CORNEAL TRANSPLANTATIONS AND INTRACORNEAL IMPLANTS

Isabelle Saelens

Corneal transplantations and intracorneal implants

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CONTENTS

General introduction

I. THE HUMAN CORNEA

The cornea, conjunctiva, and intervening transition area known as the limbus comprise the tissue at the ocular surface. The cornea functions as a protective membrane and a "window" through which light rays pass to the retina.

In the ideal refractive state, emmetropia, an image is focused directly on the retina, resulting in the perception of a sharp image in a healthy visual system. In myopia (nearsightedness), the image appears focused anterior to the retina, producing a sharp image at near and a blurred image at distance. The inverse is true in hypermetropia (farsightedness), where the image is projected posterior to the retina, producing a blurred image at near and a sharper image at distance. Astigmatism (cylindrical error) is the result of two different refractive powers between two perpendicular meridians. As an object is brought nearer to the eye, focus is maintained by an increase in the power of lens of the eye; this is accommodation. The ability to accommodate decreases with age (presbyopia).

The human cornea is composed of five distinctive anatomical layers (Fig 1). From anterior to posterior these are: the epithelium with its basement membrane; the stroma, which is subdivided into Bowman's layer, the lamellar stroma, and Descemet's membrane; and the endothelium. Part of Descemet's membrane is in fact the basement membrane of the endothelium, and, therefore it is sometimes considered to be part of the endothelium. The endothelium lines the posterior corneal surface and is derived from the neural crest during embryologic development.[1] Human endothelial cell density is approximately 6000 cells per mm^2 during the first month of life, but decreases to about 3500 cells per mm^2 by the age of 5 years. Growth of the cornea accounts for some of this decrease in density, but a decrease in the number of cells also occurs.[2] Central

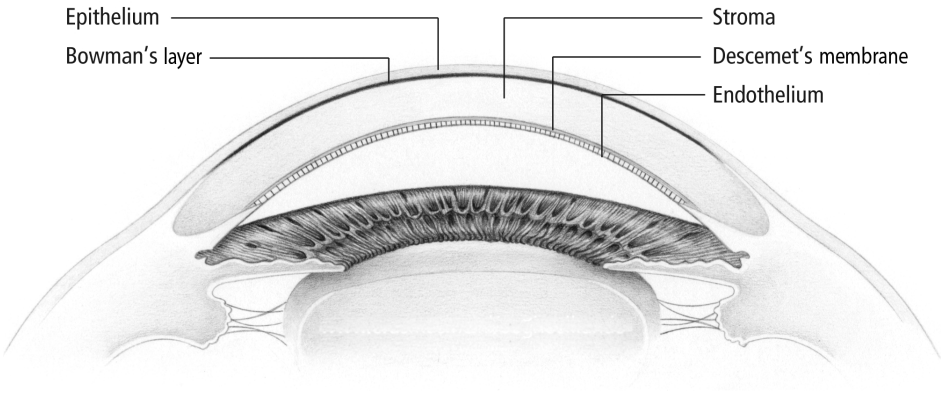

Fig 1. Corneal structure. (J Leenen)
(See also Colour Figures, p. 135.)

endothelial cell density decreases throughout life at an average rate of about 0.6% per year, so that the mean cell density is found to decrease from 3400 cells per mm^2 at the age of 15 to 2300 cells per mm^2 at the age of 85.[3] There is no evidence that human endothelial cells divide under normal circumstances, although they can divide in cultured corneas.[4] With the decrease of cell density, there is, to a certain extent, a corresponding increase in cell surface area to maintain the barrier properties of the endothelial mosaic.

II. CORNEAL TRANSPLANTATION

1. Indications of corneal transplantation

Improvement of vision is the usual indication for corneal transplantation. However, preservation of the globe, cosmetic appearance of the eye and reduction of pain due to corneal pathology are other indications. The indications for corneal transplantation vary along with geographic location and institute. The last three decades, the leading indications for keratoplasty were failed grafts, pseudophakic bullous keratopathy (PBK), Fuchs' endothelial dystrophy (FED), keratoconus (KC) and corneal scars.[5]

PBK is a corneal disorder caused by endothelial decompensation secondary to trauma (for instance by surgery), glaucoma or congenital abnormalities. Clinically, the disorder results in corneal oedema and epithelial or subepithelial bullae. For the symptomatic stage of the condition, keratoplasty is the most effective therapy to relieve pain and restore visual acuity.

FED is a slowly progressive, bilateral primary disorder of the corneal endothelium. In the asymptomatic stage of the disease, slit-lamp examination discloses irregularly scattered excrescences in the posterior cornea, called guttata.[6] Later, the patient's visual acuity can decrease because of stromal oedema. The increasing number of corneal and intraocular surgeries performed (with the risk of an additional loss of endothelial cells) together with a longer life expectancy, elevate the risk for developing symptomatic FED.[7] When a patient's visual acuity deteriorates significantly, a keratoplasty is indicated.

KC is a non-inflammatory corneal ectasia, which usually starts at puberty and progresses until the third to fourth decade of life.[8] The corneal ectasia results in a (myopic) irregular astigmatism, impairing the quality of vision. In the early stages, the refractive error can be corrected with glasses or contact lenses. In more advanced forms of KC or in case of contact lens intolerance, several surgical options are available to improve visual acuity. Ten to 20% of patients eventually require keratoplasty due to scarring in the visual axis, insufficient visual acuity with contact lens correction, or contact lens intolerance.[9]

2. History and epidemiology of corneal transplantation

The first visual successful human penetrating corneal transplantation was performed by Eduard Zirm in 1906.[10] Great advances in surgical instrumentation and techniques and the introduction of antibiotics and corticosteroids over the last century, significantly improved the success rate of corneal transplantation. Corneal transplantation is one of the most widely practised form of clinical transplantation. In the Netherlands, 995 corneal transplantations were performed in 2009 and 1165 in 2010.[11]

3. Recent developments in penetrating and lamellar keratoplasty

3.1. Penetrating keratoplasty

In traditional penetrating keratoplasty (PKP), the diseased cornea is replaced by full-thickness corneal donor tissue. The full-thickness graft gives a superior visual outcome. However, regardless the fit of the donor and recipient cornea, this vertical wound type requires relatively tight sutures. Healing of a vertical corneal wound requires a minimum of 6 months and typically 1 year in adults. In a relatively high number of patients, stable vision is not achieved until many months after traditional PKP surgery. Mean postoperative astigmatism after PKP ranges from 2.5 to 3.5 diopters (D) or higher.[12 13] Despite clear grafts, a significant number of patients have inadequate visual acuity because of a high or irregular postoperative astigmatism, often requiring selective suture removal or secondary procedures.[14] Factors that may affect postoperative astigmatism include type and size of trephines[15], time of suture removal or adjustment, apposition of donor and recipient tissues, and graft size.[16] Larger graft size results in a higher degree of topographic regularity and a higher quality of vision. However, larger grafts involve a higher risk of graft rejection.[17 18]

The postoperative course of a PKP procedure can be complicated by suture-related problems. These can involve a cascade of events such as suture loosening, sterile infiltrates, secondary infections, corneal ulceration, wound dehiscence with wound leakage and allograft rejection.[19] Incomplete wound healing after PKP leads to a structurally weaker globe, and wound dehiscence occurs with a frequency of 0.6% to 5.8%. [20]

To obtain a stronger corneal wound, many graft configurations have been proposed. In 1921, Carrel suggested a two-level, rectangular graft.[21] The square graft was characterized by a different size of the anterior and posterior layer of the graft. Afterwards, Franceschetti and Busin proposed this stepping wound configuration in a circular graft.[22 23] They called it the mushroom graft. The principle of this keratoplasty type is a full-thickness central donor graft with a lamellar wound configuration in the periphery. The full-thickness graft provides a superior refractive and visual outcome in the center, the lamellar graft increases the surface area for stromal wound healing at the margins. A potential drawback of this type of grafting is that the procedure, compared with a

traditional PKP, is more time-consuming due to the difficulty of trephination. The availability of a femtosecond laser for trephination of the graft configuration might simplify the preparation of the corneal tissue.[24 25] Corneal graft survival in the long term has been reported to range from 50% to 80% after ten years, with significant variation associated with indication for transplantation, socioeconomic and geographical factors.[26 27] Graft failure is defined as an irreversible loss of graft clarity. Risk factors for graft failure include post-graft uveitis or microbial keratitis, corneal vascularisation, recipient age and diagnosis, graft size, and occurrence of a rejection episode.[28 29] Other important reasons for graft failure in PKP are (secondary) glaucoma, ocular surface problems, and late endothelial failure.[30 31]

3.1.1. Late endothelial failure

Late endothelial failure is gradual graft decompensation without apparent cause, unresponsive to corticosteroids, and without history of a rejection episode. It is the most common cause of late graft failure.[32] After PKP, the graft loses endothelial cells at a faster than physiological rate, even in the absence of endothelial allograft rejection. Although especially marked in the short term, this abnormal cell loss persists, albeit at a much lower rate, for many years after transplantation.[33 34] Armitage et al were the first to demonstrate that chronic endothelial cell loss after PKP can be approximated using a biexponential decay model.[35]

3.1.2. Graft rejection

The ability of the cornea to survive in the absence of systemic immune-suppression led to the proposition that the cornea is an immune privileged tissue at an immune privileged site.[36] The avascularity of the cornea is an important mechanism that attributes to this corneal immune privilege. Therefore, HLA-matching of donor tissue to the recipient status is usually not performed in low risk cases. Nevertheless, good graft survival rates are obtained.

The epithelial, stromal and endothelial layers of the cornea may each show its own characteristic rejection pattern. An epithelial rejection is seen as an elevated line, starting in the periphery and progressing towards the center of the graft. The rejection reaction of the stromal layer is characterized by subepithelial infiltrates, which are whitish infiltrates in the anterior stroma, randomly positioned in the graft.[37] Like epithelium, the typical rejection of corneal endothelium is characterized by an advancing front of densely infiltrated and dying cells. This endothelium rejection line is known as a Khodadoust line.[38] The endothelial rejection is the most common and has the worst prognosis since, once affected endothelial cells do not regenerate.

The immune privilege is not absolute and can be lost. Classic risk factors of corneal transplant rejection include previous corneal transplants, corneal neovascularisation

and inflammation.[39] A loose or broken suture, associated with both inflammation and neovascularisation, has been identified as the leading associated risk factor for transplant rejection after PKP.[40] Reported rejection episodes after normal risk-PKP (patients with KC, FED, PBK) range from 7 % to 13 % within the first two years after surgery.[41 42] Fortunately, only a minority of the rejection episodes evolve to a failure of the graft. However, the survived graft has lost a substantial number of endothelial cells. Nguyen et al found that longer-term low-dose topical steroid treatment protects patients with normal-risk PKP against immunological graft rejections.[43]

3.2. Anterior lamellar keratoplasty

In anterior lamellar keratoplasty only the diseased epithelium, Bowman and anterior corneal stroma are removed and transplanted, leaving the unaffected Descemet and endothelium of the patient in place. Indications for anterior lamellar keratoplasty include KC, epithelial and (anterior) stromal corneal dystrophies, and partial-thickness corneal scars.

In the 1970s, there was an increased interest in lamellar corneal transplantation.[44] As a result of the technical difficulty of the procedure and the reduced postoperative visual acuity typically following lamellar keratoplasty, PKP remained the dominant corneal transplant procedure for the optical correction of corneal disease. A large part of these poor results were due to the irregular scattering of light at the recipient-donor wound interface. The need for a very smooth recipient and host surface at the wound interface, which was to be obtained more readily at a deeper corneal plane, was recognized early on.

Deep anterior lamellar keratoplasty (DALK) has several advantages over PKP surgery; no "open sky" related complications, no endothelial rejection, minor loss of endothelial cells, a better wound stability and less need for corticosteroids.[45] Moreover, the time to suture removal and the time necessary to achieve stable results is considerably shorter.[46] There is no advantage to DALK for refractive error outcomes. Although improved graft survival in DALK has yet to be demonstrated, postoperative data indicate that DALK is superior to PKP for preservation of endothelial cell density. Endothelial graft rejection cannot occur after DALK, which may simplify long-term management of DALK eyes compared with PKP eyes.[47]

The main parameter for good visual function after anterior lamellar keratoplasty is the thickness of residual recipient stromal bed. An eye with a DALK with a residual bed of less than 20 µm has been shown to achieve a similar visual result as an eye with a PKP.[48 49] In order to obtain a thin residual bed, various techniques have been used to accomplish baring of the Descemet's membrane. These include intrastromal air injection,[50] hydrodelamination,[51] viscoelastic dissection using an air-to-endothelium interface,[52] and blunt spatula delamination.[53] However, the technique is difficult and Descemet's

membrane perforation remains a major complication of DALK.[54] If a large perforation of the Descemet's membrane occurs, conversion to traditional PKP or a mushroom graft is necessary.

3.3. Posterior lamellar keratoplasty

The first human posterior lamellar keratoplasty (PLK) with a corneal limbal approach was performed by Charles Tillet in 1956.[55] This technique was revised by Ko et al in 1993.[56] In 1998, Melles provided a breakthrough development when he demonstrated that donor tissue could self-adhere with the aid of an anterior chamber air bubble.[57] Terry and Ousley subsequently modified this technique, renaming it deep lamellar endothelial keratoplasty (DLEK).[58] This surgery required manual lamellar dissections within the deep corneal stroma of both recipient and the donor corneas. Despite excellent postoperative results, visual acuity after DLEK rarely exceeded 20/30 due to optical aberrations at the graft-host interface.[59 60] To provide a smoother recipient interface, Melles et al introduced Descemet Stripping Endothelial Keratoplasty (DSEK).[61] Instead of performing a lamellar dissection, the Descemet's membrane was peeled off. This method accomplished two key objectives: it produced an easier procedure and it provided a possibly better optical interface. In Descemet Stripping Automated Endothelial Keratoplasty (DSAEK), the donor dissection is carried out using a mechanical microkeratome, which makes a smooth dissection deep within the corneal stroma.[62]

In the last few years, DSAEK has replaced PKP as the golden standard surgical treatment for corneal endothelial diseases. Its main advantage includes the lack of sutures, resulting in less induced astigmatism and a faster visual recovery. Furthermore, it is a safer technique with less risk of expulsive hemorrhage and suture-related complications.[63] The most common complications after DSAEK include posterior graft dislocations, primary graft failure, iatrogenic glaucoma, and endothelial graft rejection.[64] Studies suggested that corneal transplant rejection is less frequent and less severe after DSAEK than after PKP.[41] However, it is not clear yet whether these differences reflect an inherent reduction in the susceptibility to corneal transplant rejection in DSAEK or whether these are a product of extended postoperative topical steroid use. Moreover, some of these studies had a selection bias towards FED cases in the DSAEK-group. Compared to PBK, FED has a better transplant outcome.

The main concern for the DSAEK technique remains the rate of postoperative endothelial cell loss, reported in various series to be in the range of 25% to 50% at six to 12 months postoperatively.[65-67] Recently, Price et al[68] showed that overall graft success was comparable for the DSAEK and PKP procedure. However, the endothelial cell loss was higher with DSAEK at 6 and 12 months postoperatively, consistent with the endothelial trauma due to more donor tissue manipulation. Factors associated with lower endothelial cell loss are single-point fixation forceps, absence of secondary donor reattachment

procedure and larger incision width (5.0 mm versus 3.2 mm).[69 70] Folding of the corneal tissue during DSAEK causes more endothelial damage than glide insertion.[71] The post-operative decline of endothelial cell density is not significantly correlated with storage time or donor age.[72]

In Descemet Membrane Endothelial Keratoplasty (DMEK) the thickness of the transplanted layer of stroma is further reduced. Different techniques were developed by Melles, Price, and Kruse.[73 74 75] The donor material consisting of endothelium, Descemet and a very thin layer of posterior stroma, spontaneously forms a roll, with the endothelium on the outside. The donor roll can be introduced into the recipient eye using an inserter. After manipulation of this roll the donor disc is made to adhere to the recipient posterior stromal surface using an air bubble – as in DSAEK. The advantage of DMEK is an even quicker and improved visual rehabilitation than after DSAEK. However, donor preparation, positioning, and attachment are more challenging with DMEK, resulting in more donor tissue loss and a higher re-graft rate than typically experienced with DSAEK.[75]

III. INTRACORNEAL IMPLANTS AND CROSS-LINKING

1. Intracorneal ring segments

Intracorneal ring segments (ICRS) are made of polymethyl methacrylate (PMMA) and are implanted in the deep corneal stroma to modify the corneal curvature. The tunnel is created at 80% of the corneal thickness and can be made by mechanical dissection using a manual semicircular dissector or by photodisruption of lamellar tissue using the femtosecond laser technology. Two types of ICRS are available: Intacs (Addition Technology Inc, Des Plaines, Illinois) and Ferrara (Mediphacos, Belo Horizonte, Brazil) rings. The differences between these ring segments are a triangular cross-section and a smaller internal diameter of the Ferrara ring compared to the hexagonal cross-section of the Intacs.[76] The corrective result varies in direct proportion to the thickness of the ICRS and in inverse proportion to their diameter.

The ICRS are initially designed to correct low myopia in normal eyes. The last decade, ICRS have been shown effective in reducing corneal steepening and refractive errors in KC,[77] pellucid marginal degeneration,[78 79] and post-LASIK ectasia.[80 81] They might improve contact lens tolerance and prevent the need for keratoplasty in some of these patients.[82]

Patients with corneal ectasia are suitable for this treatment if they have clear central cornea, and a corneal thickness of 400 µm or more at the location where ICRS are to be placed. Reported results in patients with KC, show an increase in mean uncorrected visual acuity (UCVA) and best corrected visual acuity (BCVA) of two to four lines and a decrease in mean keratometry (K)-reading of 3 to 4 D.[83] Alio et al reported that ICRS

Table 1. Outcomes of ICRS for keratoconus.

Study*	Eyes	Intacs/ Ferrara	FU	Mean change SE (D)	Visual Acuity Change	Mean change in keratometry (D)
Alió[86]	13	Intacs	48 mo	1.45	BCVA increased from o.46 to 0.66	Mean K decreased with 3D. However, there was a significant increase in K-values between 36 and 48 mo
Colin[107]	100	Intacs	2 y	2.9	80.5% and 68.3% gained lines of UCVA and BCVA, respectively	Mean K decreased with 3.4D
Ertan[108]	118	Intacs	1 y	3.5	81.3% and 73.7% gained lines of UCVA and BCVA, respectively	Mean K decreased with 4D
Kwitko[77]	51	Ferrara	13 mo	1.45	86.4% gained lines of UCVA and BCVA	Mean K decreased with 5D. Eyes with central KC had significant better results than eyes with inferior cones.
Kanellopoulos[109]	20	Intacs	12 mo	2.2	UCVA improved from 20/154 to 20/28, BCVA improved from 20/37 to 20/22	Mean K decreased with 3.2D
Torquetti [83]	35	Ferrara	5y	-	UCVA and BCVA increased with 2 lines	Mean K decreased with 5 D

(BCVA = best corrected visual acuity; FU = follow-up; SE = spherical equivalent; UCVA = uncorrected visual acuity; mo = months; y = years; d = diopters. * First author only.)

insertion provides better results in visual acuity and corneal topography in eyes with mild to moderate KC (mean K-value ≤ 53 D) with a relatively low spherical equivalent (SE).[84] There is only one study that suggests that patients with paracentral scarring or a cornea steeper than 57 D would benefit from ICRS insertion.[85] Although most long-term follow-up studies show refractive and topographic stability, Alio et al reported a further significant increase in K-values 36 months after surgery.[86] A summary of published studies on ICRS for KC, with a follow-up of 12 months or more, are listed in table 1.

Peroperative complications during ICRS implantation are rare; anterior and posterior corneal perforation has been described.[87] The most common postoperative complication is ring segment extrusion or migration due to shallow placement.[77] A common finding after ICRS implantation is the presence of intrastromal deposits that accumulate in the lamellar channel after implantation. These channel deposits consist of intracellular lipids. However, they do not tend to grow and do not decrease visual performance.[88]

2. Intracorneal lenses

The use of corneal inlays to correct myopia or hyperopia involves creation of a lamellar flap and placement of synthetic material in the stroma. This modifies the optical power

of the cornea by changing the shape of the anterior corneal surface. Another possibility to correct refractive errors is implantation of a lens with a higher index of refraction than the corneal stroma.

Synthetic stromal inlays or intracorneal inlay implants have been investigated for nearly half a century. In 1966, Barraquer proposed synthetic intrastromal implants to alter the anterior corneal curvature as a way to correct refractive errors.[89] However, his results were unsatisfactory due to the impermeable nature of the implant lens material. Various types of synthetic inlays have been tested, including polysulfone,[90] PMMA,[91] and hydrogel.[92] The materials used in the first implants caused anterior stromal necrosis because they were impermeable to water and nutrients.[93] New materials such as high water content hydrogel showed biocompatibility and good corneal tolerance when implanted in primates.[94] Hydrogel lenses are permeable for water and nutrients, so normal corneal physiology can be maintained. Because of its refractive index (1.376), the lens has no refractive power of its own within the cornea. The refractive effect of the device is achieved through alteration of the corneal shape. Another advantage of intracorneal hydrogel lenses is that the technique is reversible. However, in the few decades these implants were superseded by laser refractive surgery and the implantation of phakic intraocular lenses. The intracorneal lenses fell into disuse.

Recently, interest in intracorneal implants has resurfaced, especially for the correction of presbyopia.[95 96]

3. Corneal cross-linking

In 2003, Wollensak et al developed a new technique for the treatment of progressive KC using corneal cross-linking (CXL) by ultraviolet A-light (UVA) and the photosensitizer riboflavin. Recently, it has been introduced to reduce the progression in pellucid marginal degeneration and post-LASIK ectasia,[97] and to treat patients with therapy-refractory infectious keratitis with corneal melt.[98]

The irradiation of the cornea is performed from a distance of 1 cm for 30 minutes, using a UV-A double diode, at 370 nm, and an irradiation of 3 mW/cm^2. Before beginning UVA-irradiation, photosensitizer riboflavin in a 0,1% solution is applied to the cornea every 3 minutes for 30 minutes to achieve adequate penetration of the solution. The maximum effect of the treatment is in the anterior 300 µm of the cornea. As for the endothelium, a cytotoxic level for endothelium has been found to be 0.36 mW/cm^2 which would be reached in human corneas with a stromal thickness of less than 400 µm.

Reported results in KC show an average flattening effect of 2 to 3 D. Visual acuity improves slightly with one or two lines.[99-101] In all of the treated eyes, the progression of KC was stopped. A preoperative maximum K-reading of more than 58 D is a significant risk factor for failure.[102]

CXL treatment has a relative low complication rate. Described complications are central stromal scars, bacterial keratitis,[103] and Acanthamoebe keratitis.[104] Diffuse lamellar keratitis has been reported after treatment in a case of post-LASIK ectasia.[105] Age over 35 years and a preoperative BCVA better than 20/25 are identified as significant risk factors for complications.[102] Haze is a common finding after CXL and extends into the anterior stroma. The nature of this haze is unclear but it is associated with the depth of CXL and loss of keratocytes.[106] Usually, the haze decreases during the first postoperative year.

OBJECTIVE AND OUTLINE OF THIS THESIS

The first part of this thesis aims to evaluate the clinical results of mushroom keratoplasty. Chapter 1 discusses the clinical outcome of the posterior mushroom (top-hat) keratoplasty in Fuchs' endothelial dystrophy and pseudophakic bullous keratopathy. Chapter 2 analyzes the visual and topographic results of mushroom keratoplasty in patients with advanced keratoconus.

The second object of this thesis is to discuss the technique of Descemet Stripping Automated Endothelial Keratoplasty (DSAEK). We evaluate the endothelial cell loss after both top-hat keratoplasty and DSAEK using a biexponential decay model (Chapter 3). Furthermore, we describe two complications of the DSAEK technique. Chapter 4 is a report on an endothelial rejection episode with the presence of a posterior Khodadoust line. Chapter 5 describes the introduction of epithelial cells, originating from the donor tissue, in the flap-graft interface.

The third part of this thesis discusses intracorneal implants and corneal cross-linking. Chapter 6 shows the usefulness of the combined treatment with Ferrara ICRS and CXL in patients with progressive KC to prevent the need for corneal grafting. Chapter 7 shows the long-term results of hydrogel intracorneal lenses in 2 aphakic eyes. The two patients were considered unsuitable candidates for intraocular lens implantation at the time.

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Part 1

Mushroom keratoplasty

Chapter 1

Posterior mushroom keratoplasty in patients with Fuchs' endothelial dystrophy and pseudophakic bullous keratopathy; transplant outcome

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ABSTRACT

Purpose: To report the outcome of posterior mushroom or top-hat shaped keratoplasty for patients with Fuchs' endothelial dystrophy (FED) or pseudophakic bullous keratopathy (PBK), concerning postoperative astigmatism, visual improvement, and endothelial cell density.

Methods: Twenty-two eyes of 21 patients who received a posterior mushroom keratoplasty for FED (n = 19) or PBK (n = 3) between March 2003 and January 2006 at the Erasmus MC, Rotterdam, The Netherlands, were included. Visual acuity, refraction and keratometry were measured at 6, 12, 24, and 36 months. Specular microscopy was performed annually.

Results: Best-corrected visual acuity (BCVA) significantly ($p < 0.05$) improved from 0.25 preoperatively to 0.47 at 6 months and 0.65 at 1 year postoperatively. Visual acuity remained stable with a BCVA of 0.62 2 years and 0.69 3 years after keratoplasty. At the last follow-up, the BCVA measured 0.5 or more in all but 2 (91%) eyes. Mean postoperative astigmatism at 1, 2 and 3 years measured 2.72 ± 1.48, 2.69 ± 1.85, and 1.75 ± 1.76 D. Endothelial cell density averaged 2143 ± 428 cells/mm^2 at one year, 1539 ± 573 cells/mm^2 at two years, and 1920 ± 474 cells/mm^2 at three years. All studied transplants remained functional and clear throughout the follow-up period.

Conclusion: The posterior mushroom keratoplasty in FED and PBK results in a relatively good visual acuity, a low astigmatism, and good endothelial cell density. The short-term results of our limited series of patients are encouraging.

INTRODUCTION

Fuchs' endothelial dystrophy (FED) is a slowly progressive, bilateral corneal dystrophy, which is typically observed clinically in patients older than 50 years of age. Because of a progressive loss of endothelial cells, the endothelial function deteriorates, and corneal thickness increases while visual acuity declines.[1-3] The increasing number of corneal and intraocular surgeries performed, together with a longer life expectancy, elevate the risk for developing symptomatic FED.[4] When a patient's visual acuity deteriorates significantly, a keratoplasty is indicated.

Pseudophakic bullous keratopathy (PBK) is a corneal disorder caused by endothelial decompensation secondary to trauma, glaucoma or congenital abnormalities. Clinically, the disorder results in corneal oedema and epithelial or subepithelial bullae. For the symptomatic stage of the condition, keratoplasty is the most effective therapy that can relieve pain and could restore visual acuity.[5]

PBK and FED are common indications for a penetrating keratoplasty (PK).[6-8] Although PK can provide good clinical results for these disorders, postoperative visual recovery is often prolonged because of high or irregular astigmatism.[9-11] Moreover, the preoperative corneal oedema in FED or PBK elevates risk of wound dehiscence after PK.[12] The mushroom shaped wound configuration, first described by Franceschetti in 1951,[13] was reintroduced by Busin[14] as "top-hat" keratoplasty using a manual approach. By fashioning a donor button with a shaped edge, a larger area of surface contact between the donor graft and the host was created. Advantages of this approach include a better and faster wound healing. With a larger posterior diameter, a higher percentage of the diseased layer is replaced, possibly leading to a lower rate of slow endothelial decompensation after transplantation. Moreover, the self-sealing posterior lip provides a better wound stability during surgery. After 4 sutures have been placed, the anterior chamber is already formed. Consequently, the risk of a possible expulsive haemorrhage is reduced and further suturing can be done in a symmetric way, which might reduce the surgery-induced irregular astigmatism. Also, a posterior mushroom keratoplasty is easier to perform in phakic eyes than a posterior lamellar keratoplasty (PLK). Recently, an artificial anterior chamber has become available and can help in the accurate trephination of the mushroom configuration.

We studied the outcome of a posterior mushroom keratoplasty in patients with FED or PBK on visual acuity, astigmatism and endothelial density.

MATERIAL AND METHODS

Patient and donor details

Between March 2003 and January 2006, a posterior mushroom keratoplasty was performed in 25 eyes of 24 patients for FED or PBK at the Erasmus MC, Rotterdam. The medical history of each patient was recorded preoperatively, and a complete eye examination was performed. All patients were scheduled for a PK because of insufficient visual acuity. Only primary transplants performed in FED or PBK patients with a follow-up of more than 6 months were included. Eyes with associated other corneal pathology were excluded. All of the procedures were performed by one of two corneal surgeons. The patient was, in consideration with the anaesthetist, free to choose between general and local anesthesia. Thirteen patients preferred general anesthesia and 9 patients underwent surgery under local anesthesia.

The donor corneas were preserved in an organ culture medium without dextran up to three weeks. The donor tissue was evaluated for a second time and subsequently transported in a transport medium with dextran. All donor corneas were obtained from Bio Implant Service, Leiden, The Netherlands.

Trephination and suturing techniques

The donor tissue was mounted on an artificial anterior chamber (Moria, Paris, France) after a viscoelastic substance (Healon®) was placed on the endothelium. The geometric center of the cornea was marked. A 7.5 mm Barron suction trephine was used to make a circular incision of 0.3 mm depth. A lamellar stromal dissection was carried out from the base of the incision to the limbus. The cornea was removed from the artificial anterior chamber and placed on a Barron suction punch (Katena) with the endothelial side up. A 9.0-mm donor button was punched out. The previous lamellar dissection allowed a superficial annular stromal lamella, 0.3 mm in thickness, which was removed in the area between 7.5 and 9.0 mm in diameter. (Fig 1)

The recipient bed was prepared to correspond with the shape of the donor button. The 7.5 mm Barron suction trephine, already used to the donor trephination, was used to cut a circular incision 0.3 mm in depth. The cornea was marked with a 9 mm trephine and a lamellar stromal dissection was carried out from the incision to the 9.0 mm mark. The anterior chamber was entered, and corneal scissors were used to complete the excision of the corneal button at the peripheral end of the posterior lamellar stromal dissection. (Fig 2)

An iridectomie was made at 12 o'clock. The donor button was positioned by sliding the peripheral wing under the 0.75 mm-wide superficial stromal lip of the recipient bed. The transplant was sutured with 8 interrupted sutures and 1 running 10-0 nylon suture,

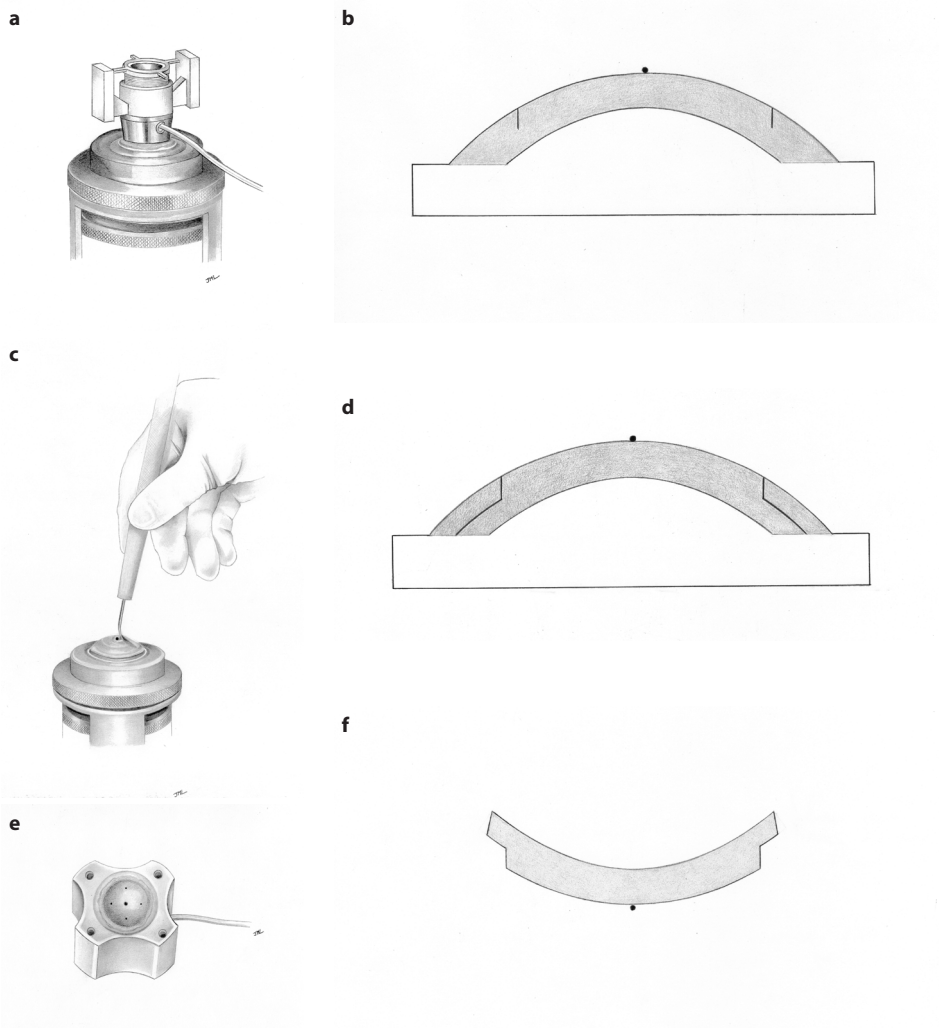

Fig 1. The trephination technique of the donor cornea. (J Leenen)
A-B: The geometric centre of the donor cornea is marked and the Barron suction trephine (7.5 mm in diameter) is used to make a circular incision of 0.3 mm depth.
C-D: A lamellar stromal dissection was carried out from the base of the incision to the limbus.
E-F: The cornea was removed from the artificial anterior chamber and placed on a Barron suction punch (Katena) with the endothelial side up. A 9.0-mm donor button was punched out.
(See also Colour Figures, p. 136.)

or with 16 interrupted 10-0 nylon sutures. Additional procedures performed in combination with mushroom keratoplasty were noted.

At the end of the procedure, all patients received a subconjunctival injection of 1 ml of betamethasondinatriumphosphate (4 mg/ml) and 0.5 ml of gentamicin (40 mg/ml). A simple handheld keratoscope was used for qualitative keratometry.[15] Sutures were

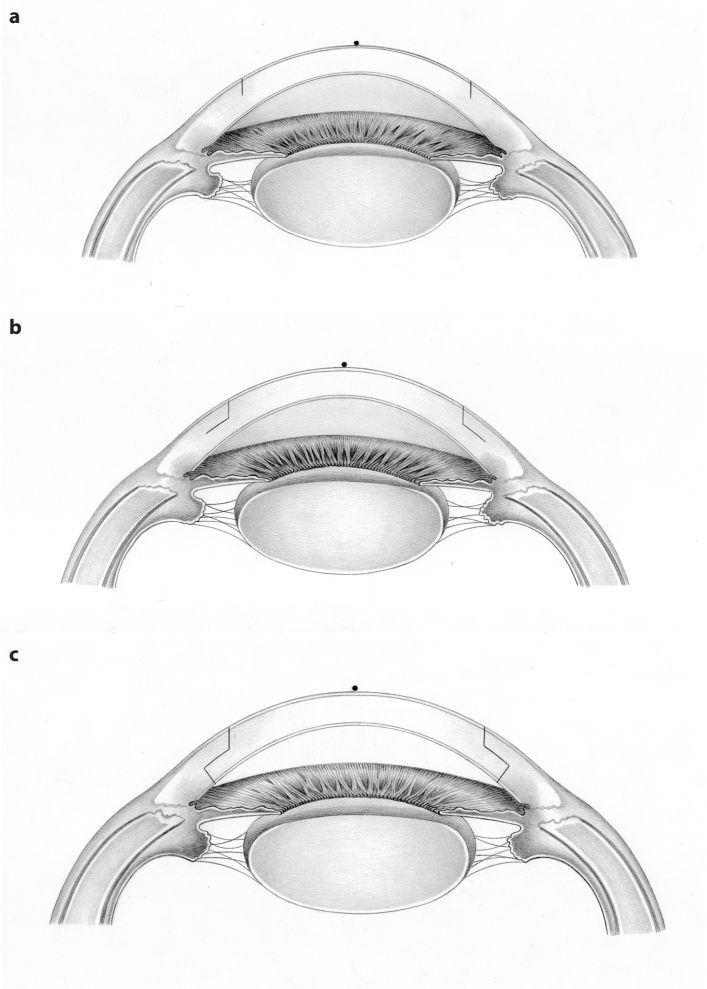

Fig 2. The trephination technique of the recipient cornea. (J Leenen)
A: The geometric centre of the recipient cornea is marked and the Barron suction trephine (7.5 mm in diameter) is used to make a circular incision of 0.3 mm depth.
B: The cornea was marked with a 9 mm trephine and a lamellar stromal dissection was carried out from the incision to the 9.0 mm mark.
C: The anterior chamber was entered, and corneal scissors were used to complete the excision of the corneal button at the peripheral end of the posterior lamellar stromal dissection.
(See also Colour Figures, p. 137.)

adjusted to correct astigmatism at the end of the procedure. Postoperatively, dexamethasone 0.1% eye drops were given 6 times a day for 2 months and subsequently tapered off during 1 year. Chloramphenicol 0.4% eye drops were administered 3 times a day for 2 weeks. Selective suture removal was performed postoperatively.

Main outcome measures and statistical methods

Main outcome measures included: visual acuity, astigmatism (Javal keratometer, Haag Streit, Bern, Switzerland), and endothelial cell density (cells/mm^2, non-contact Topcon SP 2000P specular microscope). Visual acuity was defined as best-corrected visual acuity measured by the Snellen visual acuity chart. Total surgically induced refractive change in astigmatism was calculated with the use of vector analysis using keratometric results.[16] Preoperative and postoperative comparisons were made using the paired-sample t-test, and statistical significance was set at $p = 0.05$. The Kaplan-Meier plot was used to calculate the average time until all sutures were removed.

RESULTS

Donor details

The mean age of the donors was 69.5 ± 11.1 years and the average endothelial cell count of the grafts measured 2653 ± 257 cells per mm^2.

Patient population

Two patients died within 6 months after surgery. A third patient developed a corneal ulcer, caused by a fungal infection with *Exophiala*, and a second graft was needed. All together, we studied 22 eyes of 21 patients with FED (n = 19) or PBK (n = 3). Patient age averaged 70.9 ± 10.3 years, and mean follow-up in this study was 2.3 ± 0.9 years.

Keratometry

Mean preoperative astigmatism measured 1.23 ± 0.94 D. After keratoplasty, the mean absolute keratometric astigmatism was 2.76 ± 1.98 D at 6 months (n = 21), 2.72 ± 1.48 D at 12 months (n = 22), 2.69 ± 1.85 at 2 years (n = 15), and 1.75 ± 1.76 at 3 years postoperatively (n = 8). Both vector analysis and t-test, showed no significant change in surgery-induced astigmatism during postoperative follow-up. Mean preoperative and postoperative corneal power is shown in table 1.

Visual acuity

The preoperative BCVA was 0.25 ± 0.15. Postoperatively, the BCVA improved significantly ($p < 0.05$) to 0.47 ± 0.20 and 0.65 ± 0.20 at 6 months (n = 20) and 12 months (n = 21) respectively. During follow-up, visual acuity remained stable ($p = 0.57$) with an average BCVA of 0.62 ± 0.24 at two years (n = 17) and 0.69 ± 0.25 at three years (n = 8, Fig. 3). At the last follow-up, all but 2 patients had achieved a BCVA of 0.5 or more. The low visual acuity in the 2 patients (0.3) was both due to age-related macular disease. Without considering these 2 patients, mean BCVA measured 0.69 ± 0.20 at one year, 0.69

Table 1. Pre- and postoperative patient data.

Variable	Preoperative (n = 22)	Postoperative			
		6 Mo (n = 20)	12 Mo (n = 20)	24 Mo (n = 17)	36 Mo (n = 8)
Mean age of donor ± SD (Y)	69.5 ± 11.1	-	-	-	-
Mean endothelial cell count (cells/mm^2)	2653 ± 257	-	2143 ± 428	1539 ± 573	1920 ± 474
Mean BCVA ± SD (range)	0.25 ± 0.15 (0.02 – 0.5)	0.47 ± 0.20 (0.2 – 0.8)	0.65 ± 0.20 (0.2 – 1.0)	0.62 ± 0.24 (0.1 – 1.0)	0.69 ± 0.25 (0.3 – 1.0)
Mean keratometric astigmatism (D)	1.23 ± 0.94	2.76 ± 1.98	2.72 ± 1.48	2.69 ± 1.85	1.75 ± 1.76
Mean flat/steep K-reading (D)	43.17/44.25	41.18/43.94	41.20/43.92	41.51/44.20	42.56/44.31
Mean keratometry (D)	43.79 ± 42	42.56 ± 3.0	42.56 ± 3.01	42.86 ± 2.85	43.4 ± 2.73

(BCVA, best corrected visual acuity; SD standard deviation; D: diopters; Y: years)

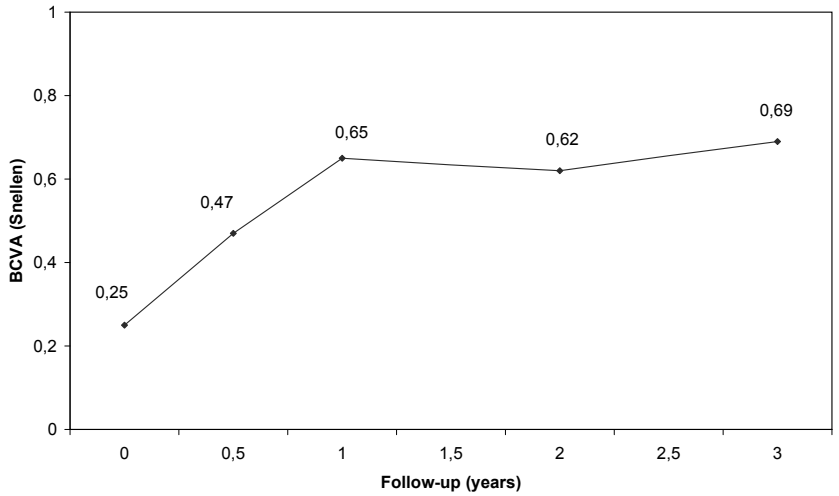

Fig 3. Pre-and postoperatively BCVA. (N = 22)

± 0.18 at two years, and 0.74 ± 0.15 at three years postoperatively. Compared to the FED patients, lower visual acuity both preoperatively and postoperatively was seen in the patients with PBK. Due to the low number of patients with PBK, these findings could not be confirmed statistically.

Endothelial cell loss

Although there was a statistically significant decline ($p = 0.0001$) in average endothelial cell count during the first year after surgery (2143 ± 428, n = 17), endothelial cell count remained stable ($p > 0.05$) during further follow-up, with 1539 ± 573 cells per mm^2 (n = 8) at two and 1920 ± 474 (n = 7) at three years after surgery.

Suture adjustment and suture related problems

Our policy is to remove all sutures 2 to 3 years postoperatively. In case of astigmatism, the suture at the steep axis was removed. A loosen or broken suture was removed immediately. In 6 eyes, we recorded loosened sutures and in one eye cheese wiring. In one patient, we placed an extra suture 6 months postoperatively to correct high astigmatism (7.5 D). At the end of the study, all sutures were removed in 6 eyes (on average at 1.48 years postoperatively). (Fig. 4)

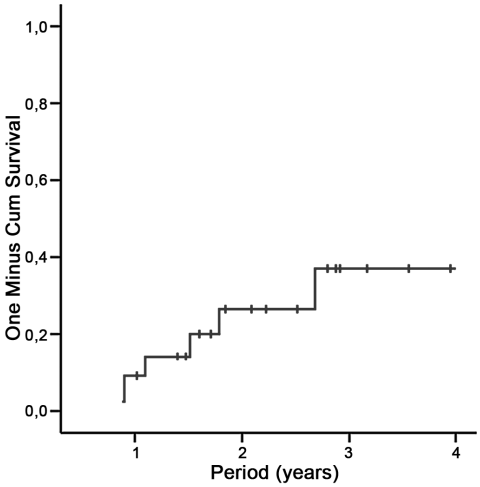

Fig 4. Cumulative percentage of patients with all sutures out.

Peroperative and postoperative complications

In all but one eye, the surgical procedure was uneventful. The patient of this one eye woke up during surgery, and a choroidal haemorrhage with vitreous prolaps occurred. An anterior vitrectomy was performed and the intraocular lens had to be removed. The trephination of the first three corneal grafts was a little more eccentric than the others. Epithelialization occurred within 2 weeks after the procedure. All included keratoplasties remained functional and clear until last follow-up.

DISCUSSION

In our study, patients with FED or PBK obtained satisfying visual acuity after posterior mushroom keratoplasty, which remained stable up to 3 years after surgery. All grafts remained clear and functional.

In most cases of FED and PBK, a keratoplasty is the only effective therapy for the symptomatic stage of the condition. Graft survival for FED is good, with a long-term graft survival rate of 89%.[9,17] Although the short-term graft survival rate in PBK is good (74%

at 5 years[18]), visual improvement is often poor.[5] One of the major limitations of visual rehabilitation after PK is a high or irregular postoperative astigmatism, often requiring selective suture removal or secondary procedures. A faster visual rehabilitation may be expected after a PLK, where only the diseased endothelium is replaced.[19,20] However, the presence of a lamellar interface may reduce the quality of vision after PLK compared with that obtained after PK.[21] Moreover, a PLK is inadvisable in phakic eyes because of the working space that is needed.[22-24]

To improve the postoperative outcome of PK, several modifications of the wound edge have been described.[25-28] In 2003, Busin described the posterior mushroom or "top - hat" keratoplasty.[14] The principal of this keratoplasty type is a full-thickness central donor graft with a lamellar wound configuration in the periphery. The full-thickness graft gives a superior refractive and visual outcome in the center; the lamellar graft increases the surface area for stromal wound healing at the margins. This self-sealing wound configuration could have major advantages. First, less tight sutures are needed, which might reduce suture-induced astigmatism. In our group of patients, selective suture removal was used. The postoperative astigmatism is relatively low and regular, with a mean keratometric astigmatism at all follow-up times of < 3 D. Our results are in line with the reported results after laser trephination of a mushroom keratoplasty.[30,31] Second, the posterior peripheral wing of deep stroma and endothelium (0.75 cm in width) creates a horizontal surgical wound. The healing of this type of wound requires less time than the edge-to-edge wound in conventional PK, allowing earlier postoperative suture removal and the risk of wound dehiscence might be reduced.[14 31] We did not notice wound dehiscence in any eye during follow-up. Another advantage of posterior mushroom keratoplasty is that the self-sealing posterior lip provides a better wound stability during surgery. Because of the intraocular pressure that pushes the lamellar wing against the recipient cornea, the anterior chamber is formed after the first 4 sutures have been put in place. Further suturing can be done in a very symmetric way, which might decrease the surgery-induced irregular astigmatism.

A potential drawback of this type of grafting is that the procedure, compared with a conventional PK, is more time-consuming because of the difficulty of trephination. The availability of a programmable femtosecond laser might simplify the preparation of the corneal tissue and could create a more comparable wound configuration. Although the endothelial cell density 3 months after laser trephination was excellent, a longer follow-up and larger series are required to definitively demonstrate the potential advantage of laser trephination.[32] Moreover, except for an artificial anterior chamber, no expensive instrumentation is required for the manual technique. The postoperative astigmatism and the visual acuity in our study are in line with recently published results of laser trephination.[32]

Visual acuity after keratoplasty is mainly a function of both retinal macular potential and the optical quality of the cornea. One year after the posterior mushroom kerato-

plasty, 82% (18/22) of our patients gained a vision of 0.5 or better. In 2 of the 4 eyes with a BCVA less than 0.5 age-related macular disease was present. In the 2 other patients, the visual acuity improved with time. In line with previously reported studies,[37] the postoperative visual improvement in our patients with PBK was lower compared to patients with FED. Our relatively good postoperative visual acuity might be explained not only by the low amount of surgery-induced astigmatism, but also by the greater corneal surface regularity after the posterior mushroom keratoplasty. Compared with reported results on visual acuity after conventional PK in patients with FED or PBK (BCVA ≥ 0.5 in 64% of the patients)[9], our results are more favourable.

Corneal endothelial cells do not divide in humans and there is a well-documented, gradual decline in endothelial cell density with increasing age. The loss of endothelial cells is greatly exacerbated after PK. The number of donor cells that survive the transplant procedure depends on the adequacy of the donor preservation and the amount of trauma during surgery.[34] The decline in endothelial cell density is especially marked in the short term, but persists at a reduced rate during many years after transplantation.[35] Patients undergoing a posterior mushroom keratoplasty usually receive more donor endothelium (9.0 mm diameter) than patients with a conventional PK (7.5-8.5 mm diameter). In our study, endothelial cell density remained relatively high after up to three years postoperatively, with an average endothelial cell density of more than 1500 cells per mm^2 at all follow-up periods. After a significant decrease the first year after surgery, the endothelial cell density in our study remained stable during further follow-up, and compares favourably with conventional PK. The rise in endothelial cell density during the third year can be explained by the fact that the patients with endothelial cell measurements in the second year are not the same as those in the third year.

A longer follow-up period is needed to study the effect of the wound configuration on the endothelium density. Although more endothelial cells are transplanted, the smaller anterior graft surface (7.5 mm in diameter) maintains a safe distance from the corneoscleral limbus. Consequently, the development of postoperative corneal neovascularisation and associated risk of graft failure might be reduced.[35] There was no rejection noted during our mean follow-up of 2.3 years, which compares favourably to other reports of PK in FED or PBK.[9,36]

This study provides a longer follow-up and a higher number of patients compared to other studies of posterior mushroom keratoplasty.[14,30-32] With inclusion of only FED or PBK patients, we evaluated the technique in a very homogeneous group. In addition, all corneal transplantations were performed by only 2 surgeons. Our results support the value of posterior mushroom keratoplasty in patients with FED or PBK. However, the obtained positive results require confirmation in a larger study group with a follow-up period beyond 3 years.

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Chapter 2

Manual trephination of mushroom keratoplasty in advanced keratoconus

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ABSTRACT

Purpose: To evaluate the technique of anterior mushroom keratoplasty for patients with advanced keratoconus, with respect to postoperative astigmatism, visual improvement and stability, and endothelial cell count.

Design: A retrospective observational study.

Methods: Sixteen eyes of 15 patients with advanced KC received an anterior mushroom keratoplasty at the Erasmus Medical Center at Rotterdam between April 2003 and May 2006. The donor cornea had a larger anterior stromal lamella (9.0 mm in diameter) and a smaller posterior part (7.0 mm in diameter). Visual acuity and keratometry were measured at 6, 12, 24, and 36 months. Specular microscopy was performed annually.

Results: Best-corrected visual acuity (BCVA) improved significantly ($p = 0.003$), from 0.25 ± 0.20 preoperatively to 0.62 ± 0.22 at 6 months postoperatively. Visual acuity remained stable with a BCVA of 0.70 ± 0.20 from 1 year onward. At all follow-up periods, mean postoperative astigmatism remained < 3.5 D. Mean postoperative astigmatism at 1, 2 and 3 years measured 2.67 ± 1.95 ($n = 15$), 2.54 ± 1.86 ($n = 6$), and 3.30 ± 2.12 D ($n = 5$). The average endothelial cell count at 1 and 2 years was 1755 ± 678 ($n = 12$) and 1573 ± 546 ($n = 5$), respectively. At the end of follow-up, all transplants remained functional and clear.

Conclusion: The mushroom-shaped graft can combine the benefits of a good visual acuity, a low astigmatism, and rapid postoperative healing. The short-term results of our limited series of patients are encouraging. Further research is necessary to draw stronger conclusions regarding the long-term effects.

INTRODUCTION

Keratoconus (KC) is a non-inflammatory corneal disorder characterized by localized conical protrusion and thinning of the corneal stroma. Depending on the diagnostic methods, the incidence varies between 4 and 230 per 100,000.[1] The diagnosis is made on the basis of progressive myopic astigmatism identified by refraction, keratometry, or corneal topographic analysis.[2] If treatment with spectacles or contact lenses is insufficient, different treatment options are possible, such as intracorneal rings,[3,4] a toric Artisan lens,[5] lamellar keratoplasty or penetrating keratoplasty (PK).[7,15] Ten to 20% of patients eventually require keratoplasty due to scarring in the visual axis, insufficient visual acuity (VA) with contact lens correction, or contact lens intolerance.[6]

KC is a common indication for corneal transplantations.[7] Although PK in KC generally has a good prognosis, it can be complicated by a high or irregular astigmatism, resulting in a delayed or poor visual recovery.[9,10] Many factors can affect postoperative astigmatism. Astigmatism decreases with increasing graft size.[11] However, larger grafts are shown to be associated with a higher risk of rejection.[12,13] PK in a cornea with a thin periphery, as seen in advanced KC, carries the risk of a high postoperative astigmatism. Besides postoperative astigmatism, graft decompensation caused by endothelial rejection is the cause of poor visual outcome in up to 9% of the KC eyes after transplantation.[14]

To avoid the risk of endothelial graft rejection, an anterior lamellar keratoplasty (ALK) can be performed. Advantages of ALK over PK surgery are the extra-ocular procedure, the preservation of recipient healthy endothelium, a better wound stability and less need for corticosteroids.[8] Moreover, the time necessary to achieve stable results is considerably shorter.[15] However, the presence of a lamellar tissue interface may reduce the quality of vision after ALK.[16-18] Although optical results are shown to be better with microkeratome - assisted LK, this procedure can only be performed if a minimal corneal thickness of >380 µm is present. A recent study suggested that the main parameter for good visual function after ALK for KC is the thickness of residual recipient stromal bed. An eye with a deep anterior LK with a residual bed of < 20 µm has been shown to achieve a similar visual result as a PK.[15] However, the technique is difficult, and Descemet perforation is a well-described complication of deep ALK.[19]

To combine the optical superiority of a large-diameter PK with some of the wound-healing advantages of a LK, a mushroom shaped keratoplasty has been proposed.[20-22] A central full-thickness donor graft with a lamellar wound configuration in the periphery is used. Recently, an artificial anterior chamber has become available and can help in the accurate trephination of the mushroom configuration. We studied the outcome of anterior mushroom keratoplasty in KC patients on visual acuity, refraction and endothelial cell density.

PATIENTS AND METHODS

Patients and donor details

Between 2003 and 2006, a mushroom keratoplasty was performed in 16 eyes of 15 patients for KC at the Erasmus MC, Rotterdam, The Netherlands. The medical history of each patient was recorded preoperatively, and a complete eye examination was performed. Seven patients had an allergic constitution. All patients were scheduled for PK because of insufficient VA or contact lens intolerance. Only primary transplants performed in KC patients with a follow-up of > 6 months were included. Eyes with associated other corneal pathology were excluded. All of the procedures were performed by one of two corneal surgeons.

The donor corneas were preserved in an organ culture medium without dextran at 31°C up to 3 weeks. The donor tissue was evaluated a second time and subsequently transported in a transport medium with dextran. All donor corneas were obtained from Bio Implant Service, Leiden, The Netherlands.

Surgical technique

The donor cornea was mounted on an artificial anterior chamber (Moria, Paris, France) after a viscoelastic substance (Healon®) was placed on the endothelium. The geometric center of the cornea was carefully marked, and a 9.0 mm Barron suction trephine (Katena) was used to make a circular incision of 0.3 mm depth. The Barron trephine provides suction indentations that are inked centrally on the donor in the quadrants to provide guidance for the first 4 suture placements. A central circle of 7.0 mm was marked with a non-disposable trephine and inkpad. A lamellar stromal dissection was carried out from the circular incision up to the 7.0 mm mark. The artificial anterior chamber was entered with a 15° razor blade knife, and curved Vannas corneal scissors were used to complete the excision of the corneal button. The upper side of the Vannas scissors was held slightly inwards to prevent oblique wound edges. The donor button obtained consisted of a large anterior stromal lamella (9.0 mm in diameter) and a small posterior diameter (7.0 mm). (Fig 1)

If there was superficial vascularisation of the recipient cornea, a fornix-based conjunctival flap was performed in these quadrants. The geometric center of the recipient cornea was carefully marked. The center of the pupil tends to be located slightly nasally. Whenever this was the case, we marked the cornea ~ 0.3 mm nasally from the center of the cornea to obtain a better centration of the graft with the pupil. The recipient bed was prepared to correspond with the shape of the donor button. The 9.0 mm Barron suction trephine, already used for the donor trephination, was used to cut a circular incision of 0.3 mm in depth. The indentations of the suction trephine were also inked. The central cornea was marked with a 6.5 mm trephine. A lamellar dissection to the central 6.5 mm

Fig 1. Trephination technique of donor cornea. (J Leenen)
A, The Barron suction trephine (9.0 mm in diameter) is used to make a circular incision of 0.3-mm depth.
B, A lamellar stromal dissection was carried out from the circular incision up to the 7.0-mm mark.
C, The donor cornea with a larger anterior stromal lamella (9.0 mm in diameter) and a smaller posterior part (7.0 mm in diameter)
(See also Colour Figures, p. 138.)

mark was carried out. (Fig 2) The excision of the corneal button was completed conform the method of the donor cornea. An iridectomy at 12 o'clock was made. Because this wound configuration is not self-sealing, the corneal graft was sutured in place. Eight single, superficial 10-0 Nylon sutures and 8 single, deep 10-0 Nylon sutures through the full thickness of the donor cornea and the peripheral lamellar wing were used to position the donor button.

At the end of the procedure, all patients received a subconjunctival injection of 1 ml betamethasondinatriumphosphate (4 mg/ml) and 0.5 ml gentamicin (40 mg/ml). A simple handheld keratoscope was used for qualitative keratometry. If needed, sutures were adjusted to correct astigmatism at the end of the procedure. There were no other procedures performed in combination with the mushroom KP. Postoperatively, dexamethasone 0.1% eye drops were given 6 times a day for 2 months and subsequently tapered off during 1 year. Preservative-free chloramphenicol 0.4% eye drops were

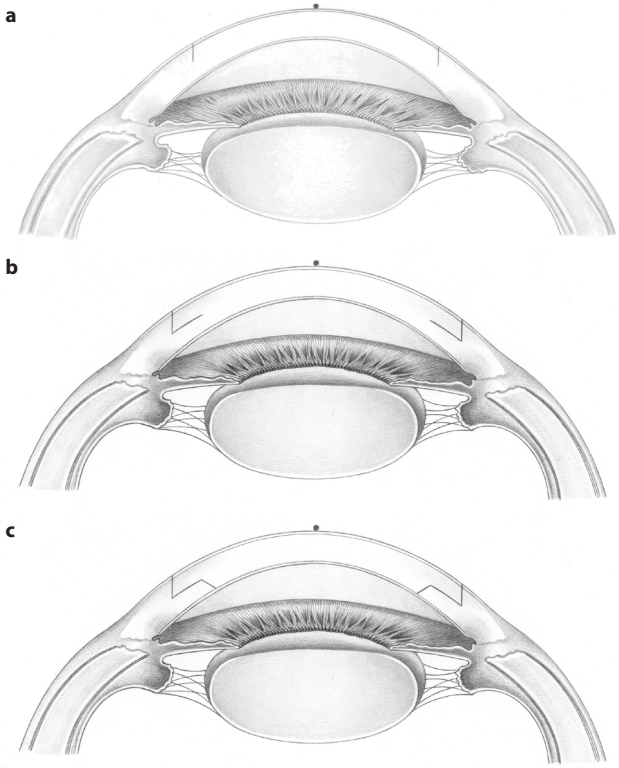

Fig 2. Trephination technique of recipient cornea. (J Leenen)
A, The geometric center of the recipient cornea is marked. The Barron suction trephine (9.0 mm in diameter) is used to make a circular incision of 0.3-mm depth.
B, A lamellar stromal dissection was carried out from the circular incision up to the central 6.5-mm mark.
C, Vannas corneal scissors were used tocomplete the excision of the corneal button.
(See also Colour Figures, p. 139.)

administered 3 times a day during 2 weeks. Selective suture removal was performed postoperatively.

Methods and main outcome measures and statistical methods

Main outcome measures included VA, astigmatism (Javal keratometer; Haag Streit, Bern, Switzerland), and endothelial cell density (cells/mm^2; non-contact Topcon SP 2000P specular microscope). VA was defined as best-corrected VA (BCVA) measured by the Snellen chart.

To compare means, Student t-test was used. $P < 0.05$ was considered statistically significant.

RESULTS

Donor details

The mean age of the donors was 62 ± 10 years (range, 43-76 years), and the average endothelial cell count of the grafts measured 2757 ± 168 cells/mm^2 (range, 2500-3100 cells/mm^2).

Patient data

From July 2003 to May 2006, there were 16 eyes of 15 patients with a diagnosis coded for both advanced KC and mushroom keratoplasty. One eye was excluded because a second graft was needed at day 6 due to a severe keratitis with hypopion. *Mycobacterium chelonae* was cultured from the donor cornea and from the culture medium at the laboratory. All in all, we studied 15 eyes of 14 patients, with an average age of 36 ± 10 years. Mean follow-up in this study was 24.47 ± 12.22 months.

Keratometry and visual acuity

Of 15 eyes, preoperative refraction was obtainable only in 7 eyes because of high and irregular astigmatism. Mean preoperative keratometric astigmatism, measured with the Javal keratometer, was 5.45 ± 3.22 D (n = 13). Further preoperative values are summarized in Table 1. After keratoplasty, mean keratometric astigmatism was 2.92 ± 1.27 D at 6 months (n = 15), 2.67 ± 1.95 D at 12 months (n = 15), and 2.54 ± 1.86 at 2 years (n = 6) and 3.30 ± 2.12 at 3 years postoperatively (n = 5).

The BCVA preoperatively measured 0.25 ± 0.20, with a best spectacle-corrected VA of 0.17 ± 0.16 (n = 8) and a best contact lens-corrected VA of 0.40 ± 0.19 (n = 5). Postoperatively, the BCVA improved significantly (p = 0.003) to 0.62 ± 0.22 and 0.70 ± 0.22 at 6 months and 12 months, respectively. During follow-up, VA remained stable with an average BCVA of 0.72 ± 0.22 (p=0.70) at 2 years and 0.80 ± 0.14 at 3 years. During the last follow-up, all but 2 patients had achieved a BCVA of 0.6 or more. Four patients used contact lenses. The low visual acuity in one of the two patients (finger counting) was due to subretinal neovascularisation, associated with high myopia. The second patient

Table 1. Pre- and postoperative patient data.

Variable	Preoperative	Postoperative			
		6 Mo	12 Mo	24 Mo	36 Mo
Mean BCVA ± SD	0.25 ± 0.20	0.62 ± 0.22	0.70 ± 0.22	0.72 ± 0.22	0.80 ± 0.14
Mean keratometric astigmatism (D)	5.45 ± 3.22	2.92 ± 1.27	2.67 ± 1.95	2.54 ± 1.86	3.30 ± 2.12
Mean endothelial cell count (cells/mm^2)	2757 ± 168		1755 ± 678	1573 ± 546	950 ± 494

(BCVA: best-corrected visual acuity; SD: standard deviation; D: diopters)

was known to have amblyopia in the operated eye. At the last recorded follow-up of all the patients, BCVA measured 0.72 ± 0.19. Without considering the 2 patients with extracorneal pathology, best spectacle-corrected VA measured 0.78 ± 0.12.

Corneal endothelial cell density

We measured an average endothelial cell count of 1755 ± 678 cells/mm^2 at 1 year, 1573 ± 546 cells/mm^2 at 2 years, and 950 ± 494 cells/mm^2 at 3 years after mushroom keratoplasty.

Suture adjustment and suture related problems

Our policy is to remove all sutures 2-3 years postoperatively. In case of astigmatism, the suture at the steep axis was removed. A loose or broken suture was removed immediately. Seven patients had at least 1 loosened suture and 1 developed a sterile infiltrate. Additional sutures were placed in 3 patients.

Intra - and postoperative complications

All of the patients underwent surgery under general anesthesia. Surgery was uneventful in all patients. Epithelialization occurred within 2 weeks after the procedure. Postoperatively, 1 patient needed an extra suture because of wound leakage. Three years postoperatively, another patient developed an intraepithelial cyst of unknown origin that was removed. All keratoplasties remained functional and clear until last follow-up.

DISCUSSION

In our study, patients with advanced KC obtained satisfying visual acuity after mushroom keratoplasty, which remained stable up to 3 years after surgery. All grafts remained clear.

Because only primary transplants for KC patients were included, we evaluated the technique in a homogeneous group of patients. In addition, all corneal transplantations were performed by the same 2 surgeons. To our knowledge, this is the first study to provide the results of the mushroom keratoplasty using the disposable Hessburg-Barron suction trephine and the artificial anterior chamber. A drawback of this study is the overall few included patients and the relatively restricted follow-up period. Because it is a retrospective study, patient data can be incomplete.

The mushroom keratoplasty is not a new technique. As far back as 1950, Franceschetti published results of the "greffe-champignon".[20,21] At that time, the indications for this procedure were unsatisfactory result of previous lamellar keratoplasty or PK, severe burns, trophic corneal disorders of aphakia, unfavourable cases with irregular cornea surface, staphylomas, descemetocele, and Fuchs' endothelial dystrophy. Unfortunately,

his mechanical cutter for the donor graft was traumatizing for the corneal endothelium and difficult to use. Afterwards, Stocker tried to refine the trephination technique, but it resulted in imperfect matching between the graft and the recipient bed.[23] Also, Roberts with the mechanical suction trephine, and Keates et al with the fixation clamp, tried to optimize the trephination of the donor cornea.[24,25] Despite all efforts, accurate trephination was still difficult to perform. Because better surgical devices have recently become available, there is new interest in mushroom keratoplasty. The disposable Hessburg-Barron suction trephine and the artificial anterior chamber facilitate a punctual trephination with minimal trauma to the endothelial layer.[26]

Transplantation of a larger anterior diameter, like the one in a mushroom keratoplasty, results in a higher degree of topographic regularity and a higher quality of vision.[27] Close proximity of the anterior layer to the limbus of the recipient may lead to suture loosening and wound healing problems. This is particularly true for allergic patients with vascularized corneas. To avoid this, we made a fornix-based conjunctival flap and placed sutures into the sclera in these quadrants. Although the diameter of this posterior graft was only 7 mm, there were no complaints about glare or halos. The elevated risk for graft failure, seen in larger graft diameters, is probably avoided because of the smaller posterior lamella in mushroom keratoplasty.[28,29]

The first 2 years after surgery, the endothelial cell density in our group of patients remained relatively good. However, endothelial cell loss after PK is an ongoing process even years after surgery.[30-32] Our data showed a rise in endothelial cell loss 3 years after keratoplasty. Because we have data of only 30% (n = 5) of our patients in that follow-up period, more patient data are needed to draw stronger conclusions regarding the long-term effects of manual mushroom trephination in KC patients.

Although the corneas of our patients were steep, with an average steep K-reading of 60D and a thin periphery, our results are in line with the reported success rate (range, 70% - 91%) of PK in general KC patients, defined as a VA of > 0.5 at 18 months postoperatively.[33-36] Suturing a conventional full thickness penetrating corneal graft in patients with a zone of marked inferior corneal thinning is more prone to develop a higher postoperative astigmatism and thus a lower BCVA because of wound healing problems (Fig 3A). Because of the particular shape of the mushroom-shaped graft, the donor and the recipient are locked to each other. This way, the graft is unlikely to dislocate (Fig 3B).

In patients with a zone of marked inferior corneal thinning within 2 or 3 mm of the inferior corneal limbus, we used to perform a large (9.0-9.5 mm) lamellar graft first. Many of these patients had a central corneal scar. To obtain a good VA, a conventional full-thickness PK of 8 mm was performed into the lamellar graft 1 year postoperatively. The mushroom keratoplasty requires only 1 procedure, and the results are much better than our results of conventional keratoplasty in patients with KC with marked inferior corneal thinning

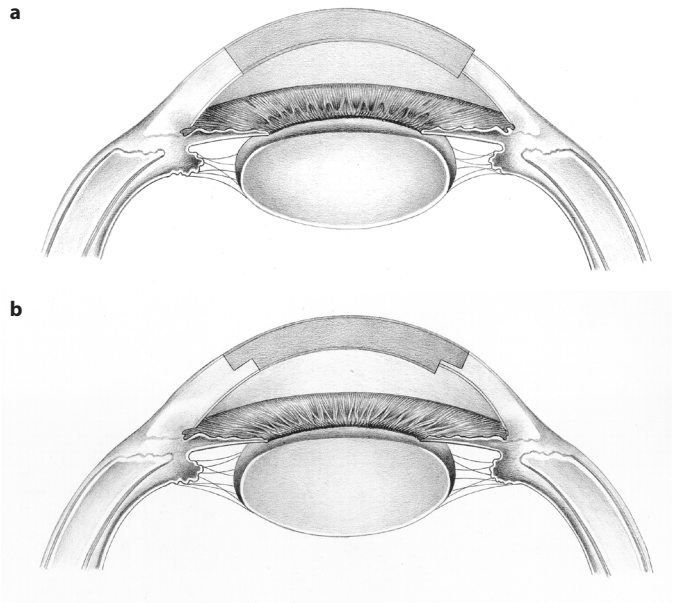

Fig 3. Keratoplasty in a recipient cornea with a thin periphery. (J Leenen)
A, Problematic wound healing of a normal thickness donor cornea to a 1-sided thin recipient in conventional keratoplasty with a vertical cut.
B, Mushroom graft to a 1-sided thin recipient; no dislocation occurs.
(See also Colour Figures, p. 140.)

The reported graft rejection rates are relatively low in patients with KC, with a 5-year graft survival that varies around 97%.[37,38] All of our grafts remained clear, and no rejection periods were noted.

During follow-up, we noted a minor increase in keratometric astigmatism. The tendency towards a myopic shift is confirmed by published data of PK in KC patients.[39] This slight increase was not significant ($p = 0.34$), and the postoperative corrected VA remained stable. Despite the fact that most of the BCVAs were obtained by using spectacles ($n = 11$) instead of contact lenses ($n = 4$), our results concerning BCVA are comparable with previous reported results of PK and lamellar keratoplasty for KC.[18,40]

Although the prevalence of KC is generally reported to be identical in male and female patients, higher rates of PK have been reported in male subjects.[42] In our study, 73% (11/15) of the patients were men.[43]

The mushroom technique does not resolve all problems in corneal grafting. Although we obtained a high VA in our study, even with spectacles, the magnitude of the induced astigmatism still remains unpredictable.[7] Because early suture removal may result in unpredictable changes in astigmatism, our policy is not to remove all sutures until 2 to 3 years after surgery. The second potential disadvantage of this mushroom keratoplasty is the difficulty of the technique, which requires a skilled surgeon. Compared with PK,

the procedure time of a mushroom keratoplasty is longer. To facilitate the procedure, a femtosecond or excimer laser might be used to incise the cornea. Laser trephination could have the potential to improve the visual outcome by creating congruent cut edges parallel to the optical axis, resulting in a higher regularity of the corneal topography.[31,43-48] No significant difference in endothelial cell density was found between the mechanical and nonmechanical technique.[46]

In conclusion, the mushroom keratoplasty can be used in patients with advanced KC. This technique is especially useful in steep corneas with a thin periphery in the recipient cornea. Our short-term results in this limited series of patients are encouraging. The extent of the corneal astigmatism after the removal of all sutures must be studied further. More studies with larger patient groups are needed to draw strong conclusions regarding the long-term effects of mushroom keratoplasty in KC patients.

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Part 2

Posterior lamellar keratoplasty

Chapter 3

Endothelial cell decay after Descemet Stripping Automated Endothelial Keratoplasty and Top-Hat Penetrating Keratoplasty

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ABSTRACT

Purpose: To analyze endothelial cell density (ECD) decay after Descemet's Stripping Automated Endothelial Keratoplasty (DSAEK) and top-hat keratoplasty (THPK) in patients with Fuchs' endothelial dystrophy (FED) and/or pseudophakic bullous keratopathy (PPBK).

Methods: Patients underwent either THPK (n = 33) or DSAEK (n = 39) at the Erasmus Medical Center, Rotterdam. For each non-randomized cohort a bi-exponential regression model for ECD decay was fitted. Factors associated with higher ECD decay were evaluated.

Results: Median follow-up was 31.2 months (range 11 - 91) in the THPK cohort, and 23.4 months (range 6 – 61) after DSAEK. The early ECD decay was much higher after DSAEK (half time 2.2 months) compared to THPK (half time 12.8 months). The late ECD decay after DSAEK was less steep (half time 75.5 months) than after THPK (half time 62 months). The 1-, 3- and 5-year endothelial cell losses derived from the models were after DSAEK: 56%, 66% and 73%, respectively; and after THPK: 24%, 50% and 64%, respectively. For both cohorts PPBK as indication for surgery was associated with significantly higher late-phase decay rates. Fuchs endothelial dystrophy and same-session cataract surgery were confounding variables in the DSAEK cohort. Regarding DSAEK, postoperative re-bubbling was not found to have significant effects on early or late ECD decay rates. However, limited sample size and other limitations related to our method of evaluation may have influenced these findings.

Conclusions: After DSAEK early ECD decay was stronger, as opposed to late decay that was faster after THPK and in case of PPBK.

INTRODUCTION

Penetrating keratoplasty (PK) has been the standard of care for treating endothelial failure for many years. The posterior mushroom or top-hat penetrating keratoplasty (THPK) is a type of PK with a stepped wound configuration, in which the posterior donor diameter of the transplant (and the recipient wound bed) is larger than the anterior donor and recipient diameter.[1] This technique has successfully been used for the treatment of Fuchs' endothelial dystrophy and pseudophakic bullous keratopathy.[2] Due to the large posterior diameter of the transplanted cornea, a higher percentage of diseased endothelium is replaced compared to standard PK. Also, the self-sealing posterior lip provides a better wound stability.

Currently, Descemet's Stripping Automated Endothelial Keratoplasty (DSAEK) has replaced PK as the gold standard surgical treatment for corneal endothelial disorders.[3] Its main advantages include the lack of sutures, less induced (irregular) astigmatism and hence a faster visual recovery. Furthermore, it is a safer technique with less risk for expulsive hemorrhage and no suture related complications. However, a main concern with this technique, especially regarding long-term results and long-term graft survival, remains the rate of postoperative endothelial cell loss. [4-6]

Recently Price et al[7] showed that overall graft success was comparable for DSAEK and PK procedures. However, the endothelial cell loss was higher after DSAEK at 6 and 12 months postoperatively, consistent with endothelial trauma caused by more donor tissue manipulation.[8] In this study, relative endothelial cell loss was found to be 34 ± 22 % for DSAEK vs. 11 ± 20 % for PK at 6 months postoperatively, and 38 ± 22 % vs. 20 ± 23 % at 12 months.

The first aim of our present study was to evaluate endothelial cell loss and analyze endothelial cell decay patterns in two groups of patients operated in our own institution (Department of Ophthalmology, Erasmus Medical Center, Rotterdam, The Netherlands). Patients underwent either THPK or DSAEK. We set out to fit bi-exponential endothelial regression models for endothelial cell decay, comparable with the biphasic models previously described by Armitage et al.[9] and Böhringer et al.[10] The second aim was to identify factors associated with faster or slower endothelial cell decay in both the early and late postoperative phases after both types of corneal transplantation. Therefore, additional analyses were performed to evaluate the effect of several pre-, intra- and post-operative factors on the decay rate coefficients of the early and late phases of both models.

METHODS

THPK has been performed in the Department of Ophthalmology of the Erasmus Medical Center Rotterdam since March 2003. DSAEK has been performed in our department since October 2004. After October 2004, indications for THPK were: endothelium-related corneal disorders with concomitant stromal opacities, endothelial disorders in patients without any crystalline lens opacities, and pseudophakic bullous keratopathies (PPBK) in case of an absent or ruptured posterior capsula (because of the risk of air dislocation to the posterior segment when performing DSAEK). Patients with persistent stromal and epithelial edema resulting in the formation of microcysts with or without bullae are both called PPBK. Only patients with persistent stromal edema were operated. Preoperative pachymetry measurements were not routinely performed.

The medical records of all patients that had undergone DSAEK or THPK, for either Fuchs' endothelial dystrophy (FED) or PPBK or both, at the Erasmus Medical Center, Rotterdam, The Netherlands, were reviewed. The few cases with other indications (e.g. re-graft) and/or more extensive concomitant pathology besides FED or PPBK were excluded from this study, as were cases with a completed follow up of less than 6 months were excluded. Our study includes the initial experiences with each respective surgical technique of both surgeons who started performing these techniques (GVR and MCB).

The main outcome measures in this study were endothelial cell densities (ECD), obtained at different (flexible) follow up time points. The endothelial cell densities had been prospectively and longitudinally measured after transplantation, using a Topcon SP3000P non-contact specular microscope and semi-automated endothelial cell analysis with Topcon Imagenet Endothelial Cell analysis software (Topcon Europe Medical B.V., The Netherlands).[11]

Two cohorts, DSAEK and THPK, were identified and data were separately analyzed for each cohort. Besides ECD, also surgeon, date of surgery, whether or not simultaneous cataract extraction had been performed (either with a routine phacoemulsification (MB) or a Blumenthal manual extracapsular technique (GVR)), and postoperative complications were recorded.

Cases with clinically manifest endothelial rejection episodes during the postoperative follow-up window were excluded before analysis.

DSAEK surgical technique

DSAEK cases were performed under either general or retrobulbar block anesthesia, by 2 surgeons (GVR and MCB). The surgical technique is in many ways comparable to the technique used by Price et al.[12] A superiorly located corneoscleral tunnel incision of 5.5 to 6.0 mm was used in all cases. The incision was sutured water-tight at the end of the procedure and covered with conjunctiva. In all cases, the donor posterior lamella

was cut intra-operatively from a corneoscleral donor disc, using a Moria Evolution 2 Microkeratome system with a 350 µm head on an artificial anterior chamber (Moria, Paris, France). All donor corneas had been preserved in organ culture medium at 31 degrees Celsius and were obtained from Bio Implant Service, Leiden, The Netherlands. A donor lamella of 8.5 mm was transplanted. In all cases, an anterior chamber maintainer was used during Descemetorhexis and during donor lamella insertion, to prevent anterior chamber collapse. A drop of viscoelastic substance (Healon®) was applied on to the donor endothelium prior to introduction of the donor lamella into the anterior chamber. No additional viscoelastic material was used. Donor insertion was performed with a pull-through technique using a prolene 10.0 nylon suture on Sabreloc Straight Transchamber needles (Ethicon, Auneau, France). The very first two donor insertions had been performed with forceps and in one later case the Busin glide and forceps were used.[13] Both cases with forceps insertion and the case with Busin glide insertion were excluded from this study before analysis, in order to provide a more homogeneous data set before analysis.

Additional DSAEK data

For the DSAEK group, other parameters included in the analysis were: indication for surgery (FED, PPBK, or both); concomitant cataract surgery with the DSAEK-procedure (yes or no); duration of high-pressure complete air fill (10 vs. 15 minutes) for donor attachment; suturing of the posterior lamella with one suture at 12 o'clock onto the anterior stroma (yes or no); late post-operative elevated intraocular pressure (IOP) (yes or no); whether or not a post-operative "re-bubbling" had been performed for (partial) donor detachment (yes or no); and a newly devised score to evaluate intra- and early post-operative manipulation of donor material. Using the "DSAEK-score", we evaluated intra-operative and post-operative donor lamella manipulation in DSAEK surgery. This score is an ordinal summation score, which had specifically been devised by us (GVR and MCB) for this purpose. In this score, points are added when more than normal manipulation of donor tissue occurred during preparation and insertion of the donor lamella. More points are added when post-operative "re-bubbling" procedures had to be performed for donor lamella detachment. (Table 1)

THPK surgical technique

All THPK cases were performed under general or retrobulbar block anesthesia by 2 surgeons (GVR and MB). The top hat surgical technique has been described in detail.[2] Either 16 interrupted, or 8 interrupted and 1 running 10-0 nylon sutures were used. No other intra-operative complications occurred in the included cases.

Table 1. DSAEK score

Microkeratome cutting problems	1
Insertion of donor lamella for a second time	1
Insertion of donor lamella more than 2 times	2
Manipulation needed for unfolding the donor lamella	1
Graft upside down, reversal of graft performed.	2
Remaining air bubble (partial fill) in AC after the procedure	1
Insufficient intraoperative adherence after initial air fill, further air fill	1
First postoperative re-bubbling	2
Second postoperative re-bubbling	3

(AC: anterior chamber)

Additional Top Hat data

Donor endothelial diameter was looked into; however all included cases had an endothelial diameter of 9.0 mm except 5 cases in which the endothelial diameter was slightly smaller (8.5 mm n=2; 8.75 n=3). Statistical evaluation of these 2 tiny subgroups was deemed not feasible.

Analyzed parameters included: suturing technique (16 interrupted vs. 8 interrupted plus 1 running suture), concomitant cataract surgery (same types as in DSAEK) during the THPK (yes or no), and later post-operative suture related procedures (either suture addition or complete suture removal; yes or no for any "suture procedure").

Statistical model

Longitudinal ECD-measurements (number of cells per square mm) were taken at various times within patients after cornea transplantation. In order to analyse ECD-decay over time a two-compartment exponential decay model was used. The parameters of that model were estimated using nonlinear mixed modelling (PROC NLMIXED of SAS, version 9.2, SAS, Gary, In). NL MIXED uses an improved maximum likelihood estimation method and can therefore deal with missing values; there is no need for imputing data.

The variable to be explained by that model is defined as the remaining ECD-fraction at time t in patient : ECD_{ti} / ECD_{0i}, with t measured in months. This fraction by definition equals 1 at time 0.

The two-compartment exponential decay model is specified as follows:

$$ECD_{ti} / ECD_{0i} = c_i \times \exp(at) + (1 - c_i) \times \exp(bt) + u_{ti}$$

For numerical purposes the term c_i is re-parameterized as a logistic function:

$$c_i = \exp(\delta_i) / (1 + \exp(\delta_i)),$$

so that $0 < c_i < 1$. The parameters $a < 0$ and $b < 0$ are the monthly decay rates (coefficients) that are assumed constant in time and between subjects. For each patient i the

term d_i is supposed to be drawn from a normal distribution with mean δ and variance V_d, representing the between-patient variability of the total decay functions. At each time t and within each patient i the residual term u_{ti} is supposed to be drawn from a normal distribution with mean 0 and variance V_u, representing the within-patient variability of ECD-fractions around the patient's own total decay function.

The parameters to be estimated are a, b, δ, V_d and V_u. A 95% reference interval for the c_i-terms across subjects is given by $\exp(\delta \pm 1.96\sqrt{V_d}) / (1 + \exp(\delta \pm 1.96\sqrt{V_d}))$.

The effect of possibly influencing factors, so-called explanatory variables x_i of patient i on his total decay function is exploratively analyzed by specifying the monthly decay rates a and b as linear functions of x_i:

$$a(x_i) = a_0 + a_1 x_i \text{ and } b(x_i) = b_0 + b_1 x_i.$$

Due to scarcity of data (small number of patients and small numbers of measurements per patient relatively to the number of parameters to be estimated), those effects are estimated for each explanatory variable x_i and for either parameter a and b separately.

RESULTS

Patient data

In the THPK-cohort eventually 33 cases were included (Table 2). In 6 out of 33 cases, concomitant cataract surgery was performed in the same session, either immediately prior to or during THPK surgery. In 11 cases only interrupted sutures were used, in the remaining 22, 8 interrupted and 1 running suture were used. In 5 out of 33 cases, late post-operative suture-related procedures (either suture addition or complete suture removal) were performed.

In our DSAEK cohort eventually 39 cases were included (Table 2). In 20 cases, concomitant cataract surgery was performed in the same session, immediately prior to DSAEK. In 7 out of 39 cases, complete air fill was performed for 15 minutes, in the remaining 32 cases, complete air fill was performed for 10 minutes. Suturing of the posterior lamella

Table 2. Patient data

Patient group	Diagnosis
THPK (n = 33)	FED (n = 17) PBBK (n = 10) FED/PBBK (n = 6)
DSAEK (n = 39)	FED (n = 22) PBBK (n = 10) FED/PBBK (n = 7)

occurred in 9 out of 39 cases. In 11 cases, one re-bubbling procedure was necessary, and in 2 more cases a second re-bubbling was performed; for purpose of statistical analysis we evaluated a group of 13 cases in which one or more re-bubblings were performed. Out of 39 cases, 21 cases had a DSAEK-score of 1. Seven cases had a score of 2, 8 cases had a score of 3, 2 cases had a score of 4, and 1 case had a score of 5. Postoperatively elevated IOP was observed in 6 out of 39 cases.

In our THPK cohort, in two cases a secondarily failed graft (not related to endothelial rejection) occurred: in one patient 4 years after transplantation and in the other patient at 2 years after transplantation. These cases were included in the model with an assumed last ECD of 0 cells/mm^2. In the DSAEK group, no secondary failed grafts occurred. Primary graft failures were not observed at all.

Median follow-up for the THPK cohort measured 31.2 months (range 11 - 91) and 23.4 months (range 6 – 61) for the DSAEK cohort.

Regression models of ECD decay and descriptive statistics

The estimated parameters of the global two-compartment exponential decay model are presented in table 3 for DSAEK and THPK.

$$ECD_{ti} / ECD_{0i} = c_i \times \exp(-at) + (1 - c_i) \times \exp(-bt) + u_{ti}$$

with $c_i = \exp(\delta_i) / (1 + \exp(\delta_i))$,

The estimated means and 95 % reference intervals of the c_i-terms across subjects for DSAEK and THPK are given by 0.472 (0.159-0.808) and 0.636 (0.447-0.791) respectively.

In DSAEK, a = -0.00918 is the estimated monthly decay-rate coefficient of the late phase, and b = -0.3102 is the estimated decay rate of the early phase. ECD decay half times for DSAEK were calculated as ln(2)/0.00918 = 75.5 months for the late phase and as ln(2)/0.3102 = 2.2 months for the early phase.

In THPK, a = - 0.01030 is the estimated decay rate of the late phase, and b = -0.04222 is the estimated decay rate of the early phase. ECD decay half times for THPK were calcu-

Table 3. Estimated parameters of the global two-compartments exponential decay model for DSAEK and THPK.

Parameter	DSAEK estimate (95 % CI)	THPK estimate (95 % CI)
a	–0.00918 (–0.01239 to –0.00598)	–0.01030 (-0.02114 to +0.000530)
b	–0.3102 (–0.4258 to –0.1945)	–0.04222 (-0.07873 to – 0.00571)
δ	–0.1135 (–0.4378 to 0.2108)	0.5585 (0.02073 to 1.0962)
V_d	0.6278	0.1542
V_u	0.001193	0.007536

(CI: confidence interval)

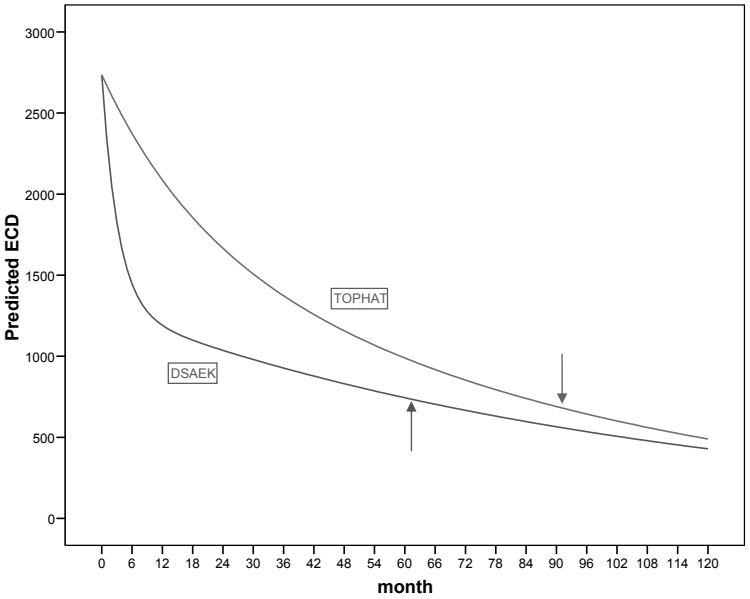

Fig 1. ECD decay graphs for DSAEK and THPK.
(Arrows indicate longest actual follow-up time in both groups; 91 months in the THPK group and 61 months in the DSAEK group.)

lated as ln (2)/0.01118 = 62.0 months for late phase, and as ln(2)/0.05401 = 12.8 months for early phase.

Using the models with estimated parameters *a, b* and *δ,* ECD-decay graphs depicted in Figure 1, starting at an average ECD-level set at 2736 cells/mm^2. Both graphs were extrapolated to a follow- up of 10 years postoperatively. Our longest actual follow-up durations were 91 months in the THPK group and 61 months in the DSAEK group.

From these extrapolated models it was calculated that on average our THPK cohort would reach the 500 cells/mm2-mark at 118 months postoperatively, and the DSAEK cohort at 103 months post-operatively.

Using the estimated models also the 1, 3 and 5-year endothelial cell loss can be predicted. The 1, 3 and 5-year endothelial cell loss after DSAEK measured 56%, 66% and 73%, respectively. The 1, 3 and 5-year endothelial cell loss after THPK measured 24%, 50% and 64%, respectively.

Possibly influencing factors

The effect of possibly influencing factors (explanatory variables) x_i of patient *i* on his total decay function was explorative analyzed by specifying the monthly decay rates *a (late phase)* and *b (early phase)* as linear functions of x_i:

$$a(x_i) = a_0 + a_1 x_i \text{ and } b(x_i) = b_0 + b_1 x_i.$$

These effects were estimated for each explanatory variable x_i and for either parameter *a* and *b* separately. For example, when $x_i = 0$ or 1, respectively denoting no or yes FED-indication, then a_0 is the late ECD decay rate without FED-indication and the sum $a_0 + a_1$ is the late decay rate with FED-indication, the difference in late decay rates being represented by a_1.

a) THPK

The effect of the indication FED for keratoplasty was neither significant on early- or late-phase ECD decay rates. However, when PPBK was the indication surgery, the decay rate of the late phase was significantly weaker (i.e., less negative) than that of non-PPBK THPKs (–0.00570 vs –0.01464; p=0.001), whereas PPBK as indication for surgery had no significant effect on the early phase decay rate. When PPBK combined with FED was the indication, again the late phase decay rate was significantly weaker (–0.00362 vs –0.01409; p = 0.0002); this was not so for the early phase decay rate.

Neither suturing technique nor late post-operative suture-related procedures had a significant effect on either early or late ECD decay rates.

Simultaneous cataract surgery during THPK had no significant effect on late phase ECD decay; however the early-phase ECD decay rate was found to be significantly weaker in cases with simultaneous cataract extractions (–0.02586 vs –0.05265; p= 0.0044).

b) DSAEK

When FED was the indication for DSAEK, the late phase ECD decay rate was significantly weaker (i.e., less negative) than in non-FED DSAEKs (–0.00852 vs –0.01962; p=0.0001). The effect of FED as indication for DSAEK on the early phase decay rate could not be estimated, probably because none of the 10 non-FED subjects had more than three visits. Late phase decay rate was significantly stronger (i.e., more negative) in PPBK DSAEKs compared to non-PPBK DSAEKs (–0.01524 vs –0.00784; p=0.0026); no significant effect of PPBK as indication for surgery was found on early phase decay. Simultaneous FED and PPBK as indication had no significant effects on either early or late phase ECD decay rates.

A difference in the duration of complete air fill for donor lamella adherence of 5 minutes (10 vs. 15 minutes complete fill) did not cause a significant difference in early or late ECD decay rates. Neither did the fact whether the donor lamella was sutured to the anterior recipient stroma or not.

Same-session cataract surgery (cataract surgery immediately prior to DSAEK) caused a significantly weaker late-phase ECD decay rate (–0.00785 vs –0.01512; p=0.0039), but no significant difference in early-phase ECD decay rate. (Late) post-operative IOP elevation had no significant effects on either late or early phase ECD decay rates.

The fact whether one or more re-bubbling procedure had to be performed to obtain definitive donor lamella adherence, was not found to have significant effects on either early or late phase ECD decay rates. Each additional point in our DSAEK score (i.e. small increases in the perceived amount of donor tissue manipulation before, during, and/or after donor lamella insertion) increased the ECD decay rate coefficients of both the early and late phases a little bit, but these effects were not significant.

DISCUSSION

In our study we were able to fit a single regression equation for endothelial cell decay to the complete data set of each technique. Our regression model took both repeated intra-patient measurements, and inter- and intra-patient variance into account. We did not have to use imputed data, such as was done in the study by Böhringer et al.[10] Even although the patients in our study had not been randomly assigned to one of each transplantation techniques - rather our cohorts were each other's historical control groups – comparing the different decay coefficients still allowed for in-depth comparison of endothelial cell decay between both groups.

Our results show that the early-phase postoperative endothelial cell loss in DSAEK is much higher and faster (decay half time was 2.2 months) compared to THPK (half time 12.8 months). This finding is in line with previously reported results, and is consistent with the conclusion that more endothelial trauma occurs in endothelial keratoplasty, due to more donor tissue manipulation.[14] The opposite is true for the late-phase endothelial cell loss; after DSAEK the late phase decay of endothelial cells was less steep than after THPK (Fig 1). Late phase ECD decay half time in DSAEK was 75.5 months vs. 62.0 months for late phase THPK half time. This also is consistent with earlier findings. [15]

Compared to other reported results on ECDs after DSAEK, the early phase endothelial cell losses in our study appeared to be higher. This may be explained by our learning curve; our data included the very first DSAEK cases performed in our center. In contrast, in our study no significant effect on ECD decay of more donor tissue manipulation (the "DSAEK score") could be demonstrated. However, it should be noted that we were only able to test the effect of a 1-point-increment on the DSAEK-score. Such a small increment in score in fact did result in a slightly higher decay rate coefficient (either early or late phase), but this was not a significant effect. Stronger effects of higher manipulation scores may be expected and were suggested by our raw data, but this could not be tested with our statistical analysis methods. We could also not demonstrate that re-bubbling procedures were detrimental to the endothelial cell decay rates. Similarly, other authors have also not been able to demonstrate higher endothelial cell losses after re-bubbling procedures.[16] The re-bubbling rate in our study was remarkably high

(up to 30%). Since these re-bubblings occurred predominantly in the first 15 cases, a learning curve in surgical technique and/or intra-operative donor tissue preparation is highly likely. There were no differences regarding donor tissue handling or preservation techniques in the non-attached cases.

In the THPK cohort, as expected, no relation was found between early or late endothelial cell loss rates and suturing techniques (continuous vs. interrupted). Late post-operative suture related procedures (additional suture placements or removal of all sutures) were also not found to be detrimental on ECD decay rates, possibly because no endothelial rejections or other major events such as dehiscence were induced.

Performing simultaneous cataract extraction caused a significantly lower early endothelial cell loss rate in THPK. This is not an easy finding to explain. Deepening of the anterior chambers may occur after recent cataract surgery, which in turn may lead to a decrease in early phase endothelial cell loss rate. Circumstantial evidence for this possible explanation can be found in increased endothelial cell loss when shallow anterior chambers after trabeculectomy surgery occur,[17] or increased endothelial cell loss after hypermetropic anterior chamber iris attached phakic IOLs,[18] or when glaucoma drainage tubing is in close proximity to the endothelium.[19] However, anterior chamber depths were not measured and hence this explanation is merely conjecture.

In both the DSAEK and THPK cohorts, the diagnosis of PPBK was associated with a significantly higher late phase endothelial cell loss rate. In addition, after DSAEK (but not after THPK) the late phase ECD decay rate was significantly lower when FED was the indication for surgery. In 20 of the 22 patients with FED, DSAEK was combined with same-session cataract extraction. The variable "intra-operative cataract surgery" therefore is highly confounded with the indication FED. Interpretation of separate effects of both variables on ECD-loss after DSAEK therefore is not possible.

These findings support the hypothesis that the often reported higher than physiological late–phase ECD loss after corneal transplantation might be caused by the phenomenon of endothelial cell redistribution onto the recipient cornea. Redistribution of endothelial cells onto the recipient cornea is supposedly stronger in cases of PPBK where the recipient endothelium has been largely or completely depleted, than in (phakic) cases with FED with better peripheral recipient endothelium. In the recent study by Böhringer et al., PKP for keratoconus (with healthy recipient endothelium), was shown to have lower late phase ECD losses than cases with PPBK. [10] These findings are also consistent with earlier findings that (PK) graft survival in PPBK is lower compared to keratoconus and FED.[15]

As Böhringer et al. pointed out, redistribution of donor endothelial cells to the recipient cornea is not only responsible for high late phase endothelial cell decay rate, i.e. for higher than physiological loss, but (hence) also for the development of Late Endothelial Failure of grafts. The concept of Late Endothelial Failure of a corneal graft was first

recognized by Polack [20] and defined by Bourne.[21] Bourne and Armitage described Late Endothelial Failure as a function of the higher than physiological ECD decay in the late phase after transplantation, and also of the level of ECD at which this higher than physiological decay starts.[10, 17] When initial ECDs are already low, increased decay will cause Late Endothelial Failure sooner. The results in our DSAEK cohort present us with some concerns in this respect.

The rate of endothelial cell loss tapered off more after DSAEK than after THPK. The reason for this difference between late endothelial cell loss rates is yet unknown. The inflammatory response might be less after DSAEK due to the smaller wound,[22] and this may be akin to the theory that chronic subclinical inflammation and/or a chronically broken-down blood-aqueous barrier may the cause of increased late cell loss after (TH) PK.[10] The differences between the wounds may also cause differences in mechanical stability of the globe and hence differences in mechanical forces causing endothelial attrition. Finally, the edge of the DSAEK onlay graft protruding into the anterior chamber might function as physical barrier that inhibits endothelial cell migration onto the recipient.[23] Endothelial cell migration does occur after DSAEK, as was recently demonstrated by Stewart et al.,[24] but due to this physical barrier it may be slower and more fragile than after PK. After THPK such a physical barrier for endothelial migration may be less of a factor and the endothelial cells may migrate more freely.

In the near future, analyses of longer-term results of other recent studies published on ECD loss after DSAEK[15] will further elucidate patterns of prolonged increased endothelial cell loss and the rate of Late Endothelial Failure after DSAEK. It will be interesting to also investigate late phase endothelial cell loss rates and Late Endothelial Failure rates after the newly emerged Descemet's Membrane Endothelial Keratoplasty (DMEK) technique. After DMEK, early endothelial cell loss due to pre-operative and intra-operative tissue manipulation appears to be similar compared to DSAEK,[25] but much less of a physical barrier for late endothelial migration may be present than after DSAEK.[26]

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Chapter 4

A posterior Khodadoust line in a graft rejection episode after Descemet Stripping Automated Endothelial Keratoplasty

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Cornea 2011; 30:245-246.

ABSTRACT

Purpose: To describe the presence of an endothelial rejection line in immunological graft rejection after Descemet Stripping Automated Endothelial Keratoplasty (DSAEK).

Methods: Case report.

Results: A 77-year-old woman underwent DSAEK procedure of the left eye because of visual limiting bullous keratopathy. One week postoperatively, the donor lamella was partially detached and an additional air bubble was injected into the anterior chamber. Thereafter, complete adherence of the lamella was seen. Four months after surgery vision deteriorated and a transient immunological graft rejection episode with endothelial rejection line was seen.

Conclusion: In contrast to other reports, an endothelial rejection line (Khodadoust line) can be seen during the endothelial rejection episode after DSAEK.

INTRODUCTION

Microkeratome-assisted Descemet Stripping Automated Endothelial Keratoplasty (DSAEK) is a form of lamellar corneal surgery that allows for the selective replacement of diseased endothelium. The most common complications after DSAEK include posterior graft dislocations, primary graft failure, iatrogenic glaucoma, and endothelial graft rejection.[1] A recent study compared immunological graft rejection episodes after DSAEK and penetrating keratoplasty; no endothelial rejection line (Khodadoust line) was observed in 54 eyes that experienced a graft rejection episode after DSAEK.[2] We present a case of an immunological graft rejection episode four months after DSAEK with an endothelial rejection line.

CASE REPORT

A 77-year-old woman was referred to our cornea clinic with complaints of reduced visual acuity of the left eye. Biomicroscopy showed endothelial guttata in both eyes and central corneal edema in the left eye. Funduscopy revealed no abnormalities. Her ocular history included a refractive amblyopia of the left eye, a cataract extraction in both eyes with Worst claw lens implantation in the right eye, and a standard posterior chamber intraocular lens in the left eye. The best-corrected visual acuity of her right eye was 0.4 and of her left eye was 0.16. A DSAEK was performed under general anesthesia at the left eye. Endothelial cell count of the donor cornea measured 2700 cells/mm^2. The patient was asked to use dexamethasone phosphate 0.1% (Dexamethason) eye drops 6 times daily for 2 months and chloramphenicol 0.5% (Chloramphenicol) eye drops 3 times daily for 1 week.

One week postoperatively, the posterior lamellar graft was detached; an additional air bubble was injected into the anterior chamber 13 days postoperatively. Thereafter, complete adherence of the lamella was seen. Four weeks after the DSAEK-procedure, visual acuity measured 0.2 with a spherical correction of +2 diopters (D). The patient was asked to continue the dexamethasone eye drops 6 times a day. The patient was referred to her own ophthalmologist for further follow-up visits.

Our patient was instructed to use the dexamethasone phosphate 0.1% eye drops 6 times daily for the first 8 weeks postoperatively, and then switch to 4 times daily. Hereafter, drops are tapered off by 1 drop per month until 1 drop a day is reached. We advise patients with pseudophakia to use 1 drop a day lifelong.

Four months postoperatively, the patient was again referred to our department with progressive blurring of vision and a mild irritation of the left eye. Visual acuity of the left eye was hand movements only. Slit-lamp examination showed severe and extensive

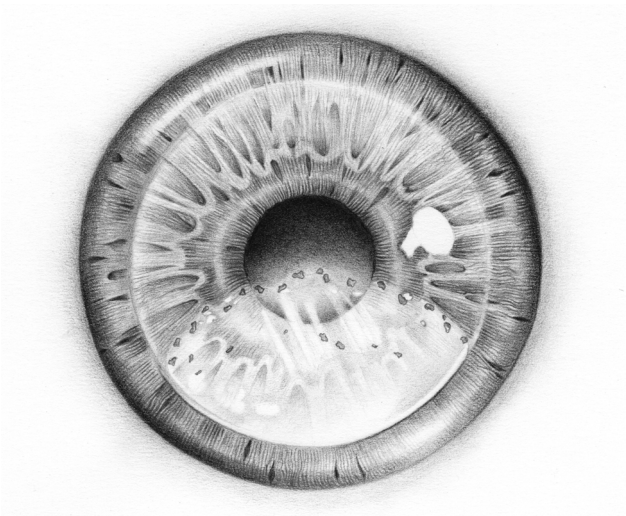

Fig. 1. Khodadoust line
(See also Colour Figures, p. 140.)

stromal edema and keratic precipitates, limited to the graft, forming a typical posterior Khodadoust line (Fig 1).[3] The posterior lamella was attached. The patient revealed that she had been avoiding further follow-up visits with her own ophthalmologist and had discontinued the dexamethasone eye drops 4 weeks after the DSAEK. Therapy with prednisolone acetate 10% (Pred Forte) eye drops 6 times daily, atropine 1% once daily, and prednisolone pivalate 5% (Ultracortenol) eye gel at night time was started. One week later, the Khodadoust line had disappeared; stromal edema and keratic precipitates were still present. One month later, the corneal graft partially cleared with stromal edema at the central 1.5 mm. Best-corrected visual acuity measured 0.2 (S +2D).

CONCLUSION

In our patient, the DSAEK was complicated by an immunological graft rejection episode with endothelial rejection line (Khodadoust line). The site of origin of an endothelial rejection line has been associated with the vascularization of the cornea.[4] Jordan et al[2] attributed the absence of endothelial rejection lines in his group of patients to the lack of stromal vessels extending into the graft tissue. They suggested that the immunological rejection process in DSAEK might be initiated via the anterior chamber instead of stromal vascularization. In our patient, no vascularization was present. When steroids are suddenly stopped shortly after surgery, the few lymphoid cells in the aqueous might multiply and form an endothelial rejection line. Our case illustrates that an endothelial rejection line can be observed after DSAEK, independent of graft vascularization.

We would like to emphasize the importance of clear patient instructions concerning the use of eye drops and possible rejection signs, especially when topical steroids are tapered off. In general, initial graft rejection leads to mild symptoms only, which can contribute to an important patient delay.

In conclusion, we describe a case of immunological graft rejection with endothelial rejection line 4 months after DSAEK. Contrary to our instructions, the patient had stopped using the steroid eye drops at 1 month postoperatively, and she did not visit her own ophthalmologist.

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Chapter 5

Introduction of epithelial cells in the flap-graft interface during Descemet Stripping Automated Endothelial Keratoplasty

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Arch Ophthalmol 2009; 127:936-7.

INTRODUCTION

Microkeratome-assisted Descemet Stripping Automated Endothelial Keratoplasty (DSAEK) is a form of lamellar corneal surgery that allows for the selective replacement of diseased endothelium. [1-3] The DSAEK procedure is relatively new. So far, few complications have been described; these include graft detachment and graft failure.[4] Recently, epithelial ingrowth in the flap-graft interface after DSAEK has been reported.[5] We report a case of introduction of epithelial cells, originating from the donor tissue, in the flap-graft interface.

REPORT OF A CASE

In May 2005, a 67-year-old woman visited with pseudophakic bullous keratopathy in the right eye. Best-corrected visual acuity was 0.08 OD. Because of the vision-limiting bullous keratopathy in the right eye, a DSAEK was performed under general anesthesia. A cornea from a male donor was used.

One week postoperatively, the posterior lamellar graft was detached inferiorly and nasally. An additional air bubble and two 10-0 nylon sutures were placed to fixate the transplant. Thereafter, partial adherence of the lamella was seen.

Four months postoperatively, the graft was attached and best-corrected visual acuity improved to 0.63 OD, with −1.00. Between the 3-o'clock and 6-o'clock positions, anterior synechia were present and the interface was slightly hazy. During regular follow-up, the abnormalities remained stable under fluorometholone, 1%, eye drops. The intraocular pressure remained between 10 and 17 mmHg during follow-up.

One year after DSAEK, the patient had progressive blurring of vision to hand movements. We observed total corneal decompensation with bullae and Descemet membrane folds. Therapy with prednisolone acetate, 10%, eye drops (Pred Forte) 6 times a day, prednisolone pivalate, 5%, eye gel (Ultracortenol) 3 times a day, and preservative-free chloramphenicol, 0.4%, eye drops 3 times a day proved to be unsuccessful. A penetrating posterior mushroom keratoplasty was performed. Six months after surgery, best-corrected visual acuity improved to 0.80 OD with -3.25 C-2.0 x 30°. The corneal graft in the right eye has remained clear.

Histopathologic examination of the removed corneal button demonstrated 2 cysts at the interface between the recipient cornea and the donor stroma (Fig 1A). One cyst was lined by squamous epithelium; a smaller, centrally located cyst contained eosinophilic granular material. The Descemet membrane with some excrescences was incarcerated in the surgical scar. There was bullous keratopathy of the corneal epithelium. On immunohistochemistry, the lining of the cyst as well as the granular material stained

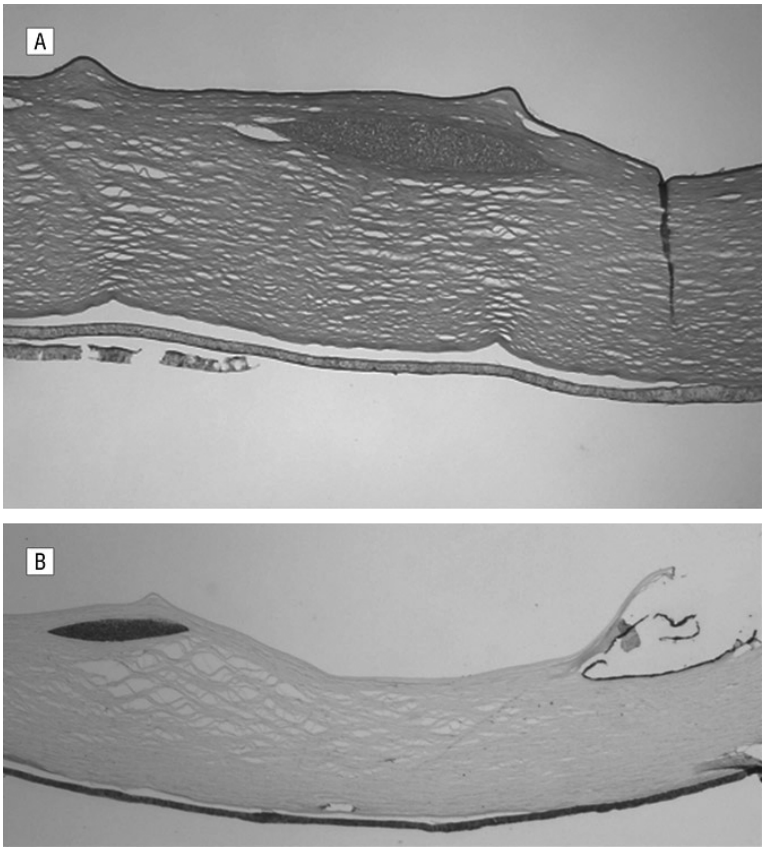

Fig 1. There are two cysts at the interface between the recipient cornea and the posterior endothelial graft tissue (A), and the lining of the cysts stains strongly positive for the epithelial markers 34Be12 and keratin 5/6 (B).
(See also Colour Figures, p. 141.)

strongly positive for the epithelial markers 34Be12 and keratin 5/6, identical to surface epithelium (Fig 1B). The granular material was consistent with degenerated epithelial cells. In situ hybridization with X and Y probes revealed 2 X chromosomes in the nuclei of the epithelium and the stroma of the recipient. The nuclei of the epithelium of the cyst wall contained 1 X chromosome and 1 Y chromosome (Fig 2).

COMMENT

In our patient, the DSAEK was complicated by introduction of epithelial cells along the interface. Her native Descemet membrane with Fuchs endothelial dystrophy was partially incarcerated in the surgical scar, similar to the earlier described case,[5] suggest-

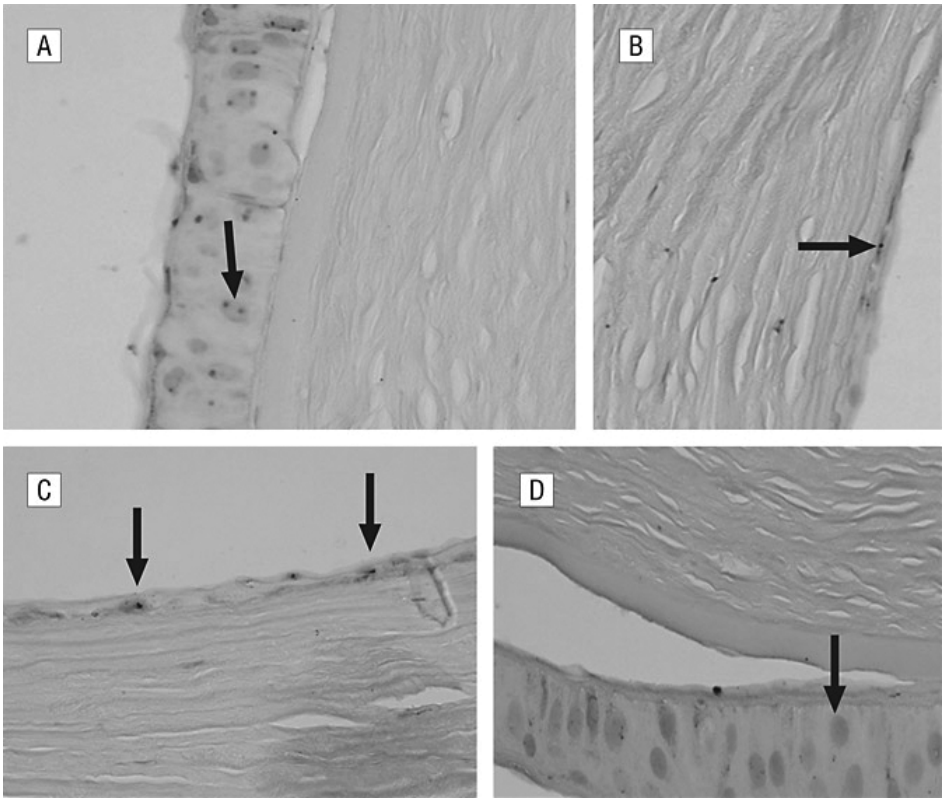

Fig 2. In situ hybridization with X and Y chromosome probes revealed 2 X chromosomes (arrow) in the epithelium and the stroma of the recipient (A) and 1 X chromosome (arrow) in the epithelium of the cyst (B). C, The epithelium of the cyst wall contained 1 Y chromosome (arrows). D, There was no staining of the Y chromosome probe (arrow) in the tissue of the recipient.
(See also Colour Figures, p. 142.)

ing an invasive postoperative ingrowth process. The X-Y karyotyping revealed that the epithelial cells were of donor origin. The technique of posterior lamellar keratoplasty is relatively new and technically difficult, with a surgeon's learning curve. The most critical step is the preparation of the posterior lamellar disc by hand or by use of a microkeratome.

We postulate that the donor epithelium was implanted during the preparation of the donor posterior lamellar disc and was introduced intraoperatively. If complete attachment of the donor posterior lamella is accomplished, ectopic epithelial cells in the interface might remain stable without proliferation. In a partially detached donor lamella, ectopic epithelial cells might proliferate along the recipient's posterior cornea.[6]

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Part 3

Intracorneal implants and corneal cross-linking

Chapter 6

Refractive, topographic and visual outcome of same-day corneal cross-linking with Ferrara intracorneal ring segments in patients with progressive keratoconus

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ABSTRACT

Purpose: To report refractive, topographic and visual outcomes 12 months after same-day treatment with corneal cross-linking and Ferrara intracorneal ring segments (ICRS) in eyes with progressive keratoconus (KC) and contact lens intolerance.

Methods: This was a case serie of 7 eyes (7 patients) with progressive mild to moderate KC and contact lens intolerance undergoing implantation of Ferrara ICRS immediately followed by corneal cross-linking. Uncorrected visual acuity, best spectacle-corrected visual acuity, refractive error, pachymetry and keratometry were evaluated at 3, 6 and 12 months of follow-up.

Results: Mean follow-up was 11.7 ± 3.6 months (range, 5 – 17 months). Mean preoperative uncorrected visual acuity and best spectacle-corrected visual acuity were 0.10 ± 0.07 (range, 0.05 - 0.2) and 0.56 ± 0.08 (range, 0.5 - 0.7), respectively. One year after treatment, mean uncorrected visual acuity and best spectacle-corrected visual acuity measured 0.60 ± 0.24 (range, 0.32 – 0.9) and 0.82 ± 0.25 (range, 0.5 - 1.2), respectively. The mean spherical equivalent decreased significantly ($p < 0.05$) with 3.5 D. Mean K-values decreased from 46.81 ± 2.13 D (range, 44 – 51 D) to 43.97 ± 2.22 D (range, 42 – 47.5D), 1 year after treatment. The average preoperative thinnest pachymetry measured 462 ± 46 µm (range, 410 ± 546 µm) and did not change significantly after treatment. In patient 1, the inferior ICRS was removed 5 months postoperatively because of implant migration.

Conclusion: The combined treatment of corneal cross-linking and Ferrara ICRS is a safe procedure that may be considered in patients with progressive mild to moderate KC and contact lens intolerance.

INTRODUCTION

Keratoconus (KC) is a non-inflammatory corneal ectasia, which usually starts at puberty and progresses until the third to fourth decade of life. In the early stages, the refractive error resulting from the KC can be corrected with glasses or contact lenses. In more advanced forms of KC or in case of contact lens intolerance, several surgical options are available to improve visual acuity, including intracorneal ring segments (ICRS),[1] toric phakic intraocular lens implantation,[2] deep anterior lamellar keratoplasty (DALK)[3] or mushroom keratoplasty.[4] However, none of these surgical procedures effectively treat KC, and progression of the cone might continue.

Ferrara ICRS have been shown to improve contact lens tolerance and visual acuity in a select group of patients with mild to moderate KC.[5] However, long-term follow-up shows that ICRS fail to provide a permanent flattening effect.[6] In 2003, Wollensak et al[7] introduced collagen cross-linking (CXL) by the photosensitizer riboflavin and ultraviolet (UV) A-light as the first treatment that changes the intrinsic biomechanical properties of corneal collagen. To obtain both the visual benefit of the Ferrara ICRS and the potential stabilizing effect of CXL on corneal ectasia, we combined these two treatment procedures. We evaluated refractive, topographic and visual outcomes at 3, 6 and 12 months after treatment in eyes with progressive mild to moderate KC with contact lens intolerance.

MATERIALS AND METHODS

Patients

Between November 2007 and November 2008, 7 eyes of 7 patients underwent UVA-induced CXL immediately after Ferrara ICRS implantation on the same day. All patients had a progressive KC with contact lens intolerance, an average keratometry of less than 53 diopters (D), and a best spectacle-corrected visual acuity (BSCVA) more than 0.4. We defined progression as a change in astigmatism or myopia of 1 D or more or a mean keratometric change of 1.5 D or more during the past 12 months. Patients with corneal scarring, corneal thickness less than 400 µm, and a mesopic pupil diameter larger than 5.5 mm were excluded. The surgical procedure and follow-up examinations were performed by the same two surgeons (M.C.B. and G.V.R.) at the Erasmus University Hospital Rotterdam. All patients were examined preoperatively and 3, 6, and 12 months after treatment using Javal keratometer (Haag Streit, Bern, Switzerland) and standard Snellen chart. Every 6 months, corneal topography was performed using the Pentacam Scheimpflug camera (Oculus Optikgeräte GmbH, Wetzlar, Germany). The Student *t*- test for paired data was used to compare preoperative and postoperative results.

Treatment procedure

The treatment procedure was conducted under sterile conditions in the operating room. Conservative-free oxybuprocaine eye drops (Chauvin Benelux, Brussels, Belgium) were applied as topical anesthesia. A circular Ferrara marker centered on the reflex of the microscope light on the central cornea was used to create two concentric circles on the cornea. The incision was made at the steepest meridian of the anterior corneal surface with a calibrated diamond knife set at 80% of the corneal thickness determined by Scheimpflug scanning slit tomography. Corneal pockets were then created with a spreader hook. One semicircular dissector was placed sequentially in the lamellar pocket and steadily advanced by rotational movement (counterclockwise and clockwise dissectors). After creation of the tunnels, the ICRS were inserted in the tunnels. Depending on the nomogram (Keraring, Mediphacos, Belo Horizonte, Brazil), one or two PMMA segments with variable thickness (150-250) were implanted in the corneal stroma.[1]

After the ICRS were implanted, riboflavine 0.1% solution in 20% dextran (Ricrolin, SOOFT Italia SRL, Montegiorgio, Italy) was applied every 3 minutes for 25 minutes on the cornea and injected in the intrastromal canals. Riboflavin penetration of the stroma was confirmed at the slitlamp. The central 8.5-mm cornea was then irradiated with UVA-light diodes (370 nm) for 30 minutes using 3mW/cm^2. During the irradiation, riboflavine was further applied every 3 minutes. Finally, a therapeutic soft contact lens was applied. Postoperative medications included preservative free diclofenac sodium 1 mg/ml, ofloxacine 3 mg/ml and dexamethason monofree 1mg/ml 3 times daily. Once complete reepithelialization occurred, the contact lens was removed and ofloxacine eye drops were discontinued. Diclofenac sodium eye drops were discontinued after one week, and dexamethason eye drops were tapered off by one drop weekly.

RESULTS

Seven eyes of 7 patients were included. The mean age of the patients was 33.31 ± 9.05 years (range, 20 – 42 years). Mean follow-up was 11.72 ± 3.6 months (range, 5 - 17 months). Ferrara ICRS implantation was uneventful in all eyes. Table 1 shows data of implanted Ferrara ICRS in each patient.

Patient 1 underwent explantation of the inferior ring segment 5 months postoperatively. Four months after treatment, the patient reported irritation in his left eye. Little migration of the inferior ring towards the incision was seen. There was a small epithelial defect at the site of the incision. Although the ring segment was not wholly extruded, we decided to explant the ring. Three months after explantation of the inferior ring, mean K-value increased with 1.7 D and remained stable until now. Compared with the

Table 1: Data of the implanted Ferrara ICRS in each patient.

Patient	Steep K (D)	Flat K (D)	Inferior ring (µm)	Superior ring (µm)
1	52.6 * 60°	47 * 150°	250	150
2	46 * 10°	42 * 100°	200	200
3	48 * 120°	45 * 25°	150	150
4	49,5 * 40°	48 * 140°	150	150
5	47 * 40°	42 * 130°	200	200
6	48.25 * 160°	44,5 * 40°	200	
7	50.5 * 30°	45 * 120°	200	200

(D; diopters)

Table 2. Pre-and postoperative data.

	Pretreatment	3 Months	6 Months	12 Months
UCVA	0.10 ± 0.07	0.40 ± 0.18	0.48 ± 0.29	0.60 ± 0.24
BSCVA	0.56 ± 0.08	0.53 ± 0.23	0.68 ± 0.22	0.82 ± 0.25
SE	-4.16 ± 2.41	-1.67 ± 1.81	-1.79 ± 1.79	-0.68 ± 1.49
K steep	48.84 ± 2.23	44.79 ± 1.30	45.73 ± 2.24	45.21 ± 2.08
K mean	46.81 ± 2.13	44.77 ± 1.66	45.01 ± 2.34	43.97 ± 2.22
K astigm	4.05 ± 1.48	2.11 ± 1.46	1.44 ± 1.11	1.58 ± 0.85

(UCVA; uncorrected visual acuity, BSCVA; best spectacle-corrected visual acuity, SE; spherical equivalent, K; keratometry) Data are means ± standard deviation.

preoperative data, mean K and steep K-values were decreased by 1 and 3 D, respectively. The corneal pachymetry did not change until now.

Mean baseline uncorrected visual acuity (UCVA) and BSCVA were 0.10 ± 0.07 (range, 0.05 – 0.2) and 0.56 ± 0.08 (range, 0.5 – 0.7), respectively. One year after treatment, mean UCVA significantly improved to 0.60 ± 0.24 (range, 0.32 - 0.9) and BSCVA to 0.82 ± 0.25 (range 0.5 – 1.2). Three of our patients wear contact lenses, with a best contact lens-corrected visual acuity of 0.87 ± 0.12. One patient was still contact lens intolerant, and two patients had no correction due to excellent UCVA. The safety index (ratio BSCVA postoperatively to BSCVA preoperatively) in this study measured 1.47 after one year. None of the treated eyes had a decrease in visual acuity. The mean preoperative spherical equivalent (SE) was – 4.16 ± 2.41 D (range 0.25 to –5.5D). One year after treatment, mean SE measured -0.68 ± 1.49 D (range 0.25 to -3.0D). Pre- and postoperative keratometric results are shown in table 2. One year after treatment, the decrease in mean K and steepest K-values were 3.0 D ($p > 0.05$) and 3.6 D ($p < 0.05$), respectively. Q-value obtained by the Pentacam software, changed from -0.76 preoperative to -0.30 one year after treatment.

DISCUSSION

This prospective study describes the combination of Ferrara ICRS implantation with CXL as a safe and effective treatment option in patients with progressive KC and contact lens intolerance. The achieved visual and refractive results remained stable during follow-up.

In all 7 eyes presented in this study, an increase in UCVA was obtained. Three months postoperatively, the mean UCVA improved significantly and remained stable. Compared with the few studies published on Ferrara ICRS, mean UCVA in our 7 cases increased with more Snellen lines after combined treatment than after Ferrara ring segments alone.[1,5,8] None of the patients in our small study group is considered for corneal transplantation.

Our results on visual acuity are in line with the reported results of combined treatment of CXL and Intacs.[10,11] However, our keratometric results are more favorable; we obtained a significant reduction of 3.6 D in steep K-value and a significant reduction of 2.4 D in keratometric astigmatism one year after treatment. Chan et al[10] reported an average reduction of 1.9 ± 1.32 D in steep K and 1.34 ± 1.27 D in average K values 3 months after CXL combined with Intacs. Ertan et al[11] showed an average reduction in keratometry of 2 D after CXL (without epithelial abrasion) combined with Intacs implantation. The larger flattening effect in our patients might be attributed by two factors. First, Ferrara ICRS provide a smaller optical zone of 5 mm, whereas Intacs are designed for an optical zone of 7 mm. Insertion closer to the center could theoretically induce a more flattening effect. Comparative studies between Intacs and Ferrara ICRS are necessary to prove that the greater reduction in keratometry readings in our study can be attributed to the type of ICRS. Secondly, El-Raggal[12] showed in his comparative study that a significant greater reduction of keratometric values is obtained when ICRS insertion and CXL are performed in one session compared to two sessions. There was no significant difference regarding the UCVA, BCVA and refractive error.

The long-term stability of visual acuity and mechanical flattening of Ferrara ICRS has been recently studied.[13] Intacs were used in previous reported long-term studies of ICRS; Alió showed an increase in keratometry readings between 6 and 36 months postoperatively.[6] In our study group, no significant change in keratometry values between 6 and 12 months postoperatively could be observed. Moreover, a further decrease of keratometry values between 6 and 12 months postoperatively is seen in our study group, although it was not significantly different in this small group of patients.

An alternative combination treatment focused on structural and refractive improvement, is simultaneous topography-guided PRK and CXL.[14,15] However, long term outcome is yet unknown. In contrast to PRK, intracorneal ring segment implantation is reversible.

Regarding postoperative complications of ring implantation, several events have been described; ring extrusion and migration,[1,5,16] channel deposits,[17] infectious keratitis[18] and corneal melting.[19] We only found one ring migration in patient with extreme eye rub-

bing. During our follow-up, no other complication of CXL or ring implantation was seen. We hypothesize that the CXL procedure immediately after ICRS implantation might be attributable to a more stable ring placement with less migration of the ring segments.

In these seven patients, we did not use any eye drops with benzalkonium chloride, neither did we perform epithelial abrasion before CXL treatment. However, epithelial defects were proven at the ring implantation side and the tunnels were irrigated with riboflavin.

In conclusion, both visual and keratometric results after combined CXL and Ferrara ICRS implantation are encouraging. However, longer follow-up and larger patient groups are necessary. Our findings show that a combined procedure of CXL and Ferrara ICRS implantation is a safe treatment option in progressive moderate KC to postpone or avoid corneal transplantation.

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Chapter 7

Long-term follow-up of hydrogel intracorneal lenses in two aphakic eyes

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INTRODUCTION

We report the outcome of hydrogel intracorneal lens implantation in two patients. The lenses were implanted at approximately 50% depth in the cornea to correct high hyperopic refractive errors of 10.5 diopters (D) and 14.0 D, respectively. Both patients were contact lens intolerant and not suitable for intraocular lens (IOL) implantation. Surgery was performed in 1988, and the patients were followed until early 2010. The patients showed good tolerance for the intracorneal lenses, but both developed opacities around the implant, leading to reduced visual acuity in one patient. Long-term patient monitoring is essential since corneal opacities can develop after many years. Removing the implant is not necessary as the lens can easily be rinsed by lifting the corneal cap.

In 1966, Barraquer[1] proposed synthetic intrastromal implants to alter the anterior corneal curvature as a way to correct refractive errors. However, his results were unsatisfactory because of the impermeable nature of the implant material. New materials such as hydrogel showed biocompatibility and good corneal tolerance when implanted in primates.[2] Hydrogel lenses are permeable to water and nutrients, so normal corneal physiology can be maintained. Because of its refractive index (1.376), the lens has no refractive power on its own within the cornea. The refractive effect is achieved by alteration of the corneal shape. Another advantage is that implantation is reversible. Hydrogel lenses were soon superseded by laser refractive surgery, and their use declined. However, the incidence of severe complications is higher after hyperopic laser in situ keratomileusis (LASIK) than after myopic LASIK.[3] In this article, we report two cases of hydrogel intracorneal lens implantation in aphakic eyes. Both patients were considered unsuitable candidates for intraocular lens implantation at that time.

CASE REPORT

Surgical Technique

The hydrogel intracorneal lens is made of lidofilcon A. This material has a water content of 68% and is therefore permeable to glucose. A 9.0 mm corneal cap is created with a microkeratome with a 300 µm depth plate. The interface is not irrigated; excess hydration is removed with a sponge. The implant is placed on the stromal bed and centered on the nondilated pupil. The corneal cap is sutured with 16 interrupted 10-0 nylon sutures. The sutures are adjusted under keratoscopic control to reduce astigmatism. Postoperative treatment consists of antibiotic and corticosteroid eye drops.

Case 1

A 38-year-old man experienced a penetrating injury with a foreign body through the cornea and lens of his left eye in 1981. The foreign body was removed through the pars plana, and extracapsular lens extraction with anterior vitrectomy was performed. One year later, surgery for a rhegmatogenous retinal detachment was performed successfully. The patient was contact lens intolerant. The corrected distance visual acuity (CDVA) was 20/25 with + 10.25 C -1.25 x 20. In 1988, a hydrogel intracorneal lens of + 10.50D with a diameter of 5.5 mm was implanted in the left cornea. On the second postoperative day, the uncorrected distance visual acuity (UDVA) and CDVA were 20/40 and 20/20 with + 1.0 C -1.0 x 120, respectively. Six months postoperatively, the CDVA was 20/20 with plano C -1.50 x 25. From the first postoperative day, the cornea was clear and the epithelium intact. At further follow-up visits, visual acuity and keratometry remained stable.

Six years after implantation, reticular opacities anterior and posterior to the implant appeared. However, visual acuity remained stable. At 13 years, the opacities increased and the CDVA declined to 20/60. The corneal cap was partially reopened, and irrigation was performed anteriorly and posteriorly to the implant to remove the opacities. The corneal cap was closed without suturing. After the procedure, the cornea was clear and visual acuity increased to 20/20. The removed substance was analyzed. It consisted of amorphous crystalline structures. No classification was possible.

Over the next 9 years, visual acuity slowly declined to 20/60. Biomicroscopy showed recurrence of the stromal opacities. No epithelial thinning or defects were observed during the 22 years of follow-up. In vivo confocal microscopy (Confoscan 4, Nidek, Inc.) was performed 22 years postoperatively, according to a previously described method. Corneal backscatter analysis was calibrated using a turbidity standard (AMCO Clear, GFS Chemicals, Inc.). The linear relationship between turbidity and image intensity made it possible to convert image intensity to backscatter expressed in "scatter units." Increased backscatter was seen in both interfaces and the anterior stroma (Fig 1). Total corneal thickness was 789 mm, including the 225 mm thick hydrogel intracorneal lens. The depth of implantation was 272 mm and the endothelial cell count (ECC), 1200 cells/mm2.

Case 2

A 42-year-old man was aphakic after congenital cataract extraction when he was three years old. Iridolenticular adhesions remained after the extracapsular extraction. He was contact lens intolerant. The CDVA was 20/50 (amblyopia) with + 11.50 C -2.0 x 105. In 1988, a + 14.00 hydrogel intracorneal lens of 5.5mm diameter was implanted in the left cornea. On the second postoperative day, the CDVA was 20/80 with +3.0 C -1.50 x 100. Six weeks postoperatively, it was 20/50 with and without correction (+2.0 C -0.50 x 90). From the first postoperative day, the cornea was clear and the epithelium intact.

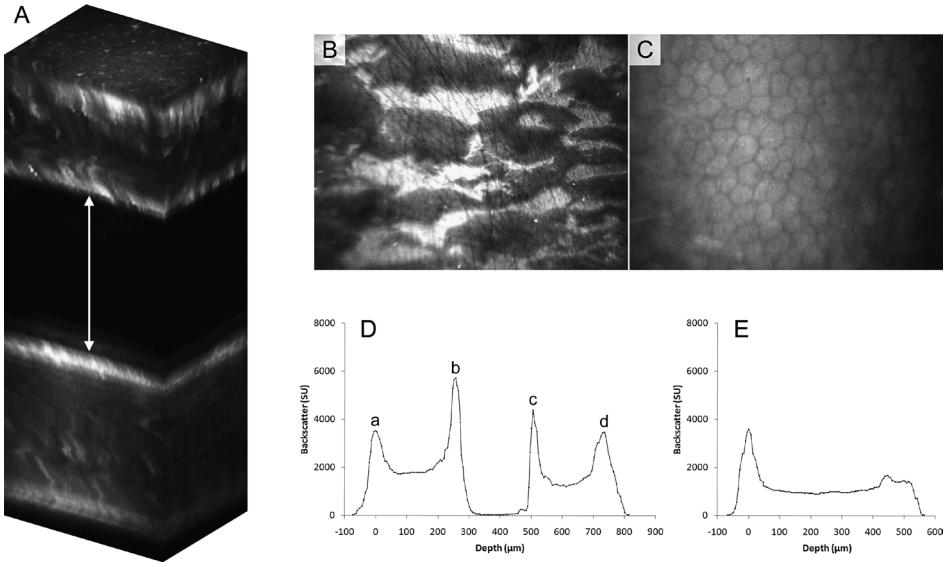

Fig. 1. Case 1: In vivo confocal microscopy performed 22 years after intracorneal hydrogel lens implantation.
A: The 3-dimensional stack of a complete corneal pass composed of 149 confocal images with approximately 6 µm intervals is shown. The intracorneal hydrogel lens (double arrow) was interspersed between the reflective anterior and posterior stroma - lens interfaces. **B:** After debridement, the anterior stroma - lens interface had a crackled aspect and multiple small scratches on the lens surface. **C:** There was endothelial cell loss (1200 cells/mm2) in the hydrogel lens - implanted eye. **D:** Four peaks could be identified in the backscatter profile of the hydrogel lens - implanted eye (a = endothelium; b = posterior interface; c = anterior interface; d = anterior stroma). **E:** The backscatter pattern in the fellow-eye was normal.

Fourteen years later, subtle corneal opacities developed anterior and posterior to the intracorneal lens. The CDVA and refraction remained stable until early 2010. No epithelial thinning or defects were observed during the entire follow-up period. Confocal imaging was performed 22 years postoperatively (Fig 2).

The backscatter profile was similar to the one in Case 1, although there was more reflectivity in the anterior interface and subepithelial layers. The thickness of the lens was 225 mm, the implantation depth 385 mm, the corneal thickness 904 mm, and the ECC 3500 cells/mm^2. Table 1 summarizes the confocal microscopy results.

DISCUSSION

The 22-year follow-up in these 2 patients shows that intracorneal lens implantation can be a safe treatment option in aphakic patients. Today, Artisan (Ophtec BV) IOL implantation would be considered for the first patient. In the second patient, implantation of

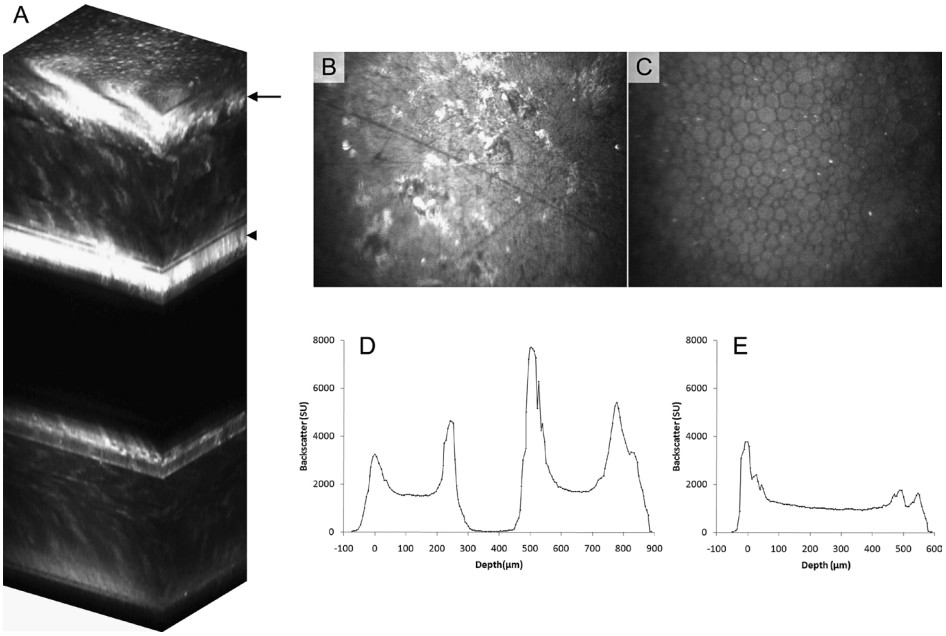

Fig 2. Case 2: In vivo confocal microscopy performed 22 years after intracorneal hydrogel lens implantation.
A: The 3-dimensional stack composed of 173 images showed pronounced backscatter of the anterior stroma (arrow) and the anterior interface (arrowhead). **B:** There was cellular debris in the posterior stroma - lens interface and minute scratches on the lens surface. **C:** The morphology of the endothelial cells in the hydrogel lens - implanted eye was normal. Endothelial cell density was higher than in the normal fellow eye (3500 versus 1270 cells/mm^2). **D:** The backscatter profile of the hydrogel lens-implanted eye was very similar to that in the first case provided that the anterior interface and the subepithelial layers showed a higher reflectivity. **E:** The backscatter pattern in the fellow-eye was normal.

Table 1. *In vivo* confocal microscopy characteristics of 2 patients 22 years after intracorneal hydrogel lens implantation.

		Mean backscatter (SU)	Pachymetry (µm)	ECD (cells/mm^2)
Patient 1	ICL	1631	789	1200
	FE	1230	543	1800
Patient 2	ICL	1742	904	3500
	FE	1295	572	1270

(ECD = endothelial cell density; ICL = eye with intracorneal lens; FE = fellow eye; SU = scatter units)

the Artisan anterior chamber IOL would be more difficult because of the iridolenticular adhesions formed after the extracapsular cataract extraction. The reported explantation rates of intracorneal lenses range from 17% to 66%.[4–6] Reasons for explantation are lens migration,[4] lens extrusion,[5] and interface deposits.[7] In both our patients, intracorneal deposits developed more than 6 years after surgery. Years later, the opacities caused

reduced visual acuity in one patient. However, implant removal is not necessary as the lens can be easily rinsed by lifting the corneal cap.

The second most frequently reported complication is lens migration. In the first patient, the intracorneal lens was implanted at a depth of 278 µm and the corneal cap was sutured with interrupted sutures. This patient developed opacities earlier than the second patient, with a decrease in visual acuity. More recent studies report an implantation depth of 120 to 180 µm.[4,5] In 1990, McCarey et al.[8] demonstrated that hydrogel lenses have to be placed between 36% and 60% of the corneal thickness for success. Deeper stromal implantation allows better movement of water and nutrients across the corneal stroma and around the edges of the alloplastic material, which might lead to fewer complications.[9] A potential drawback of a thicker corneal cap is that the cap has to be sutured. McCarey et al. observed no epithelial thinning or defects, which suggests that the glucose metabolism is sufficient in the anterior part of the cornea after implantation of a hydrogel lens at this depth.

Confocal imaging has been used to evaluate short-term effects of hydrogel intracorneal lens implantation in animals and humans.[10,11] Twenty-two years after implantation, both patients showed a similar backscatter profile, with increased backscatter at the anterior and posterior interfaces. This can be explained by a small difference in refractive index between the hydrogel lens and the surrounding stroma. Subsequent reflection at the stroma–lens interface will cause a peak in the backscatter profile, as image intensity is composed of backscatter and reflectance.[12,13] Furthermore, deposition of cellular debris and crystalline structures, as we demonstrated by relifting the cap in the first patient, contributed to this peak formation. The peak at the anterior interface was higher in the second patient, possibly because irrigation and rinsing around the implant was done in the first patient only. Both cases also showed increased backscatter at the subepithelial level. This might be explained by a chronic immune response against the foreign material. Antigen presenting cells, which can be observed in close vicinity to the subbasal nerve plexus,[14] could be mediators.

In conclusion, deep intracorneal lens implantation can be a safe treatment option in patients with high hyperopia who are contact lens intolerant and not suitable for IOL implantation. Long-term patient monitoring is essential because corneal opacities can occur after many years, with a possible reduction in visual acuity.

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General discussion

GENERAL DISCUSSION

The first visual successful human penetrating corneal transplantation was performed by Eduard Zirm in 1906.[1] Great advances in surgical instrumentation and techniques and the introduction of antibiotics and corticosteroids over the last century, significantly improved the success rate of corneal transplantation. Corneal transplantation is one of the most widely practised forms of clinical transplantation. In the Netherlands, 995 corneal transplantations were performed in 2009 and 1165 in 2010.[2]

When to choose for a mushroom keratoplasty today?

Until recently, traditional penetrating keratoplasty (PKP) was considered the standard surgical method for the treatment of keratoconus (KC). However, graft rejection of the corneal endothelial layer is one of the most important reasons of corneal transplant failure.[3] Deep anterior lamellar keratoplasty (DALK) leaves the unaffected endothelium of the patient in place, avoiding the risk of endothelial rejection. The results of the DALK technique are equivalent to PKP for the outcome measure of best spectacle corrected visual acuity, particularly if the surgical technique yields minimal residual host stromal thickness. Moreover, the time necessary to achieve stable results is considerably shorter after DALK. However, the technique is difficult, and Descemet's membrane perforation remains a major complication of DALK.[4] If a large perforation occurs, we have to convert to a full thickness graft. A mushroom shaped graft allows transplantation of a larger anterior diameter (9.0 mm) and leaves most of the healthy endothelium unaffected. The second indication for mushroom keratoplasty is a patient with deep corneal scars. The close proximity of the anterior layer to the limbus of the recipient may lead to suture loosening and wound healing problems.[5] This is particularly true for allergic patients with vascularized corneas. To avoid this, we make a fornix-based conjunctival flap and place sutures into the sclera in these quadrants.

Although the corneas of our patients were steep, with an average steep keratometry-reading of 60D and a thin periphery, our results are in line with the reported success rate of traditional PKP in general KC patients.[6 7] Suturing a conventional full thickness graft in patients with a zone of marked inferior corneal thinning is more prone to a higher post-operative astigmatism and a lower BCVA because of wound healing problems. Because of the particular shape of the mushroom-shaped graft, the donor and the recipient are locked to each other. This way, the graft is unlikely to dislocate.

Despite all the advances of posterior lamellar surgery in the treatment of corneal endothelial dysfunction, there are still some indications for a posterior mushroom or a top-hat keratoplasty. For example, a patient with endothelial dysfunction with stromal opacification needs a penetrating top-hat keratoplasty. A younger phakic patient with a

shallow anterior chamber and a pseudophakic patient with no capsule support and an anterior chamber lens might be other indications.

Posterior lamellar keratoplasty; how thin should it be?

Over the last few years, Descemet Stripping Automated endothelial keratoplasty (DSAEK) has replaced PKP as the golden standard surgical treatment for corneal endothelial diseases.[8] Its main advantage includes the lack of sutures, resulting in less induced astigmatism and a faster visual recovery. Furthermore, it is a safer technique with less risk for expulsive hemorrhage and suture related - complications. The main concern of this technique remains the rate of postoperative endothelial cell loss. [9-11] Recently, Price et al showed that overall graft success was comparable for DSAEK and PKP procedure.[12] However, the endothelial cell loss was higher with DSAEK at 6 and 12 months postoperatively, consistent with the endothelial trauma due to more donor tissue manipulation in DSAEK.[13] Our results confirm these findings; the early postoperative endothelial cell loss in DSAEK is much higher (half time 2.2 months) compared to top-hat keratoplasty (half time 12.8 months). The opposite is true for the late endothelial cell loss; after DSAEK the decay of endothelial cell loss is less steep than after top-hat keratoplasty. This is consistent with earlier findings.[14-16] In both techniques, the diagnosis of pseudophakic bullous keratopathy (PBK) was significant correlated with more endothelial cell loss in the late phase.

Recently, the thickness of the transplant layer has been further reduced. A study of Neff et al[17] showed that a thinner endothelial keratoplasty (≤ 131 µm) is correlated with a higher postoperative visual acuity compared to thicker transplants (> 131 µm). However, donor preparation, positioning, and attachment are more challenging with thinner transplant tissue, resulting in more donor tissue loss and a higher re-bubbling rate.[18] Future innovation might come of a hybrid technique that combines the visual result of DMEK and the stability of a DSAEK graft.[19]

Combined treatment options with corneal cross-linking

UV-A induced corneal cross-linking (CXL) has been shown effective in progressive KC; a decrease in maximal keratometry readings of 2 to 3 D and an increase in UCVA of 1 or 2 lines.[20-22] However, other treatment methods for KC (e.g., intracorneal ring segments) yield more significant improvement in visual acuity and keratometry.

In order to obtain both the stabilizing effect of CXL and the visual benefit of intracorneal ring segments on corneal ectasia, these two treatment procedures can be combined. Reported results in KC patients showed an average improvement in UCVA and BCVA of two lines, a significant decrease in spherical equivalent of 2 D and a decrease in maximum K value of 2 to 4 D.[23-25] Our results are more favorable; an increase in UCVA and BSCVA of 5 and 3 lines, respectively. Mean keratometry and spherical equivalent

decreased with 3 and 3.5 D, respectively. El-Raggal confirmed in his comparative study that a significant greater topographic improvement is obtained when ring segment insertion and CXL are performed in one session compared to two sessions. No significant differences were found in visual acuity outcome.[26] The second treatment option to obtain both structural and refractive improvement is the combination of CXL with topography-guided photorefractive keratectomy (PRK).[27] However, long term outcome is yet unknown. Finally, the residual refractive error after CXL can be corrected by a toric intraocular (phakic) lens. [28]

Intracorneal lenses; what is in for the future?

In 1966, Barraquer proposed synthetic intrastromal implants to alter the anterior corneal curvature to correct refractive errors.[29] However, his results were unsatisfactory because of the impermeable nature of the implant material. New materials such as hydrogel showed biocompatibility and good corneal tolerance when implanted in primates.[30] Because of its refractive index (1.376), the lens has no refractive power on its own within the cornea. The refractive effect is achieved by alteration of the corneal shape. Hydrogel lenses were soon superseded by laser refractive surgery, and their use declined.

Today, Artisan (Ophtec, BV) lens implantation would be considered in our patient (case 1) with posttraumatic aphakia. In the second patient, an Artisan anterior chamber lens implantation would be more difficult due to the iridolenticular adhesions formed after the extracapsular lens extraction.

Recent studies on intracorneal lenses report a high explantation rate (range from 17% to 66%).[31,32] Reasons for explantation are lens migration[31], lens extrusion[32] and interface deposits.[33] The implantation depth in these studies measured 120 – 180 µm.[31-33] In 1990, McCarey demonstrated that hydrogel lenses need to be placed deeper in the corneal stroma, at a depth between 36% and 60% of the corneal thickness, for success.[34] In our two patients, the depth of implantation was 272 µm and 385 µm, respectively. During follow-up, no lens migration or extrusion was seen.

In our two patients, intracorneal deposits developed more than 6 years after surgery. Years later the opacities caused reduced visual acuity in one patient. We showed that implant removal is not necessary, since rinsing of the lens can easily be done by lifting the corneal cap.

Recently, interest in intracorneal implants has resurfaced, especially for the correction of presbyopia.[35,36] The requirements for future intracorneal implants will be: thin, small diameter, high nutrient and fluid permeability, and implantation relatively deep in the stroma.[37,38]

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Summary / Samenvatting

SUMMARY

In the **General Introduction**, an overview of all types of corneal transplantations with their indications and results are presented. Secondly, a review of the literature of published studies on intracorneal ring segments (ICRS), intracorneal lenses and corneal cross-linking (CXL) is given.

In **Chapter 1** we describe the clinical outcome of posterior mushroom or top-hat keratoplasty in 22 eyes with Fuchs endothelial dystrophy and pseudophakic bullous keratopathy. One year after surgery, good visual acuity and keratometric results were obtained in our study group. During follow-up, these results remained stable. Postoperative endothelial cell loss was in line with reported results on traditional penetrating keratoplasty (PKP).

Chapter 2 analyzes the visual and keratometric results of mushroom keratoplasty in 15 patients (16 eyes) with advanced keratoconus (KC). Although our patients all had advanced KC, with an average keratometry reading of 58 diopters (D) and a thin periphery, visual results were comparable with the outcome of standard PKP in general KC patients. The relatively good postoperative visual acuity might be explained not only by the low extent of astigmatism, but also by the greater surface regularity owing to the larger anterior diameter.

In **Chapter 3** we evaluate the endothelial cell loss after both mushroom keratoplasty and Descemet Stripping Automated Endothelial Keratoplasty (DSAEK) using biexponential endothelial regression models. The models show that early postoperative endothelial cell loss in DSAEK is much higher (half time 2.2 months) compared to mushroom keratoplasty (half time 12.8 months). This finding is in line with previous reported results, and is consistent with the endothelial trauma due to more donor tissue manipulation in DSAEK. The opposite is true for the late endothelial cell loss; after DSAEK the decay of endothelial cell loss is less steep than after mushroom keratoplasty. This is consistent with earlier findings. In both techniques, the diagnosis of PBK was significantly correlated with more endothelial cell loss in the late phase.

In **Chapter 4** we describe a case of immunological graft rejection with an endothelial rejection line (Khodadoust line) four months after DSAEK. Contrary to our instructions, the patient had stopped using the steroid eye drops one month postoperatively and she did not visit her own ophthalmologist. Previously, it was generally assumed that endothelial rejection lines did not occur after DSAEK procedure due to the absence of graft vascularization. Our case illustrates that an endothelial rejection line can be observed after DSAEK, independent of graft vascularization.

In **Chapter 5** we report a case of introduction of epithelial cells in the flap-graft interface during DSAEK. A 67-year-old woman underwent DSAEK procedure of the left eye because of visual limiting bullous keratopathy. A cornea from a male donor was used. One week postoperatively, the donor lamella was partially detached and an additional air bubble was injected. Four months postoperatively, the donor lamella was attached, but the interface was slightly hazy. One year after DSAEK, total corneal decompensation was seen, and a posterior mushroom keratoplasty was performed. Histopathologic examination of the removed corneal button showed two cysts, lined with squamous epithelium, at the interface. The X-Y karyotyping revealed that the epithelial cells were of donor origin. We postulate that the donor epithelium was implanted during the preparation of the donor posterior lamellar disc and was introduced intraoperatively. If complete attachment of the donor posterior lamella is accomplished, ectopic epithelial cells in the interface might remain stable without proliferation. In a partially detached donor lamella, ectopic epithelial cells might proliferate along the recipient's posterior cornea.

Our findings in **Chapter 6** show that a combined procedure of Ferrara ICRS implantation and CXL is a safe treatment option in progressive moderate KC in order to postpone or avoid corneal transplantation. We included 7 eyes (7 patients) with progressive KC with a clear cornea, contact lens intolerance and an average keratometry of less than 53 D. Patients with a corneal thickness of less than 400 µm and a mesopic pupil diameter more than 5.5 mm were excluded. After one year, uncorrected visual acuity and best spectacle-corrected visual acuity increased with 5 and 3 lines, respectively. Mean keratometry and spherical equivalent decreased with 3 D and 3.5 D, respectively.

In **Chapter 7** we report the outcome of hydrogel intracorneal lens implantation in two aphakic patients. The lenses were implanted at approximately 50% depth in the cornea to correct high hyperopic refractive errors of 10.5 D and 14.0 D, respectively. Both patients were contact lens intolerant. At the time, both patients were considered unsuitable candidates for an intraocular lens. Surgery was performed in 1988, and the patients were followed until early 2010. The patients showed good tolerance for the intracorneal lenses, but both developed opacities around the implant, leading to reduced visual acuity in one patient. Removing the implant is not necessary as the opacities around the lens can easily be rinsed by lifting the corneal cap.

SAMENVATTING

De **Inleiding** geeft een overzicht van alle types van hoornvliestransplantaties met bijhorende indicatiegebieden en resultaten. Tevens wordt er een literatuuroverzicht gegeven van verschillende recente studies over de resultaten na intracorneale ringsegmenten, intracorneale contactlenzen en corneale crosslinking.

In **Hoofdstuk 1** evalueren we de klinische resultaten van een posterieure mushroom keratoplastiek die werd uitgevoerd in 22 ogen met Fuchs' endotheeldystrofie en pseudofake bulleuze keratopathie. Eén jaar na de ingreep hadden de patiënten een goede gezichtsscherpte en goede keratometrie waarden. Tijdens de follow-up periode bleven deze beide parameters stabiel. Het postoperatief verlies aan endotheelcellen was vergelijkbaar met het verlies na een conventionele perforerende keratoplastiek (PKP).

Hoofdstuk 2 analyseert de gezichtsscherpte en keratometrie waarden bij 15 patiënten (16 ogen) met vergevorderde keratoconus (KC) die een mushroom keratoplastiek ondergingen. Al onze patiënten hadden een vergevorderd stadium van KC, met een gemiddelde keratometrie waarde van 58 dioptrie (D) en een dun perifeer hoornvlies. Toch zijn de resultaten vergelijkbaar met patiënten die een matig steile KC hadden en daarvoor een conventionele PKP ondergingen. Er was postoperatief weinig astigmatisme. Dit verklaart de relatief goede gezichtsscherpte postoperatief. Ook geeft de grotere voorste diameter van het transplantaat een meer regelmatig oppervlak, wat de gezichtsscherpte positief beïnvloedt.

In **Hoofdstuk 3** evalueren we het endotheelcelverlies na een mushroom keratoplastiek en een posterieur lamellaire keratoplastiek (DSAEK: Descemet Stripping Automated Endothelial Keratoplasty) met behulp van biexponentiële regressiemodellen. Deze laten zien dat het vroegtijdig endotheelcelverlies na DSAEK veel hoger is (halfwaardetijd 2.2 maanden) dan na een mushroom keratoplastiek (halfwaardetijd 12.8 maanden). Deze bevinding is in lijn met voorgaande publicaties. Endotheelschade veroorzaakt door meer donorweefsel manipulatie bij DSAEK wordt als de meest logische verklaring hiervoor beschouwd. Het omgekeerde is waar voor het laattijdig endotheelcelverlies; bij DSAEK is dit endotheelcelverval minder groot. De diagnose pseudofake bulleuze keratopathie is bij beide vormen van hoornvliestransplantatie gecorreleerd met een groter laattijdig endotheelcelverlies.

In **Hoofdstuk 4** beschrijven we een immunologische afstotingsepisode met een endotheliale rejectielijn (Khodadoustlijn) na DSAEK. De patiënte was, op eigen initiatief, een maand na de ingreep gestopt met de steroïd druppels. Ook ging zij niet langer op controle bij haar eigen oogarts. Voorheen werd aangenomen dat een Khodadoustlijn

niet kan voorkomen bij DSAEK vanwege de afwezige vaatingroei in het transplantaat. Onze casus toont aan dat er wel degelijk een Khodadoustlijn kan voorkomen na DSAEK, onafhankelijk van de vaatingroei.

In **Hoofdstuk 5** rapporteren we de aanwezigheid van donor epitheelcellen in de interface na DSAEK. Een 67-jarige vrouw met bulleuze keratopathie onderging een DSAEK. Het hoornvlies was afkomstig van een mannelijke donor. Een week na de ingreep werd er een extra luchtbel in de voorste oogkamer ingebracht omdat de donor lamel niet volledig aanlag. Vier maanden postoperatief werden er lichte troebelingen in de interface gezien. Een jaar na de ingreep was er sprake van een volledige decompensatie van het hoornvlies waarvoor een posterieure mushroom keratoplastiek moest worden verricht. Pathologisch onderzoek van het verwijderde hoornvlies laat ter hoogte van de interface twee epitheelcysten zien. X-Y karyotypering toont aan dat de epitheelcysten afkomstig zijn van de mannelijke donor. Wij gaan ervan uit dat de donor epitheelcellen zijn ingebracht samen met de donorlamel. Als de lamel volledig aanligt, blijven deze ectopische epitheelcellen waarschijnlijk stabiel zonder proliferatie. Indien de donor lamel niet volledig aanligt, kunnen deze epitheelcellen vrij proliferen ter hoogte van de interface, met mogelijk falen van het transplantaat tot gevolg.

De veiligheid en doeltreffendheid van een gecombineerde behandeling met Ferrara intracorneale ringsegmenten en crosslinking bij progressieve KC worden besproken in **Hoofdstuk 6**. Wij behandelden 7 patiënten met progressieve KC met een heldere cornea, contactlens intolerantie en een gemiddelde keratometrie waarde van 53 D of minder. Patiënten met een corneale dikte van minder dan 400 µm en een mesopische pupildiameter van meer dan 5.5 mm werden uitgesloten. Eén jaar na de ingreep waren de ongecorrigeerde en gecorrigeerde gezichtsscherpte (met bril) toegenomen met respectievelijk 5 en 3 lijnen. De gemiddelde keratometrie waarde en het sferisch equivalent waren met respectievelijk 3 D en 3.5 D afgenomen.

In **Hoofdstuk 7** beschrijven we twee patiënten waarbij een hydrogel lens in de cornea werd geïmplanteerd ter correctie van hun hoge hypermetropie (10.5D en 14D). De lens werd geplaatst op ongeveer 50% diepte in het hoornvlies. Beide patiënten waren afaak en contactlens intolerant. Ze werden toen ongeschikt bevonden voor intraoculaire lensimplantatie. De operatie vond plaats in 1988 en de patiënten werden opgevolgd tot in 2010. Beide patiënten hebben een goede tolerantie voor de intracorneale lens, maar ontwikkelden beiden opaciteiten rond het implantaat. De opaciteiten zorgden bij één patiënt voor een vermindering van de gezichtsscherpte. Verwijderen van de intracorneale lens was niet nodig. De opaciteiten rondom de lens kunnen weggespoeld worden door de corneaflap te liften.

Dankwoord en Curriculum Vitae

DANKWOORD

Bij het tot stand komen van dit proefschrift waren veel mensen direct en indirect betrokken. Ik wil hier dan ook graag die mensen bedanken voor hun bijdrage.

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CURRICULUM VITAE

Isabelle Saelens was born in Bonheiden in Belgium on the 23th February 1981. She graduated from the secondary school Don Bosco College Haacht in 1999. Subsequently, she started her medical studies at the University of Hasselt, and continued at the Catholic University of Leuven. In 2006, she obtained her medical degree with distinction. That same year, she moved to The Netherlands to start with the research project, described in this thesis. Six months later, she commenced her residency in Ophthalmology at The Erasmus University Medical Center Rotterdam, headed by prof. Dr. G. van Rij. She will finish her residency in March 2012.

LIST OF PUBLICATIONS

1. Bleyen I, **Saelens IEY**, van Dooren BTH, van Rij G. Cataract surgery and Descemetorhexis as treatment for Fuchs endothelial dystrophy: a report of 8 patients. *Submitted*
2. Van Dooren BTD, **Saelens IEY**, Bleyen I, Mulder PGM, Bartels MC, Van Rij G. Endothelial cell decay after Descemet's Stripping Automated Endothelial Keratoplasty and Top Hat Penetrating Keratoplasty. *Accepted for publication in Invest Ophthalmol Vis Sci.*
3. **Saelens IEY**, Bartels MC, Bleyen I, Van Rij G. Refractive and visual outcome after corneal cross-linking combined with Ferrara intracorneal ring segments in patients with progressive keratoconus. *Accepted for publication in Cornea.*
4. **Saelens IEY**, Bleyen I, Hillenaar T, Thiadens AA, Beekhuis WH, Remeijer L, Van Rij G.Long-term follow-up of hydrogel intracorneal lenses in 2 aphakic eyes. J Cataract Refract Surg 2010; 36(12):2200-3.
5. van Rij G, Bartels MC, Bleyen I, van Dooren BT, **Saelens IE**. Femtosecond incisions. Ophthalmology 2010; 117(7):1458-9; author reply 1459.
6. **Saelens I**, Bleyen I, Bartels MC, Van Rij G. Posterior Khodadoust line in endothelial rejection episode after Descemet Stripping Automated Endothelial Keratoplasty. Cornea 2010; 30(2): 245-6.
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8. **Saelens IE**, Bartels MC, Van Rij G, Dinjens WN, Mooy CM. Introduction of epithelial cells in the flap-graft interface during Descemet stripping automated endothelial keratoplasty. Arch Ophthalmol 2009; 127(7):936-7.
9. **Saelens IE**, Bartels MC, Van Rij G. Posterior mushroom keratoplasty in patients with Fuchs endothelial dystrophy and pseudophakic bullous keratopathy: transplant outcome. Cornea 2008; 27(6):673-8.
10. **Saelens IE**, Bartels MC, Van Rij G. Manual trephination of mushroom keratoplasty in advanced keratoconus. Cornea 2008; 27(6):650-5.

Colour Figures

GENERAL INTRODUCTION

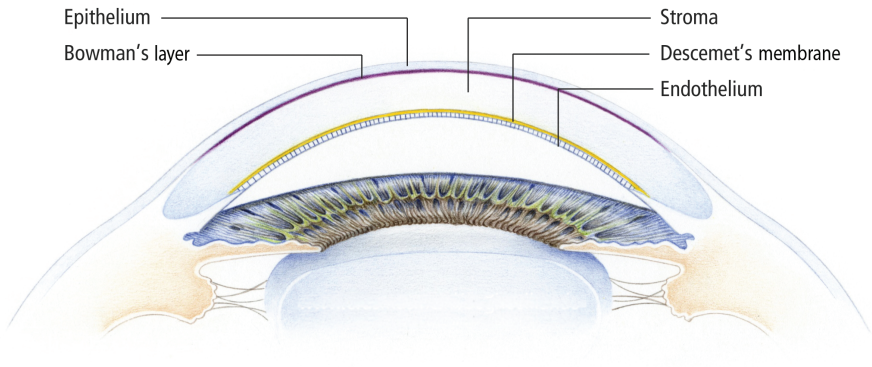

Fig 1. Corneal structure. (J Leenen)

CHAPTER 1

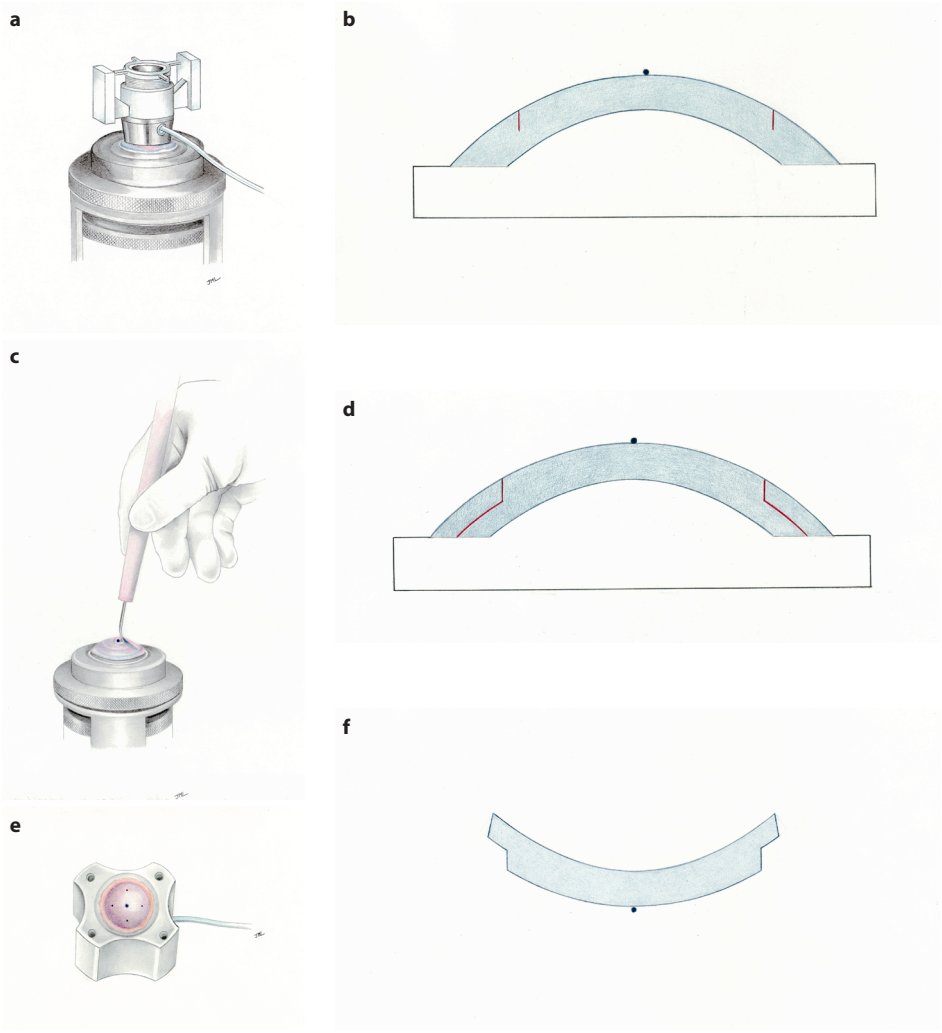

Fig 1. The trephination technique of the donor cornea. (J Leenen)
A-B: The geometric centre of the donor cornea is marked and the Barron suction trephine (7.5 mm in diameter) is used to make a circular incision of 0.3 mm depth.
C-D: A lamellar stromal dissection was carried out from the base of the incision to the limbus.
E-F: The cornea was removed from the artificial anterior chamber and placed on a Barron suction punch (Katena) with the endothelial side up. A 9.0-mm donor button was punched out.

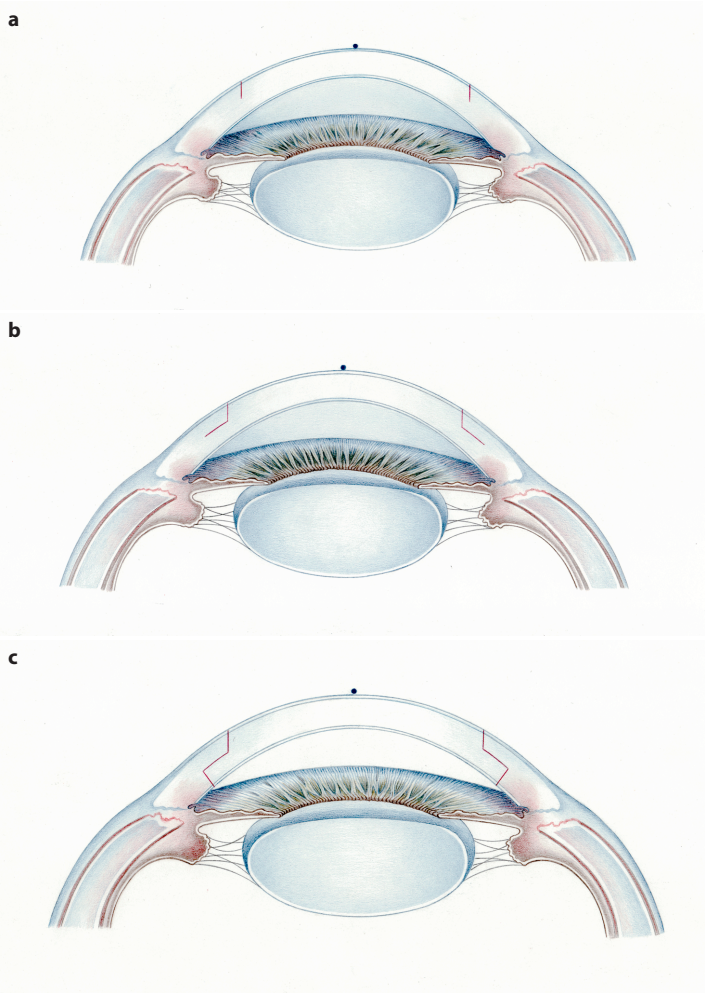

Fig 2. The trephination technique of the recipient cornea. (J Leenen)
A: The geometric centre of the recipient cornea is marked and the Barron suction trephine (7.5 mm in diameter) is used to make a circular incision of 0.3 mm depth.
B: The cornea was marked with a 9 mm trephine and a lamellar stromal dissection was carried out from the incision to the 9.0 mm mark.
C: The anterior chamber was entered, and corneal scissors were used to complete the excision of the corneal button at the peripheral end of the posterior lamellar stromal dissection.

CHAPTER 2

Fig 1. Trephination technique of donor cornea. (J Leenen)
A, The Barron suction trephine (9.0 mm in diameter) is used to make a circular incision of 0.3-mm depth.
B, A lamellar stromal dissection was carried out from the circular incision up to the 7.0-mm mark.
C, The donor cornea with a larger anterior stromal lamella (9.0 mm in diameter) and a smaller posterior part (7.0 mm in diameter)

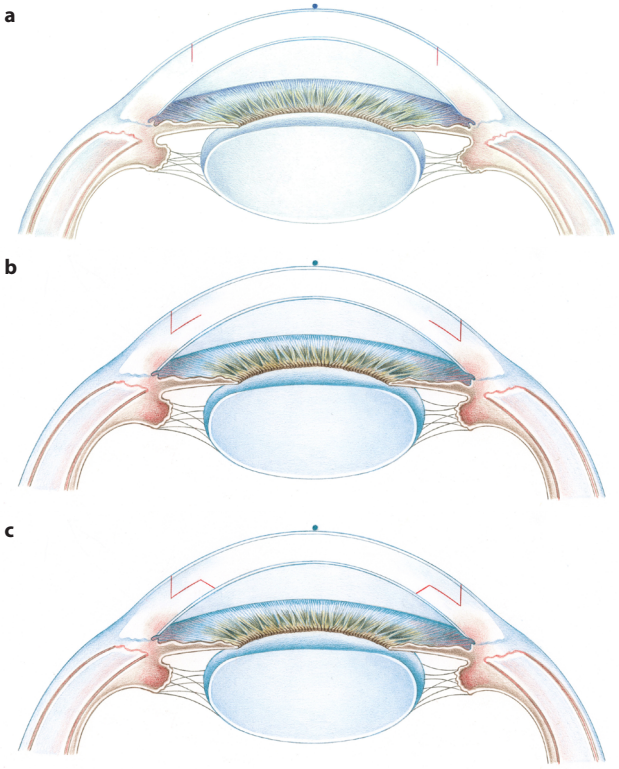

Fig 2. Trephination technique of recipient cornea. (J Leenen)
A, The geometric center of the recipient cornea is marked. The Barron suction trephine (9.0 mm in diameter) is used to make a circular incision of 0.3-mm depth.
B, A lamellar stromal dissection was carried out from the circular incision up to the central 6.5-mm mark.
C, Vannas corneal scissors were used tocomplete the excision of the corneal button.

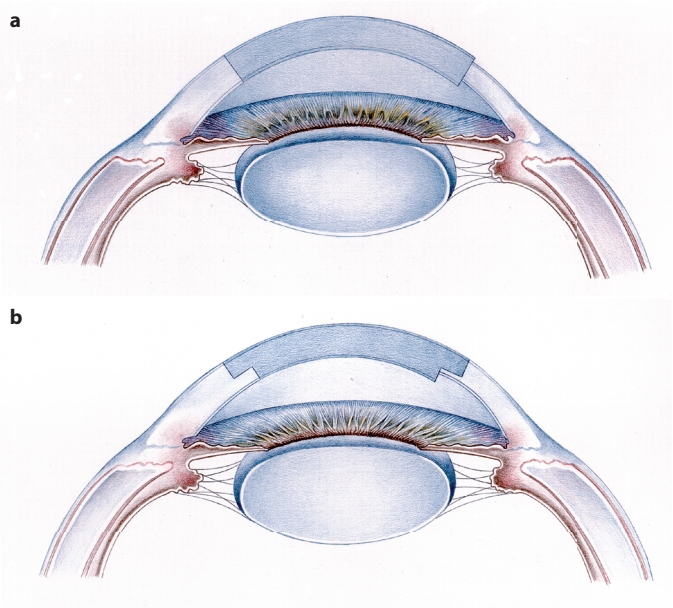

Fig 3. Keratoplasty in a recipient cornea with a thin periphery. (J Leenen)
A, Problematic wound healing of a normal thickness donor cornea to a 1-sided thin recipient in conventional keratoplasty with a vertical cut.
B, Mushroom graft to a 1-sided thin recipient; no dislocation occurs.

CHAPTER 4

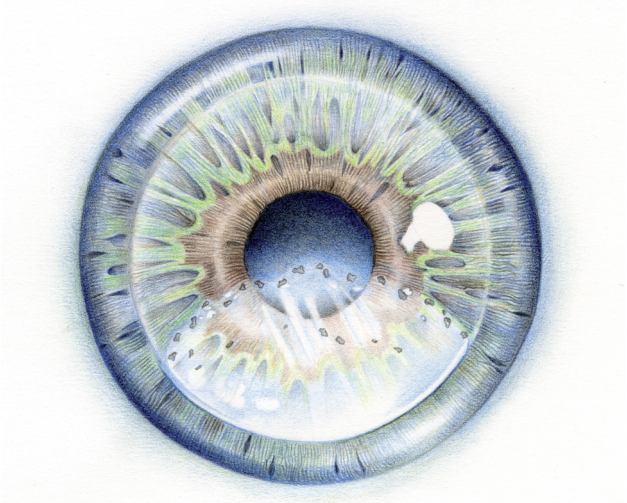

Fig. 1. Khodadoust line (J Leenen)

CHAPTER 5

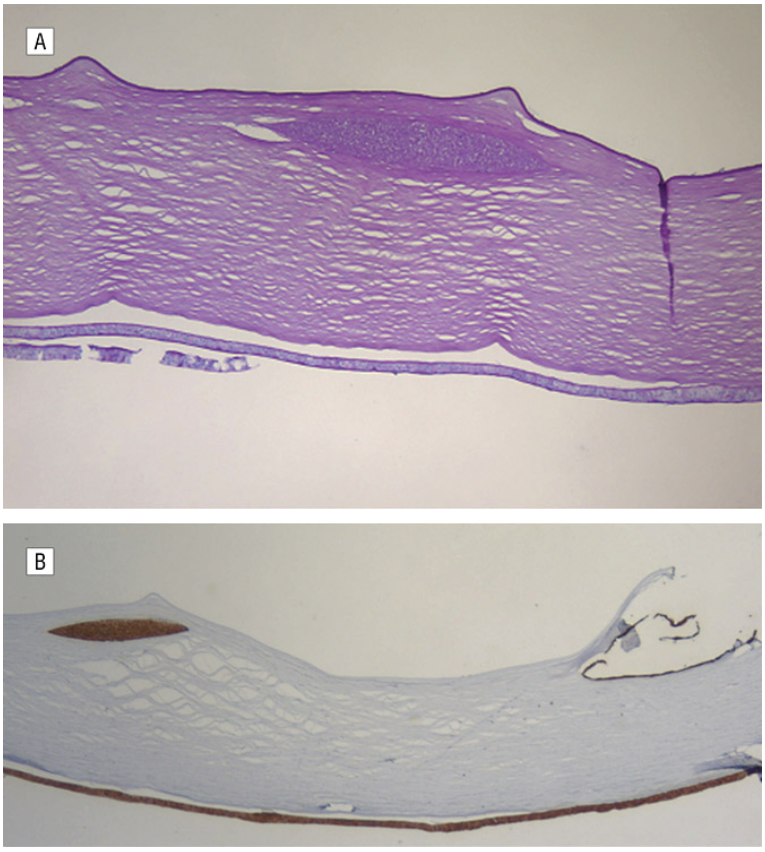

Fig 1. There are two cysts at the interface between the recipient cornea and the posterior endothelial graft tissue (A), and the lining of the cysts stains strongly positive for the epithelial markers 34Be12 and keratin 5/6 (B).

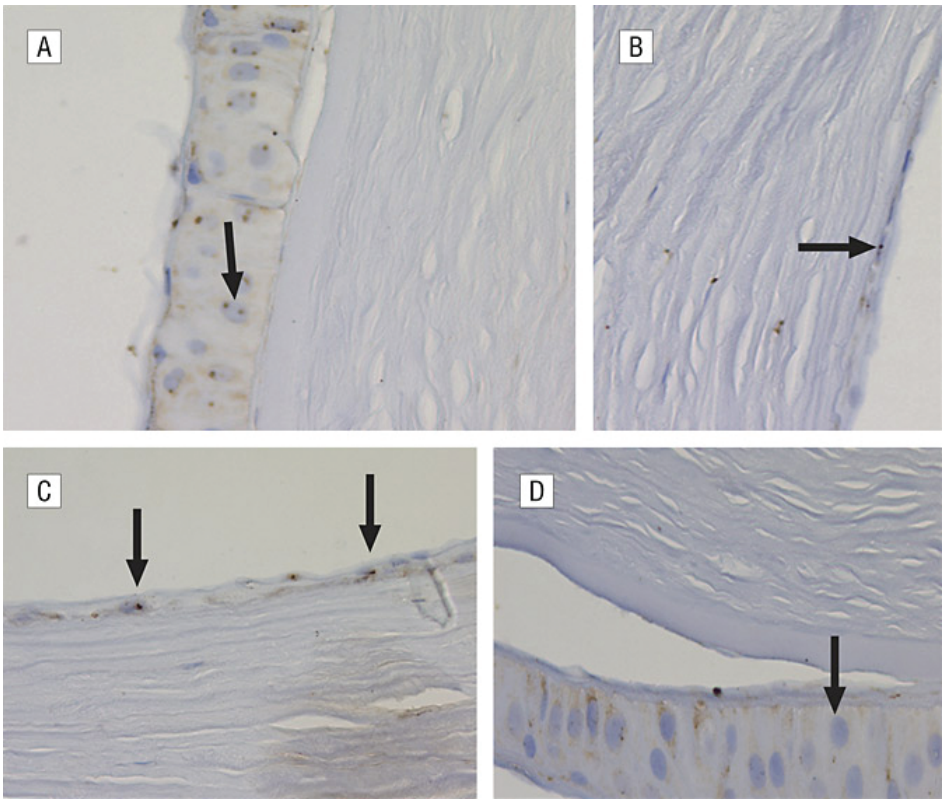

Fig 2. In situ hybridization with X and Y chromosome probes revealed 2 X chromosomes (arrow) in the epithelium and the stroma of the recipient (A) and 1 X chromosome (arrow) in the epithelium of the cyst (B). C, The epithelium of the cyst wall contained 1 Y chromosome (arrows). D, There was no staining of the Y chromosome probe (arrow) in the tissue of the recipient.