

- 4) satisfactory post-operative management and outcomes, including visual acuity and acceptable endothelial cell loss rates.

Each of these four critical steps continues to pose challenges. Little has been reported in the literature in the four years since Melles described the first case of DMEK, possibly due to on-going difficulties with the critical steps to successful DMEK, including the harvesting process, which is perhaps the most important for paving the way for future advances. It is difficult to tackle the other steps without having a reliable method for preparing tissue grafts. Furthermore, there have been no reports of a statistically validated method for reliably harvesting Descemet's membrane for DMEK. A variety of methods that have been tried and anecdotal case reports have claimed successful harvest of Descemet's membrane, but without analysis of surgical or tissue factors associated with success (57).

Our procedure describes a method for harvesting the Descemet's membrane-endothelial graft with an overall success rate of 70.0%. By itself, a 30% failure rate for one step of the surgical procedure would not be acceptable. However, we found that when donor tissue was soaked in BSS Plus and warmed to ambient temperature for at least 60 minutes, the success rate improved to 81.3%. This was the only statistically significant factor that we found to be associated with successful harvesting. We have previously reported that rinsing with saline is an important factor contributing to success in DSEK graft adherence (60). While rinsing the preservative medium from graft tissue may improve graft adherence, we hypothesize that warming and soaking the donor tissue may serve an additional purpose in rehydrating the tissue before graft harvest. This may allow for easier access to the natural tissue plane between Descemet's membrane and the

stroma. Histological studies would be needed to assess the underlying cause of this difference in tissue handling.

Regardless of the histologic reason for improved graft harvest, we believe this result to be an important step towards making DMEK a viable option for patients with endothelial dysfunction. Just as the recognition of the importance of the endothelium lead to surgeons taking more care with the manipulation of graft tissue for penetrating keratoplasty, so too might this research change the way donor tissue is prepared for dissection prior to DMEK.

### **Limitations of the study**

There are several important limitations to this case series. First, we were unable to assess the impact of our technique on endothelial cell density (ECD) due to inconsistent reporting from the source eye banks. Other studies have shown acceptable ECDs after harvesting, with a 30% decline in ECD in the 6 months after DMEK, similar to other forms of endothelial keratoplasty (52), but we were unable to assess that here.

Second, our case series includes only 40 corneal dissections, a small number which limits our ability to make statistical inferences about the factors associated with success or failure. Nonetheless, we were able to show a statistically significant association of presoaking the corneas in ambient temperature BSS Plus for 60 minutes or more with successful graft harvest. It remains unclear whether the soaking of the donor tissue with BSS Plus, the warming to ambient temperature, or the combination of the two was the most important factor associated with improved success in this technique.

Third, these 40 dissections were also our first attempts to dissect corneal tissue systematically for the purpose of preparing tissue for DMEK. There is undoubtedly a learning curve associated with these delicate dissections. Although we found no association of the attempt number with successful harvest, the learning curve nevertheless may have influenced our success with harvesting the Descemet's membrane-endothelial graft. We would expect the success rate to improve with more practice. Of note, the factor that was found to be significantly associated with harvest *failure* in this series (i.e., not warming and presoaking the tissue) occurred exclusively in the second quarter of the series, from harvest sequence number 11 to 18. This may have been a confounding factor of our findings. With more experience and a larger case series, the effects of the experiential learning curve on the study would be diminished, perhaps allowing for other true associations to emerge.

Lastly, although we attempted to adjust for as many factors as possible, there may have been other unmeasured factors that influenced our success or failure with graft harvest. In addition, we were not blinded to the characteristics of the donor corneal tissue that we dissected; this may have influenced our successful harvesting of the graft membrane.

### **Future directions: Hybrid DSEK/DMEK techniques and InjEK**

We believe that this research is an important step towards the reliable harvesting of Descemet's membrane-endothelial grafts for DMEK. However, the remaining technical challenges involved in DMEK may make it too daunting a procedure for

widespread adoption by corneal surgeons. Until those challenges are satisfactorily addressed, other methods for selective endothelial cell transfer should be considered.

Specifically, it may be advantageous to keep a small amount of adherent stroma (but not as much as in DSEK) with the endothelial graft in order to facilitate surgical manipulation. One such DSEK/DMEK “hybrid” technique has already been described by McCauley et al., who, keeping to tradition, call it *Descemet’s membrane automated endothelial keratoplasty* or DMAEK (58). Studeny et al. have described a similar technique that they call *Descemet’s membrane endothelial keratoplasty with stromal ring* or DMEK-S (61). In the first procedure, a microkeratome is used to remove the anterior cornea. Then, a big-bubble technique (as in DALK) is used to detach the central stroma from Descemet’s membrane. The central stroma is excised but a rim of stroma is left attached to the bare Descemet’s membrane. The residual stromal rim allows the tissue to be folded and inserted as in DSAEK, and provides enough elastic rebound to spontaneously unfold once inside the anterior chamber. In Studeny’s method, no microkeratome is used and the tissue is dissected manually using the big-bubble technique. They also leave a rim of stroma attached to the bare Descemet’s membrane. Potentially, these techniques may combine the visual outcomes of DMEK with the ease of tissue manipulation found in DSAEK. Unfortunately, both of these procedures remain technically challenging. Chiefly, the use of the big-bubble technique may cause breaks or tears in Descemet’s membrane, making the tissue unusable.

We also propose a hybrid technique between DSAEK and DMEK, which aims to combine the benefits of DSAEK (graft stability, ease of surgical manipulation) with some of the advantages of DMEK (small clear corneal incision, topical anesthesia, fast

recovery time). This technique uses an off-label intraocular lens injector system for inserting a precut endothelial graft into the anterior chamber. However, in this method we use a precut graft that is much thinner than in typical DSAEK. Whereas in DSAEK, the graft is approximately 150 microns thick, in this procedure the graft may be 50-75% thinner (40-70 microns). The thinness of the graft makes it possible for the tissue to be gently placed in the IOL injector and inserted instead of folded, grasped with forceps, and pushed through a scleral tunnel as in DSAEK. We have nicknamed it “InjEK” for *Injector-assisted endothelial keratoplasty* (See Figure 4).

As mentioned above, the benefits of this procedure are the better graft stability of DSAEK and the improved recovery time of DMEK. The major drawback of this approach is that it preserves DSAEK’s limitations in terms of fluid accumulation at the stromal interface, potentially leading to limited visual outcomes or graft detachment.

The most important advantage that this technique offers over all other forms of endothelial keratoplasty is the simplification of surgical technique. In contrast to DSAEK (which is often done via a scleral tunnel, leading to a longer recovery time) and DMEK (which requires extraordinarily delicate tissue dissection in all of its iterations), this procedure would require no more training than that which is needed for a basic cataract extraction and have a short recovery time.

In time, with the aide of the present study, we expect that the barriers that currently exist to widespread adoption of DMEK will be overcome. In the meantime, InjEK offers a simplified and accessible procedure for endothelial keratoplasty.



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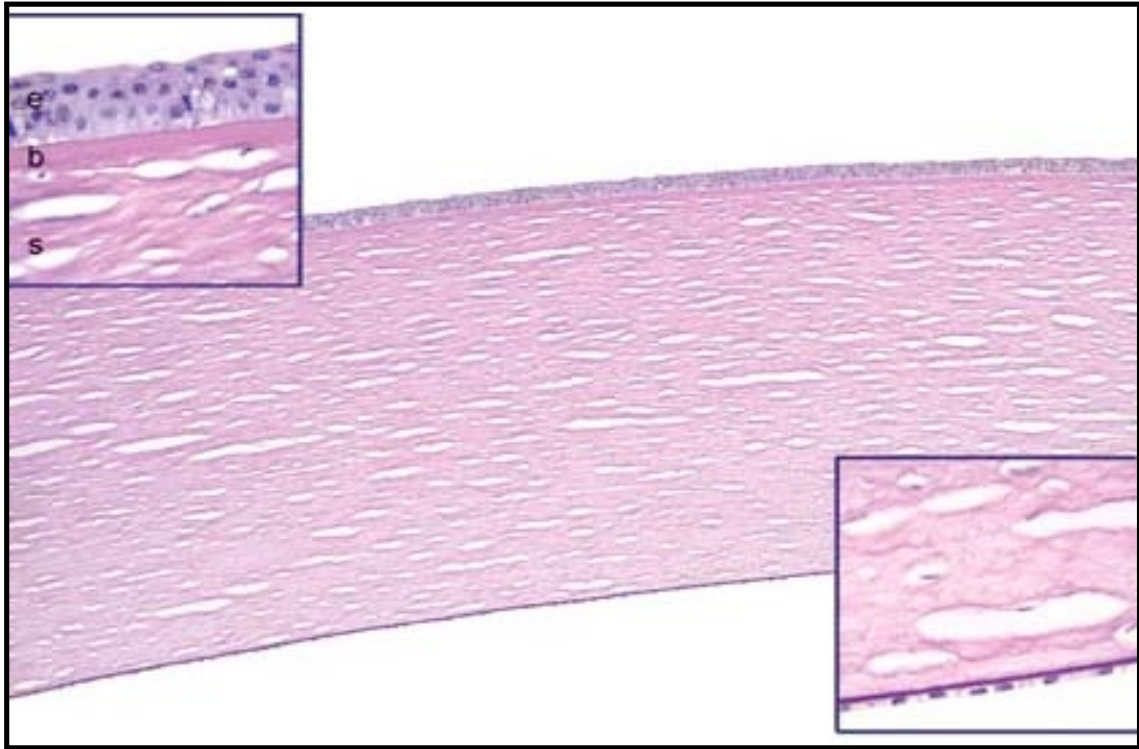
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## VII. FIGURES

Figure 1: Corneal microarchitecture in cross section, from Robbins Pathology 7<sup>th</sup> ed.



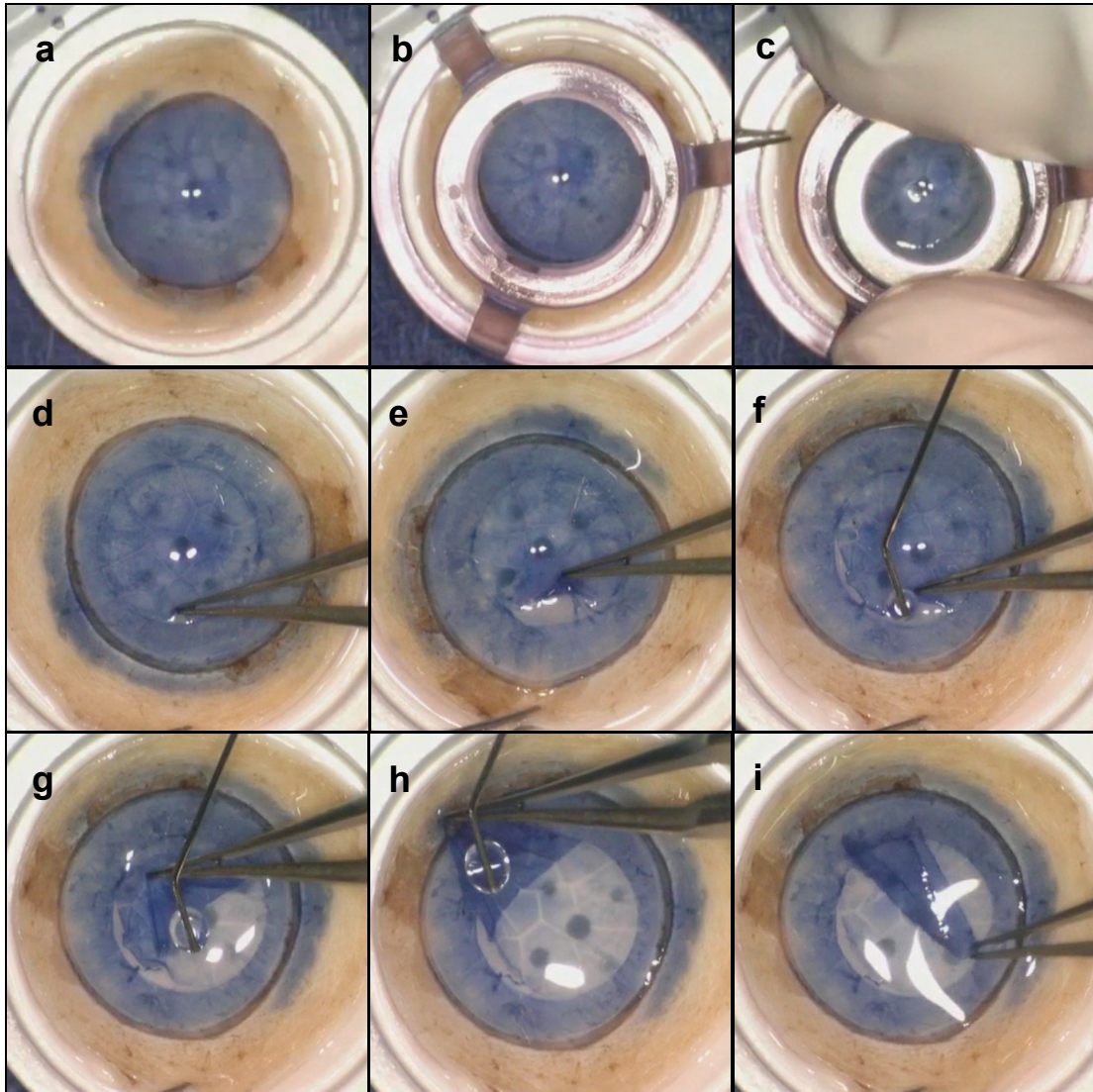
Legend: The inset at top left reveals (e) the epithelial layer of stratified squamous epithelium, (b) Bowman's layer (anterior elastic lamina) of condensed collagen fibers, and (s) the stroma (substantia propria) consisting of approximately 200 layers of collagen fibers with sparse keratocytes. The inset at bottom right reveals the dark layer of basement membrane called Descemet's membrane, and the monolayer of endothelial cells between Descemet's membrane and the aqueous humor of the anterior chamber.

Figure 2: Fuchs' Endothelial Dystrophy on slit-lamp examination, from Yanoff & Duker Ophthalmology, 3<sup>rd</sup> ed. Note guttae in the slit-lamp beam.





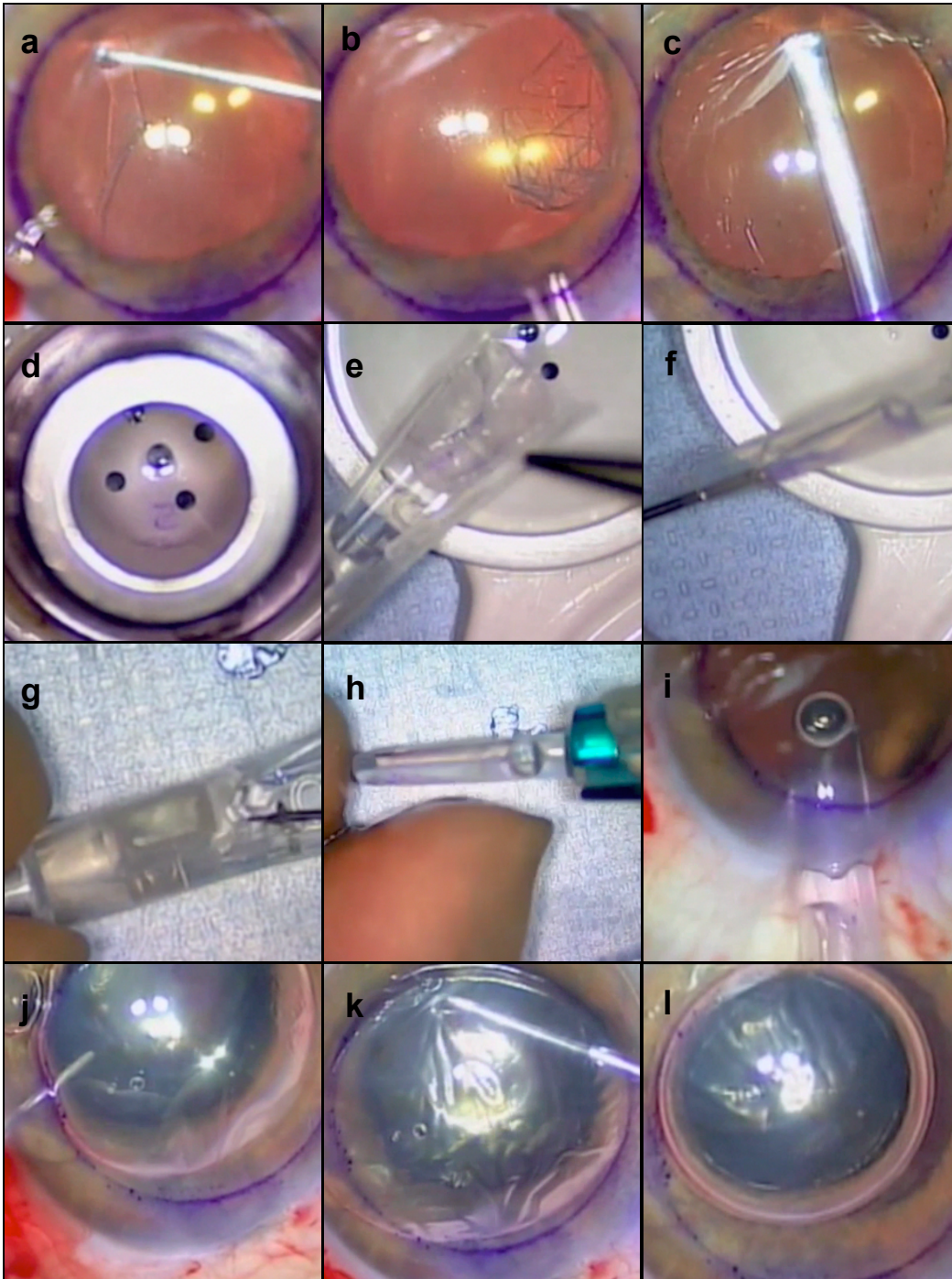
Figure 3: Descemet's membrane harvesting procedure, as described in the text.



Legend: From left to right: a) Corneoscleral button mounted on trephination block and stained with trypan blue dye. b) Tissue with scoring guide. c) Scoring tissue with 8.5 mm trephine with twisting motion, while holding tissue steady with forceps. d) Finding an edge for peeling. e) Adherent edge of graft. f) BSS Plus in dropwise fashion to assist in initiating peel. Note the 180° rotation of the cornea (from image 4 to 6) that was performed in search of a suitable dissection edge. g) Continued peel with drops of BSS Plus. h) Progressive peeling with BSS Plus. i) Successful scroll of Descemet's membrane.



Figure 4: Injector-assisted Endothelial Keratoplasty (InjEK), as described in the text.



Legend: From left to right: a) Descemetorhexis of recipient eye. b) Removal of DM from recipient eye. c) Stromal roughening. d) Trephination of thin-cut donor membrane. e) Loading of graft into IOL injector. f) Advancing graft to tip of injector. g) Loading BSS and viscoelastic behind graft. h) Graft loaded in tip of injector. i) Insertion of graft through clear corneal incision. j) Injection of air bubble. k) Centering of graft. l) Final result.

## VIII. TABLES

Table 1: Donor tissue characteristics and results of 40 trials of Descemet's membrane-endothelium harvest.

<b>Trial #</b>	<b>Death to Preservation (hh:mm)</b>	<b>Death to Dissection (days)</b>	<b>Donor Age (years)</b>	<b>Donor Sex</b>	<b>Warmed and presoaked</b>	<b>Successful Harvest</b>
<b>1</b>	4:26	18.70	62	F	Yes	Yes
<b>2</b>	6:10	15.03	77	F	Yes	Yes
<b>3</b>	5:24	17.88	49	M	Yes	Yes
<b>4</b>	6:10	15.03	77	F	Yes	No
<b>5</b>	3:08	21.89	45	M	Yes	Yes
<b>6</b>	6:11	20.82	77	F	Yes	No
<b>7</b>	3:08	21.89	45	M	Yes	Yes
<b>8</b>	3:25	18.49	76	M	Yes	Yes
<b>9</b>	6:11	20.82	77	F	Yes	Yes
<b>10</b>	3:25	18.49	76	M	Yes	Yes
<b>11</b>	11:17	18.64	72	F	No	Yes
<b>12</b>	11:17	18.64	72	F	No	No
<b>13</b>	11:31	16.21	32	F	No	Yes
<b>14</b>	21:18	20.94	19	M	No	No
<b>15</b>	21:18	20.94	19	M	No	No
<b>16</b>	11:31	16.21	32	F	No	No
<b>17</b>	9:27	4.83	57	F	No	No
<b>18</b>	9:27	4.83	57	F	No	No
<b>19</b>	12:39	13.43	74	F	Yes	Yes
<b>20</b>	12:39	13.43	74	F	Yes	Yes

<b>Trial #</b>	<b>Death to Preservation (hh:mm)</b>	<b>Death to Dissection (days)</b>	<b>Donor Age (years)</b>	<b>Donor Sex</b>	<b>Warmed and presoaked</b>	<b>Successful Harvest</b>
<b>21</b>	1:32	12.54	74	M	Yes	Yes
<b>22</b>	1:32	12.54	74	M	Yes	Yes
<b>23</b>	24:25	12.00	73	F	Yes	Yes
<b>24</b>	24:25	12.00	73	F	Yes	Yes
<b>25</b>	21:53	13.55	51	M	Yes	Yes
<b>26</b>	21:53	13.55	51	M	Yes	Yes
<b>27</b>	3:35	3.68	69	F	Yes	Yes
<b>28</b>	3:35	22.60	69	F	Yes	Yes
<b>29</b>	5:43	29.46	52	M	Yes	No
<b>30</b>	5:43	29.46	52	M	Yes	Yes
<b>31</b>	10:19	47.37	71	M	Yes	Yes
<b>32</b>	3:54	24.04	61	M	Yes	Yes
<b>33</b>	10:19	47.37	71	M	Yes	Yes
<b>34</b>	3:54	24.04	61	M	Yes	No
<b>35</b>	41:30	22.52	59	M	Yes	No
<b>36</b>	8:23	19.22	73	M	Yes	No
<b>37</b>	8:23	19.22	73	M	Yes	Yes
<b>38</b>	41:30	22.52	59	M	Yes	Yes
<b>39</b>	5:18	32.76	57	M	Yes	Yes
<b>40</b>	5:18	32.76	57	M	Yes	Yes

Table 2: Success rates for Descemet's membrane-endothelium harvest

	<b>Success</b>	<b>Percent</b>
Overall	28/40	70.0%
Warmed	26/32	81.3%
Not-warmed	2/8	25.0%

Table 3: Logistic regression analysis of 40 trials of Descemet's membrane-endothelium harvest

	<b>OR</b>	<b>95% CI</b>	<b>p-value</b>
Warmed	17.62	1.10-283.25	0.043 *
Age	1.00	0.93-1.07	0.904
Female	1.53	0.14-16.55	0.726
Time (Death to Preservation)	0.67	0.09-4.95	0.692
Time (Death to Dissection)	1.01	0.91-1.12	0.823
Trial number	0.98	0.90-1.08	0.738

\* statistically significant