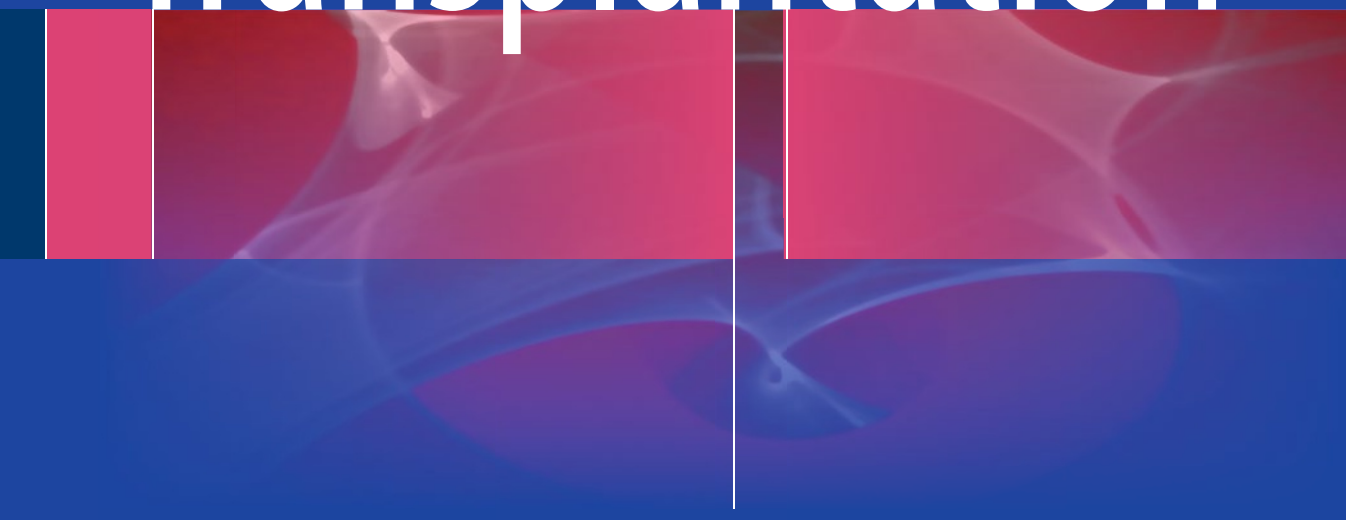
Jesper Hjortdal
Editor

Corneal Transplantation



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ISBN 978-3-319-24050-3 ISBN 978-3-319-24052-7 (eBook)
DOI 10.1007/978-3-319-24052-7

Library of Congress Control Number: 2015955555

Springer Cham Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media (www.springer.com)

Preface

Corneal transplantation has been performed for more than 100 years. Until 15 years ago the state-of-the art type of transplantation was penetrating keratoplasty, but since the start of this millennium, newly designed surgical techniques have developed considerably. Today, the vast majority of keratoplasty procedures are performed as delicate lamellar procedures either assisted with fine microkeratomes or femtosecond lasers or using skilled surgical dissection procedures.

These advancements have helped patients undergoing keratoplasty to have a much faster visual recovery and a more stable eye with less risk of rejection episodes.

Besides covering updated chapters on penetrating keratoplasty, and anterior and posterior lamellar procedures, this textbook also gives a thorough overview of the history of corneal transplantation and a detailed presentation of the microstructural components of the cornea essential to keratoplasty procedures. Corneal banking has changed over recent years as graft preparation for anterior and posterior lamellar keratoplasty now often is performed within the bank. Chapters have been devoted to description of graft registries, which are an indispensable source of information of daily practices and outcomes, and to economical evaluations of keratoplasty procedures. The optical consequences of a keratoplasty procedure, especially in relation to simultaneous or later cataract surgery, are discussed in addition to current methods for reducing post-keratoplasty astigmatism. Economic considerations on cost and benefit of medical treatment and surgical procedures are today an integrated part of the health system in many countries, and a chapter covers these aspects of corneal transplantation.

This textbook is aimed at presenting an updated review of the new techniques and to assist fellows and corneal surgeons in their advice and selection of patients for the best surgical procedure considering benefits and risks.

Aarhus, Denmark — Jesper Hjortdal

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The History of Corneal Transplantation

1

Gabriël van Rij and Bart T.H. van Dooren

Abstract

The concept of corneal transplantation is very old. However, it took many centuries before this miraculous operation could be performed with some success in both animals and humans. Knowledge of the history of keratoplasty is obligatory for a better understanding of modern corneal transplantation.

In the second half of the twentieth century, penetrating keratoplasty became the gold standard in corneal transplantation. Good results became more or less routine, due to a better knowledge of indications for treatment, a better understanding and hence prevention and treatment of allograft rejection and improvements in eye banking, operating microscopes, instruments and suture materials.

The recent two decades have once more seen a paradigm shift towards the selective replacement of only the diseased layers of the cornea. This has resulted in a rapid rise in the popularity of (deep) anterior lamellar and endothelial keratoplasty.

Keywords

History of keratoplasty • Corneal transplantation • Penetrating keratoplasty • Deep anterior lamellar keratoplasty (DALK) • Endothelial keratoplasty (EK)

History of Keratoplasty

In ancient times, cosmetical treatment of corneal scars had been performed by means of a tattoo-like coloration of the scar. Lampblack or soot was used in old Egypt (±1500 BC), and copper sulphate reduced with nutgall was applied to achieve reasonable cosmesis by Galenus (131–200 AD). In the eighteenth century, superficial removal of

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J. Hjortdal (ed.), *Corneal Transplantation*, DOI 10.1007/978-3-319-24052-7_1

scars was widely performed by surgeons in France and Germany [1]. The idea of removing scars from the cornea using a trephine was first proposed by Erasmus Darwin (the grandfather of Charles Darwin) in 1796 [2]. In 1789 Pellier De Quengsy introduced his ideas on treating corneal opacification with what would now be called keratoprosthesis, i.e. the replacement of opaque corneal tissue by man-made material. His concept entailed an artificial cornea made from glass framed in silver [3]. Attempts in the second half of the nineteenth century to actually treat patients with artificial corneas, among others by von Hippel and by Nussbaum, were not successful [4, 5]. The artificial cornea concept was in fact not developed into a useful technique until 1963, when among others Strampelli published on successful clinical application of keratoprostheses. In Strampelli's case, this was the osteo-odonto-keratoprosthesis, in which the optical element was embedded in a biocompatible carrier made out of the patient's own tooth and jawbone [1, 6]. Recently the application of keratoprostheses made of artificial materials has increased, with variable results in patient groups with significant high-risk eyes [7].

The first widely known experiments with full-thickness tissue corneal transplantations in animals, conducted in 1818, either heterologous (between species a.k.a. xenografting) or homologous (within species), are attributed to Reisinger. He also introduced the term "keratoplasty" for corneal transplantation [8]. Wars at the end of the eighteenth and the beginning of the nineteenth centuries made corneal blindness from smallpox, venereal disease and "Egyptian ophthalmia" (trachoma) prevalent. With this background, Bigger performed the first successful corneal transplantation in animals. In 1837, during his captivity in Egypt by Sahara Bedouins, he performed a homograft on his captor's pet gazelle which had been blinded by a corneal wound [9].

Heterologous transplantations of animal tissue into humans were then attempted. In 1838, the New York ophthalmologist and general practitioner Richard Sharp Kissam transplanted a pig's cornea into a human patient. Kissam operated without any anaesthesia. Ether anaesthesia was not introduced until 1846, chloroform anaesthesia in 1847 and topical cocaine anaesthesia in 1858. His patient initially received more light in his eye, but the cornea opacified and absorbed over a 2-week period [10]. The experiments on corneal transplantation in humans and animals conducted by Power, described in 1872, suffered the same fate [11].

Success in heterografting remained elusive until the first successful lamellar heterograft in a human by Von Hippel. A leucoma corneae was excised from a young girl's eye with Descemet's membrane and endothelium remaining, and a rabbit cornea was transplanted into the wound bed. This procedure was performed in 1886 and was described in 1888 as the first in a series of 8 lamellar operations, of which 4 were successful [4]. Von Hippel performed anterior lamellar keratoplasty because he felt that corneal transparency depended on the integrity of the corneal endothelium and Descemet's membrane. Therefore, he abandoned full-thickness corneal grafts.

It was not until 1905 that the first successful penetrating homologous corneal graft was performed in a human patient. The Moravian ophthalmologist Eduard Konrad Zirm transplanted a donor cornea obtained from an enucleated eye of a young boy into the eye of a 45-year-old labourer, suffering from corneal scars caused by a chemical lye injury. Zirm used general anaesthesia (chloroform) and strict asepsis [12].

Shortly thereafter, the concept of autokeratoplasty or homograft was initiated. In this concept the donor cornea was harvested from the patient itself: from the fellow, blind eye, as described by Plange [1], or as a rotational graft in which a small corneal scar can be rotated out of the visual axis in the diseased eye, as described by Kraupa [5].

Allografting, in which the donor cornea is harvested from another individual of the same species, is currently the most commonly practised form of corneal transplantation. However, it took quite some time after Zirm, before reproducible results with penetrating corneal allografts were obtained. First the operative technique and donor tissue preservation and preparation had to be further developed and standardised. Much work in

this respect was done and published in the 1920s and 1930s by Elschnig from Tsjechia, Filatov from Russia, Tudor Thomas in the UK and Castroviejo in the USA [13–18]. Improvements in lamellar transplant technique were achieved by the French ophthalmologists Paufique et al. [19] and Switzerland's Franceschetti [20], from the 1930s through the 1950s, leading to a temporarily renewed popularity of this treatment modality.

The biggest hazard to a successful penetrating corneal graft is allograft rejection. Paufique described the concept of "maladie du greffon", i.e. opacification of a previously clear cornea, which he attributed to sensitisation to the donor by the recipient [19]. This seminal concept of immunological rejection of the donor graft was proven by Maumenee in 1951 [21]. Much important work in the field of corneal allograft rejection was done by Khodadoust and Silverstein [22, 23]. The use of corticosteroids realised a breakthrough in the treatment and prevention of corneal transplant rejection and opacification. This concurred with the introduction of antibiotics, the introduction of the operation microscope, the development of microsurgical techniques and of newer suture materials that ensued. Other important developments included the better understanding of endothelial physiology and of donor cornea preservation. US-based ophthalmologists and scientists such as Paton, Troutman, Maurice, McCarey and Kaufman played important roles in these developments [24, 25]. All these developments led to a substantial improvement in the popularity of penetrating keratoplasty and hence in the number of cases operated with this technique.

Recent and Current Developments in Penetrating and Lamellar Keratoplasty

Penetrating Keratoplasty

In the past, penetrating keratoplasty was considered the gold standard in corneal transplantation. In penetrating keratoplasty (PK), a full-thickness button of diseased cornea is replaced by full-thickness corneal donor tissue. A successful outcome after a penetrating keratoplasty is a clear graft with low astigmatism, providing a good visual acuity. Irregular and high regular astigmatism are the most frequent visual acuity impairing complications after penetrating keratoplasty.

At present there are three forms of penetrating keratoplasty: traditional penetrating keratoplasty; anterior mushroom keratoplasty, with a wider anterior than posterior diameter; and top-hat (or posterior mushroom) keratoplasty, with a wider posterior than anterior diameter. A top-hat keratoplasty is indicated in patients with both endothelial failure and secondary stromal opacities. Anterior mushroom keratoplasty has better astigmatic properties and can be applied in patients with relatively healthy endothelium [26, 27].

Femtosecond lasers have recently been applied to more reproducibly fashion several types of (mushroom and other) shaped corneal incisions in both donor and recipient corneas [28].

The graft survival in all types of PK is good in low-risk cases, with a success rate of 80 % or more of having a clear graft after 10 years. The cornea enjoys a relative immune privilege being avascular tissue, and furthermore immunosuppressive treatment can be directly applied in high concentrations using eye drops. Therefore, HLA matching of donor tissue to recipient status is usually not performed in low-risk cases, and still good graft survival rates are obtained. Allograft rejection however is still one of the major causes of corneal transplant failure in PK [29]. When a cornea becomes vascularised, the risk for corneal graft rejection is elevated. High-risk cases include repeat transplantations, especially after previous allograft rejection, and corneas with extensive deep blood (and lymph) vessel ingrowth [29, 30].

Other important reasons for graft failure in PK are (secondary) glaucoma, ocular surface problems and late endothelial failure [29]. The concept of late endothelial failure is an intriguing problem. After PK, grafts lose endothelial cells at a faster than physiological rate, even in the absence of overt endothelial allograft rejection. The exact cause for the elevated endothelial cell loss rate

needs yet to be determined. Hypothetically it may arise from prolonged cell redistribution onto the recipient cornea or from chronic pro-apoptotic changes in the anterior chamber [31–33].

Anterior Lamellar Keratoplasty

In anterior lamellar keratoplasty (ALK), only the diseased epithelium, Bowman's membrane and (anterior) corneal stroma are removed and transplanted, leaving the unaffected but vulnerable endothelium of the patient in place. Indications for ALK include many cases of keratoconus, epithelial and (anterior) stromal corneal dystrophies and partial-thickness post-infective (i.e. non-active, of herpetic and non-herpetic origin) and non-infective (e.g. traumatic) corneal scars.

In the 1960s and 1970s, the frequency with which anterior lamellar keratoplasty was performed sank inversely with the increase in PK's success and hence popularity. This was mainly caused by ALK's disappointing visual results. A large part of these poor results stem from the irregular scattering of light (diffraction) 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 recognised early on. However, to attain this goal required both surgical skills and time [34]. Yet, the advantages of ALK over PK in suitable indications remained tempting. There were less complications to be expected, as ALK was not truly an intraocular surgery. There was no risk of postoperative endothelial rejection and probably less risk of late endothelial failure and open globe after traumatic wound dehiscence.

In spite of this, comparative studies from the late 1970s kept on showing that visual results were better after PK than after ALK for keratoconus – one of the most apt indications for ALK [34–36]. However, good visual results were actually shown to be obtainable, when the lamellar dissection could be made at or just above the level of Descemet's membrane which presented a natural, very smooth optical interface [37]. It was not until the introduction of the "big-bubble" technique by Anwar, however, that Descemet's membrane could reproducibly be bared. In this technique an air bubble is used to dissect through the corneal stroma and to split the stroma from Descemet's membrane. A nearly full-thickness donor cornea, devoid of donor endothelium, is sutured in. Visual results after deep ALK with the big-bubble technique proved to be as good as or even better than PK [38]. Injecting viscoelastic material into the deep stroma can also be used to bare Descemet's membrane [39].

Microkeratome and femtosecond laser-assisted approaches towards ALK have recently gained some interest. Especially with the microkeratome, both the recipient and donor lamellar interfaces can be cut very smoothly. For selected cases, the results are promising [40].

Endothelial Keratoplasty

Endothelial keratoplasty (EK) is a treatment concept aimed at replacing only the diseased endothelium and posterior corneal layers, which have caused corneal clouding through oedema. Disorders that may be treated with EK include endothelial dystrophies, especially Fuchs endothelial dystrophy, iridocorneal endothelial (ICE) syndrome and pseudophakic bullous keratopathy. The main advantage of this concept is an untouched anterior corneal curvature, resulting in much less suture-induced high and irregular astigmatism, as can be seen after PK. Other suture- and full-thickness wound-related complications such as infections and wound dehiscence can also be avoided.

Barraquer was the first to publish on the concept of selective transplantation of an endothelium-containing posterior corneal lamella for the treatment of corneal oedema. In 1951 he reported for the first time on such a design, which involved the (manual) cutting of a hinged anterior lamellar corneal flap, followed by the excision and replacement of a deep corneal stroma lamella including the endothelium [41]. In 1964 he reported on the first results obtained with this technique in two patients, who obtained clear grafts and good visual acuities. In 1983 he introduced the motor-driven microkeratome in

EK for the cutting of the anterior flap in both donor and recipient and reported a good result in one patient [42].

Apparently unaware of Barraquer's work, Tillet published a report in 1956 on the selective transplantation of a posterior donor corneal lamella with endothelium, performed successfully in a patient with Fuchs' endothelial dystrophy, in 1954. The posterior recipient disc had been excised after a manual lamellar dissection through a 180° superior corneal incision. The half-thickness donor posterior disc was positioned onto the posterior surface of the recipient's anterior cornea and fixated with silk sutures. The graft remained clear for at least 1 year. However, the visual results were disappointing because of a poorly controlled glaucoma [43].

In the late 1970s, the concept of selective endothelial transplantation gained new interest, when experimental models were developed for the transplantation of cultured human and heterologous corneal endothelial cells. Experiments were performed with seeding the endothelial cells on animal and human donor corneas, Descemet's membranes, amnion membranes and artificial carrier devices [44]. Experiments on bioengineered corneal constructs with cultured human corneal endothelial cells have continued into the present time [45]. Although progress has been made, none of these techniques has reached the clinical phase yet.

The microkeratome-assisted approach towards EK, as conceptualised by Barraquer, was revived in the 1990s. A number of patients were operated with these techniques. These attempts however suffered from very unpredictable refractive outcomes [46–50]. A quite different approach for EK, more in line with the technique described by Tillet, was initiated by Ko et al. in 1993. They used a technique of EK in a rabbit model, in which the posterior lamella was introduced through a superior limbal incision and sutured against the recipient corneal surface [51].

In 1997 and 1998, Melles reported on a model for EK or posterior lamellar keratoplasty (PLK): the transplantation of a posterior corneal lamella with endothelium through a 9 mm corneoscleral tunnel incision [52, 53]. This technique was particularly remarkable because the posterior donor disc was not kept in place by sutures. The pressure of an air bubble in the anterior chamber helps to keep the disc in place in the first postoperative hours. The supposed mechanism that maintains good donor disc apposition thereafter might be the mere pumping action of the endothelial cells. Other postulated appositional mechanisms include the inherent adhesive quality of bare stromal surfaces and fibrils, assisted by the intraocular pressure [54, 55]. In 1999 and 2000, the first encouraging results in the first seven patients in Melles' series were reported, with all transplants attached and all corneas clear [56, 57]. In the next few years, technical improvements included the use of a smaller incision combined with the insertion into the anterior chamber of a folded donor disc. Later, Descemet's membrane stripping or "descemetorhexis", instead of the previously used deep lamellar cross-corneal dissection of the recipient corneal disc, was introduced [58].

Terry introduced PLK in the USA with slight modifications under the name deep lamellar endothelial keratoplasty (DLEK) and reported on large series of patients operated successfully with this technique [59, 60]. Price adopted the technique involving the descemetorhexis. He named this technique Descemet's stripping with endothelial keratoplasty (DSEK) or Descemet's stripping automated endothelial keratoplasty (DSAEK) when a microkeratome was used to cut the donor cornea. This reproducible technique provided excellent results regarding visual acuity, speed of visual recovery, astigmatism and postoperative refractive error and showed a low donor disc detachment rate [60, 61]. Midterm donor endothelial cell survival after EK seems comparable or even favourable to PK, and graft survival is also very comparable [62]. DSAEK has currently become the most often used technique for EK worldwide. Not only EK rates but also comprehensive corneal transplant rates have gone up since DSAEK's introduction [63]. Recent improvements in DSAEK include the use of thinner and pre-cut (i.e. microkeratome dissection in eye banks instead of in the OR) donor lamellae [64, 65].

In Descemet's membrane endothelial keratoplasty (DMEK), the thickness of the transplanted layer of stroma is further reduced. Different techniques were recently developed by Melles and later Price, Kruse and others. The donor material, mainly consisting of endothelium and Descemet's membrane, spontaneously forms a roll, with the endothelium on the outside. The donor roll can be introduced into the recipient eye using an inserter. The advantage of DMEK is an even faster and better visual rehabilitation than after DSAEK, although possibly at the cost of higher dislocation rates [66–69].

So far, the application of femtosecond lasers has not led to improved outcomes in endothelial keratoplasty [70, 71].

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2 Anatomy and Physiology: Considerations in Relation to Transplantation

Ursula Schlötzer-Schrehardt
and Friedrich E. Kruse

Abstract

Over the past decade, corneal transplantation has evolved rapidly from full-thickness penetrating keratoplasty toward partial-thickness or lamellar keratoplasty. Lamellar corneal surgery is in continuous evolution, which requires an understanding of the structural, biomechanical, and histological characteristics of corneal layers involved. In this chapter, we describe the anatomy and physiology of the human cornea in order to provide the structural basis for understanding the newly developed techniques. The chapter provides detailed information on morphological, histological, ultrastructural, and physiological characteristics of the five anatomical corneal layers, i.e., epithelium, Bowman's layer, corneal stroma consisting of regularly arranged collagen fibrils interspersed with keratocytes, Descemet's membrane, and endothelial cells, in relation to corneal transplantation. In particular, it outlines regional and age-related differences in structure, biomechanical properties, mechanisms of wound healing and restoration of corneal transparency, causes of stromal haze, cleavage planes and interface characteristics in lamellar transplantation techniques, and reasons for graft failure. Patterns of corneal innervation and the molecular mechanisms of antiangiogenic and immune privileges, which determine the success of allogeneic corneal transplantation, are described in addition.

Keywords

Epithelium • Bowman's layer • Stroma • Descemet's membrane • Endothelium • Anatomy • Histology • Electron microscopy • Corneal innervation • Immune privilege

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The cornea is a transparent avascular connective tissue covering the front part of the eye. It is one of the most highly innervated tissues in the body, protects the interior eye from penetration by

J. Hjortdal (ed.), *Corneal Transplantation*, DOI 10.1007/978-3-319-24052-7_2

foreign bodies and pathogens, and contributes, together with the tear film, two-thirds of the eye's refractive power. It is nourished and provided with oxygen anteriorly by tears and posteriorly by the aqueous humor. It has to maintain the intraocular pressure and to withstand the forces applied by the extraocular muscles during eye movement. Corneal shape and curvature, which are relevant for refraction, are achieved by the specific arrangement of collagen lamellae in the stroma, and corneal transparency, which is critically important for vision, is the result of many factors including avascularity of the corneal tissue, the integrity of the corneal epithelium, and the regular arrangement of the extracellular and cellular components of the stroma, which in turn depends on the state of hydration regulated by the corneal endothelium [18].

Corneal transplantation remains the mainstay treatment for patients with corneal blindness. The success of allogeneic corneal transplantation benefits from the immunologically privileged state of the cornea [55]. Penetrating keratoplasty (PKP) has been the gold standard for corneal transplantation for almost a century. Over the past decade, corneal transplantation has evolved rapidly from full-thickness PKP toward partial-thickness or lamellar keratoplasty to only remove and replace damaged or diseased layers of the cornea allowing more rapid visual rehabilitation and reduced rates of rejection [4, 63, 69]. Current developments in lamellar keratoplasty include deep anterior lamellar keratoplasty (DALK) for anterior corneal disorders [3], such as keratoconus or stromal scars, as well as Descemet's stripping (automated) endothelial keratoplasty (DSEK, DSAEK) and Descemet's membrane endothelial keratoplasty (DMEK) for posterior corneal disorders, such as Fuchs' corneal endothelial dystrophy [47, 61]. Lamellar corneal surgery is in continuous evolution, which requires an understanding of the structural, biomechanical, and histological characteristics of corneal layers involved. In this chapter, we describe the anatomy and physiology of the human cornea in order to provide the structural basis for the subsequent chapters on corneal transplantation techniques.

Gross Anatomy and Physiology

In adults, the cornea has a horizontal diameter of 11.0–12.0 mm, a vertical diameter of 10.0–11.0 mm, and a thickness of approximately 500–550 μm at the center, which gradually increases to 600–800 μm toward the periphery [18]. The cornea has an aspheric anterior surface being steeper in the center and flatter in the periphery. Average refractive power is 43.25 diopters, average radius of curvature is 7.8 mm, and the corneal index of refraction is 1.376. It is composed of five anatomical layers, i.e., corneal epithelium, Bowman's layer, corneal stroma, Descemet's membrane, and corneal endothelium (Fig. 2.1a). Besides these conventional layers of the cornea, an additional pre-Descemet's stromal layer has been recently described [19], which has, however, been subsequently disproved by a multicenter study [66].

Confocal microscopy with the Heidelberg retina tomograph (HRT) II and Rostock Cornea Module can be used for in vivo imaging of all anatomical layers and corneal cell types including nerve plexi and immune cells (Fig. 2.2).

Corneal Epithelium

The epithelial surface of the cornea represents the physical barrier to the outer environment and an integral part of the smooth tear film–cornea interface that is critical for the refractive power of the eye. It is responsible for protecting the eye against loss of fluid and invasion of foreign bodies and pathogens and for absorbing oxygen and nutrients from the tear film.

The corneal surface is covered by a stratified, nonkeratinizing, squamous epithelium, about 50 μm in thickness, comprising 5–7 cell layers collectively. It can be structured into three layers, the superficial or squamous cell layer, the suprabasal wing cell layer, and the basal columnar cell layer (Fig. 2.1b) [18, 60]. Desmosomes promote strong adhesion between cells of all epithelial layers. The superficial layer is formed by 2–3 layers of flat polygonal cells, which form intercellular tight junctions to provide an effective barrier

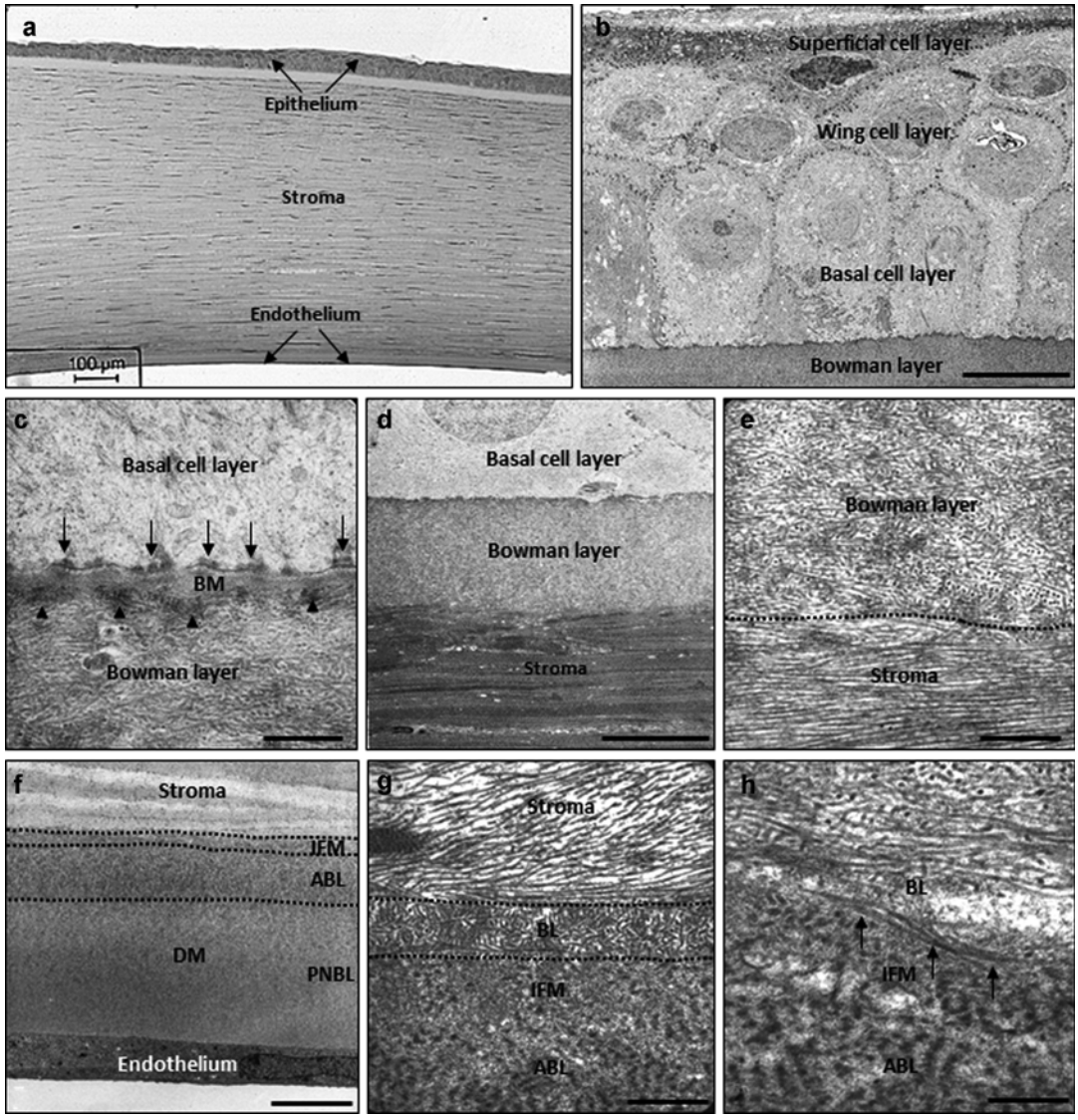

Fig. 2.1 Light (**a**) and transmission electron (**b**–**h**) micrographs of anatomical corneal layers. (**a**) Semithin cross section of the cornea showing three cellular layers, i.e., epithelium, stroma with keratocytes, and endothelium. (**b**) Corneal epithelium showing three layers, the superficial or squamous cell layer, the suprabasal wing cell layer, and the basal columnar cell layer resting on Bowman's layer. (**c**) Anchoring complexes formed by hemidesmosomes (*arrows*) and anchoring fibrils (*arrowheads*) mediating attachment of basal epithelial cells to basement membrane (*BM*) and Bowman's layer. (**d**) Bowman's layer representing the most anterior portion of the corneal stroma. (**e**) Interface (*dotted line*) between Bowman's layer and corneal stroma showing differing arrangement of collagen fibrils. (**f**) Descemet's membrane (*DM*), the basement membrane of the corneal endothelium, being composed of interfacial matrix (*IFM*), anterior banded layer (*ABL*), and posterior nonbanded layer (*PNBL*). (**g**) "Bowman's-like layer" (*BL*), a meshwork of randomly arranged collagen fibrils at the interface between Descemet's membrane and stroma. (**h**) Connecting collagen fibrils (*arrows*) projecting from "Bowman's-like layer" into the interfacial matrix zone (*IFM*) of Descemet's membrane (magnification bars = 100 µm in **a**; 15 µm in **b** and **d**; 5 µm in **f**; and 0.5 µm in **c**, **e**, **h**) (**e**, **g** Reproduced from Schlötzer-Schrehardt et al. [66], and **h** Reproduced from Schlötzer-Schrehardt et al. [64], with permission from Elsevier)

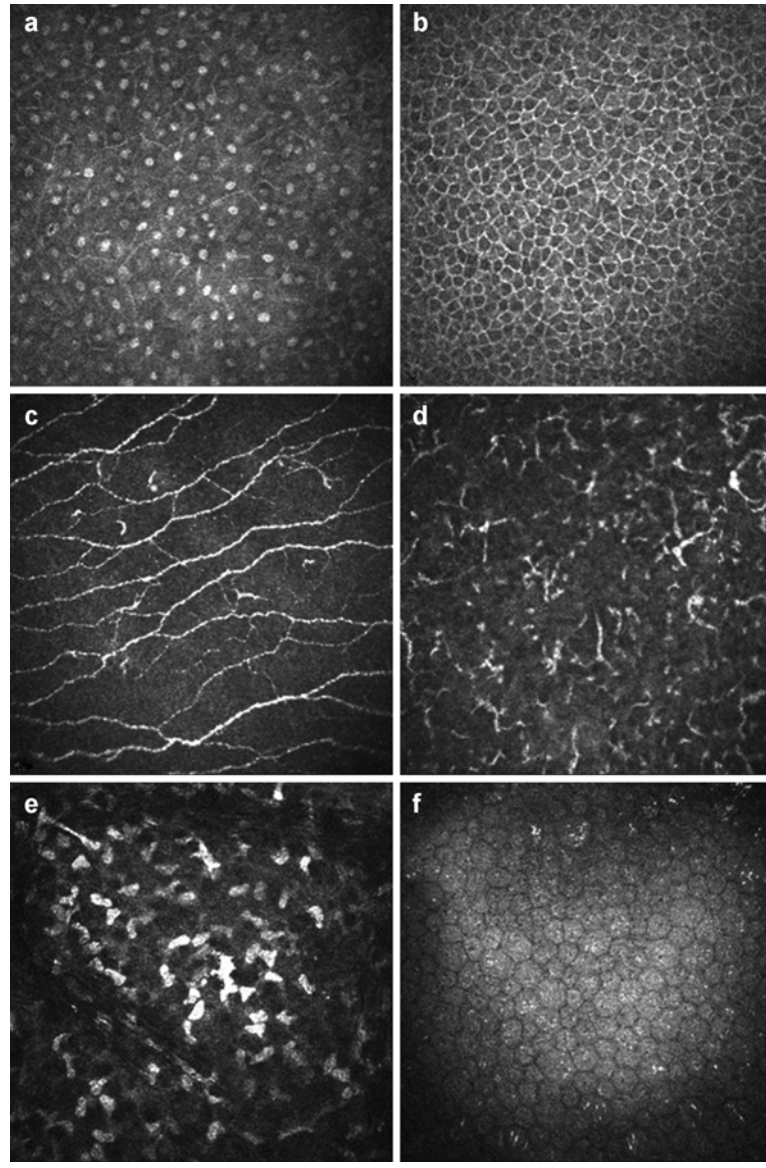

Fig. 2.2 In vivo confocal microscopy of corneal layers using the Heidelberg retina tomograph (HRT) II and Rostock Cornea Module in a 52-year-old patient at the levels of suprabasal epithelium (**a**), basal epithelium (**b**), subbasal nerve plexus (**c**), intraepithelial dendritic cells (**d**), stromal keratocytes (**e**), and corneal endothelium (**f**) (By courtesy of Christina Jacobi, Erlangen)

and numerous surface microvilli, which increase the cellular surface area and enhance oxygen and nutrient uptake from the tear film. The microvillar glycocalyx coat interacts with and helps to stabilize the pre-corneal tear film, which is composed of three layers: a superficial lipid layer to provide protection from evaporation, an aqueous layer providing nutrients and oxygen supply to the corneal epithelium, and a basal mucin layer, which interacts closely with the epithelial cell glycocalyx to allow lubrication of the ocular surface and spreading of the tear film with each eyelid blink [26, 70]. The tear film also supplies immunological and growth factors that are critical for epithelial health, proliferation, and repair, and defects in tear film, e.g., in neurotrophic keratopathy after corneal surgery, can cause epithelial wound healing problems and surface inflammation. The wing cell layer is formed by 2–3 layers of wing-shaped cells which have laterally interdigitated cell membranes with numerous desmosomes (Fig. 2.2a). The basal layer consists of a single

layer of columnar cells (Fig. 2.2b), which are attached to the underlying basement membrane by hemidesmosomes (Fig. 2.1c). The epithelial basement membrane has a critical role in corneal wound healing, because defects in this delicate layer allow penetration of growth factors from the epithelium into the stroma [71]. Corneal epithelial adhesion to Bowman's layer is maintained by an anchoring complex including anchoring fibrils (type VII collagen) and anchoring plaques (type VI collagen) (Fig. 2.1c) [25]. Abnormalities in these anchoring complexes may result clinically in recurrent corneal erosions or nonhealing epithelial defects.

Besides epithelial cells, there are numerous nerve endings in between the cells (Fig. 2.2c), which exert important trophic influences on the corneal epithelium and which have been estimated to amount to a density of 7000 nociceptors per mm^2, which is 400 times more than in the skin [52]. Mechanical stress to these nerves, such as in bullous keratopathy, can therefore cause tremendous pain. Furthermore, resident MHC class II-expressing cells, i.e., CD11c+-dendritic cells and CD207+-Langerhans cells, were identified in the human basal epithelium and anterior stroma (Fig. 2.2d), which are capable of rapidly mobilizing to the site of epithelial trauma and viral infection within the cornea [37]. The corneal epithelium itself exerts strong anti-inflammatory and antiangiogenic properties, and transplantation of donor corneas without the epithelium, e.g., after abrasion, leads to increased postoperative inflammation and neovascularization [17].

Corneal epithelial cells routinely undergo apoptosis and desquamation from the surface. This process results in complete turnover of the corneal epithelial layer every 5–7 days as deeper cells replace the desquamating superficial cells in an orderly, apically directed fashion. Two populations of cells, the basal epithelial cells and limbal stem cells, help renew the epithelial surface [7]. The epithelial stem cells and their progenitors are located at the bottom of the palisades of Vogt at the corneoscleral limbus [15]. Depletion of this stem cell reservoir, e.g., after chemical burns, can cause severe ocular surface disease and significant visual deterioration, a condition known as limbal stem cell deficiency [1]. In these cases, epithelium of conjunctival phenotype may replace the corneal surface. Transplantation of limbal autografts or allografts [35] and ex vivo expanded limbal epithelial stem cells are established therapeutic strategies to regenerate the damaged corneal surface [67].

The corneal epithelium responds to injury in three phases, i.e., migration, proliferation, and differentiation with reattachment to the basement membrane [80]. Following injury, cells adjacent to an epithelial defect migrate to cover the wound within few hours. Following wound closure, basal epithelial and limbal stem cells proliferate and differentiate to repopulate the epithelium. In the final phase, hemidesmosomes replace focal contacts in order to anchor the basal epithelial cells tightly to the basement membrane and stroma. If the basement membrane remained intact, a tight adhesion is established in only a few days. If the basement membrane was damaged, its repair can take up to 6 weeks. During this time, the epithelial attachment to the newly deposited basement membrane tends to be unstable and weak, and the regenerated epithelium is very susceptible to damage. Following PKP, re-epithelialization is usually observed within 1 week, although morphological abnormalities, detected by specular microscopy, may persist up to 6 months postoperatively [74]. Corneal grafts showed some recovery of the subbasal nerve plexus, at least in the graft periphery, but not complete recovery of function [68].

Bowman's Layer

Bowman's layer represents the most anterior, acellular portion of the corneal stroma (Fig. 2.1d). It is approximately 8–12 μm thick and structurally composed of randomly oriented collagen fibrils, 20–25 nm in diameter, consisting of collagen types I, III, V, and VI (Fig. 2.1e) [77]. Its thickness has been reported to decline with age by 0.06 μm per year, thus losing one-third of its thickness between 20 and 80 years of age [23]. Unmyelinated nerve axons penetrate Bowman's layer to terminate within the epithelium. The

functional role of Bowman's layer is not completely known, but it is believed to serve as a barrier that protects corneal stroma and nerves from traumatic injury. In addition, it has been suggested to ensure epithelial anchorage to the corneal stroma and helps to maintain the shape and tensile strength of the cornea. Bowman's layer also functions as an important UV shield protecting the inner eye and a nearly insurmountable barrier against the invasion of epithelial tumors into the corneal stroma [60].

When disrupted, Bowman's layer does not regenerate but forms a scar. Therefore, diseases or surgical procedures leading to defects in Bowman's layer increase the risk for corneal ruptures and ectasias. On the other hand, sutures have to extend through Bowman's layer to ensure tight and effective suturing [17].

Corneal Stroma

The stroma is the thickest layer of the cornea measuring approximately 500 μm in width and represents a dense avascular connective tissue of remarkable and unique regularity. It is composed of regularly arranged bundles of collagen fibrils embedded in a glycosaminoglycan-rich extracellular matrix, which are interspersed with flattened fibroblast-like cells termed keratocytes [18]. Collagen organization in the stroma is crucial to corneal functions such as light transmission and maintenance of corneal curvature, tensile strength, and rigidity [27]. The individual collagen fibrils, being mainly composed of collagen types I and V, are extremely uniform in diameter measuring about 25–30 nm [38, 44] and are organized into approximately 250–300 2 μm thick sheets or lamellae. Regular spacing of fibrils within these lamellae is maintained by interactions of collagens with proteoglycans forming bridges between the fibrils [53]. The major proteoglycans of the stroma are keratan sulfate proteoglycans, such as keratocan and lumican, and chondroitin/dermatan sulfate proteoglycans, such as decorin [27, 48], which also regulate stromal hydration by means of their ability to bind water molecules. The collagenous lamellae form a highly organized ply, with adjacent lamellae being oriented at right angles, although there are organizational differences in the collagen bundles between anterior and posterior stroma [45]. In the anterior third of the stroma, lamellae are oriented more obliquely, mediating a tighter cohesive strength and rigidity, which appears particularly important in maintaining corneal curvature [51], whereas in the posterior two-thirds, lamellae run in parallel to the corneal surface. These differences in stromal collagen organization may also explain why the anterior stroma resists changes to stromal hydration much better [46] and why surgical dissection in a particular plane is easier in the posterior depths of the stroma, e.g., in DALK. Moreover, the peripheral stroma is thicker than the central stroma, and the collagen fibrils may change direction to form a circumferentially oriented network, which is thought to be pivotal in maintaining corneal stability and curvature, as they approach the limbus [45]. Any disturbance of this fine-tuned arrangement, either by deposition of abnormal extracellular matrix, e.g., deposition of mucopolysaccharides in macular corneal dystrophy, or the irregular arrangement of collagen fibrils in stromal scars, can cause corneal opacity.

The collagen lamellae are interspersed with flattened stellate keratocytes, which are interconnected by gap junctions and arranged in a circular, corkscrew pattern forming a coherent network (Fig. 2.2e) [50, 59]. The density of keratocytes in the anterior stroma is 20,000–24,000 cells/mm^2 and the density decreases posteriorly. Keratocytes are metabolically active cells involved in synthesis and turnover of extracellular matrix components, i.e., collagen molecules and glycosaminoglycans. They contain water-soluble proteins, corneal "crystallins," which appear to be responsible for reducing backscatter of light from the keratocytes and for maintaining corneal transparency [32]. In addition, sensory nerve fibers are present in the anterior stroma, which are cut during PKP leading to a mild neurotrophic keratopathy [68], and MHC class II antigen-presenting cells, which seem to migrate out of the cornea during organ preservation, thereby explaining the reduced rates of immune rejections of longer organ-cultured grafts [17].

Following injury to the stroma, e.g., in PKP, keratocytes adjacent to the wound undergo apoptosis [71, 78, 80]. About 24 h after wounding, the remaining keratocytes begin to proliferate and transform into activated fibroblasts, which migrate into the wound region and produce extracellular matrix components, a process that may last up to 1 week. Inflammatory cells, including monocytes, granulocytes, and lymphocytes, infiltrate the stroma from the limbal blood vessels. Fibroblasts transform into myofibroblasts, which contract the wound and secrete extracellular matrix, a process which may last up to 1 month. Deposition of large amounts of disorganized extracellular matrix may lead to loss of corneal transparency causing stromal haze. Matrix remodeling by repopulating keratocytes thereby restoring transparency is the last phase of stromal wound healing and can last for years [71]. In penetrating or lamellar keratoplasty, a rather complete wound healing response is usually noted at donor-recipient interfaces. However, abnormal collagen fiber size and arrangement, indicating incomplete stromal wound remodeling and persistence of fibrotic scar tissue, have been observed within the graft margin after PKP [11]. Similarly, the presence of fibrocellular tissue, probably derived from myofibroblasts, has been found in the graft–host interface in about 20 % of corneas after DSAEK failure [79]. Therefore, stroma-to-stroma interface haze may occur in DALK or DSAEK and can degrade visual acuity, even if the microkeratome or femtosecond laser is used to achieve a smooth resection [4].

Recently, the existence of a novel, previously unrecognized layer of the pre-Descemet's corneal stroma, which can be separated by air injection into the stroma during DALK using big-bubble technique, has been reported [19]. This distinct layer was reported to measure about 10 μm in width and was characterized to lack any keratocytes and to show a pronounced immunostaining for collagen types III, IV, and VI [20]. However, the description of this hypothesized new anatomic layer was critically commented on in the literature and eventually refuted by a detailed ultrastructural reinvestigation of the human corneal stroma [66]. The findings of this three-center study provided evidence that there is no distinctive acellular pre-Descemet's stromal zone justifying the term "layer" apart from a thin (0.5–1.0 μm) intermediary "Bowman's-like zone" of randomly arranged collagen fibers at the Descemet's membrane–stromal interface (Fig. 2.1g). The collagen fibers of this intermediary layer partly extend into Descemet's membrane serving a connecting function (Fig. 2.1h). Stromal keratocytes were found to approach Descemet's membrane up to 1.5 μm (mean 4.97 ± 2.19 μm) in the central regions and up to 4.5 μm (mean 9.77 ± 2.90 μm) in the peripheral regions of the cornea. The intrastromal cleavage plane after pneumodissection, which seemed to occur at multiple stromal levels along rows of keratocytes offering the least resistance to mechanical forces, was obviously determined by the variable distances of keratocytes to Descemet's membrane. Consistently, the residual stromal sheet separated by air injection into the stroma varied in thickness from 4.5 to 27.5 μm, being usually thinnest in the central and thickest in the peripheral portions of the bubble (Fig. 2.3d). This phenomenon has been well documented as "residual stroma" in previous studies, providing evidence that the big-bubble technique in DALK is not consistently a Descemet-baring technique [31, 36, 43].

Descemet's Membrane

Descemet's membrane represents the thickened (10–12 μm), specialized basement membrane of the corneal endothelium consisting of collagen types IV, VIII, and XVIII and non-collagenous components including fibronectin, laminin, nidogen, and perlecan as well as dermatan, keratan, heparan, and chondroitin sulfate proteoglycans [64]. Apart from providing structural integrity of the cornea, Descemet's membrane has been suggested to play a role in several important physiological processes including corneal hydration, endothelial cell differentiation and proliferation, and maintenance of the corneal curvature. It is composed of an anterior banded (fetal) layer, approx. 3 μm in thickness, and a

posterior nonbanded (postnatal) layer that gradually thickens with age reaching up to 10 µm in elderly individuals (Fig. 2.1f) [33, 54]. In the periphery, Descemet's membrane forms wart-like excrescences (Hassall-Henle warts) and merges into the trabecular meshwork beams. The thickened fusion site, known as Schwalbe's line, is a gonioscopic landmark that defines the end of Descemet's membrane and the beginning of the trabecular meshwork.

Descemet's membrane is attached to the corneal stroma by a narrow (about 1 µm thick)

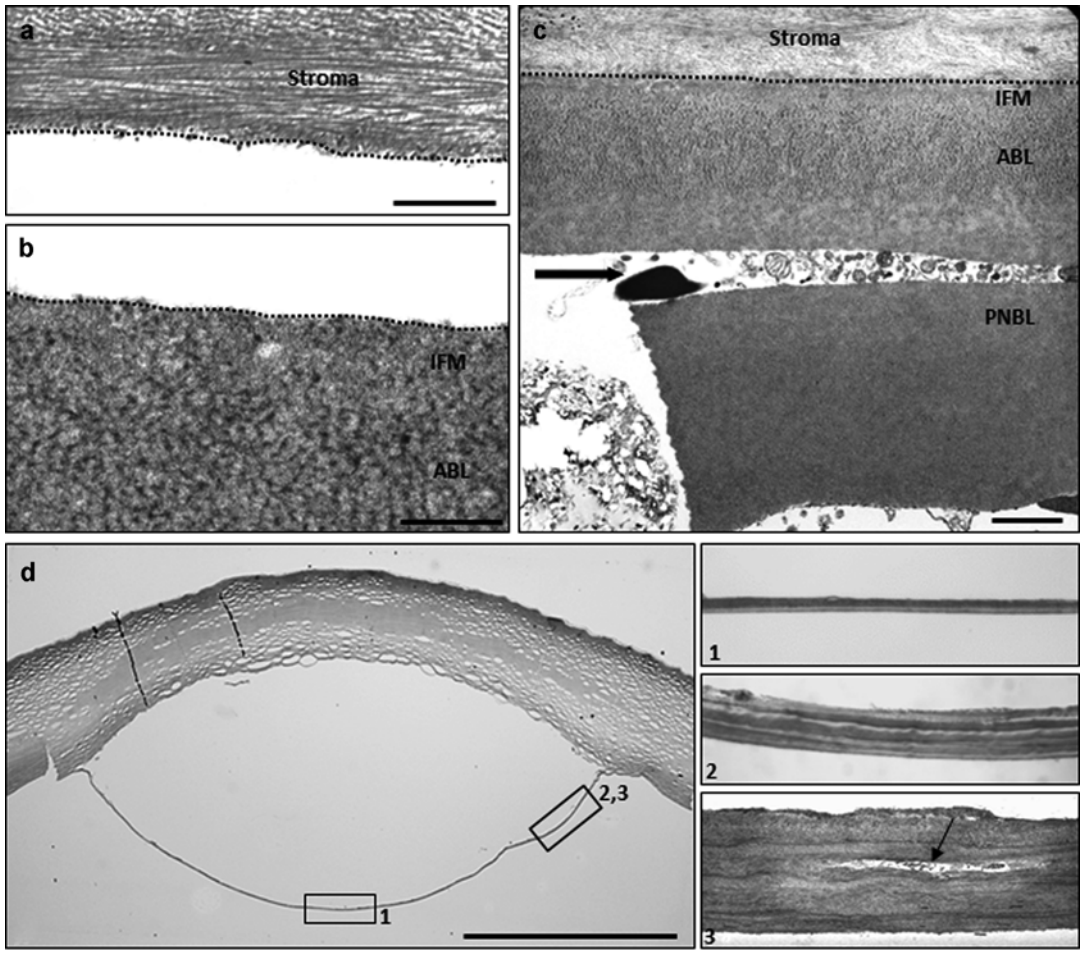

Fig. 2.3 Light (D) and transmission electron (**a**–**c**, **e**–**g**) micrographs showing cleavage planes in lamellar keratoplasty and usability of Descemet's membrane ultrastructure as indicator of endothelial function. (**a**, **b**) Physiological cleavage plane between the posterior stromal collagen lamellae (**a**) and interfacial matrix zone (*IFM*) of Descemet's membrane in DMEK. (**c**) Lamellar splitting of Descemet's membrane between anterior banded layer (*ABL*) and posterior nonbanded layer (*PNBL*) (*arrow*) of a donor cornea with unsuccessful stripping due to strong adhesion of Descemet's membrane to the corneal stroma (*dotted line*). (**d**) Semithin section of a donor cornea showing big-bubble formation after air injection into the corneal stroma; the *boxed areas* (*1*, *2*, *3*) are shown in higher magnification on the left illustrating the stromal sheet forming the bubble wall of variable thickness with remnants of keratocytes (*arrow*). (**e**–**g**) Ultrastructural analysis of Descemet's membrane showing normal structure (**e**), abnormal collagen inclusions (*arrows*) within posterior nonbanded layer (*PNBL*) (**f**), and a posterior collagenous layer (*PCL*) deposited onto a normal Descemet's membrane (**g**) (*IFM* interfacial matrix, *ABL* anterior banded layer; magnification bars = 2 mm in **d**; 2.5 µm in **c**, **e**, **f**, **g**; and 1 µm in **a** and **b**) (**a**, **c**, **e** reproduced from Schlötzer-Schrehardt et al. [65], and **c** reproduced from Schlötzer-Schrehardt et al. [66], with permission from Elsevier)

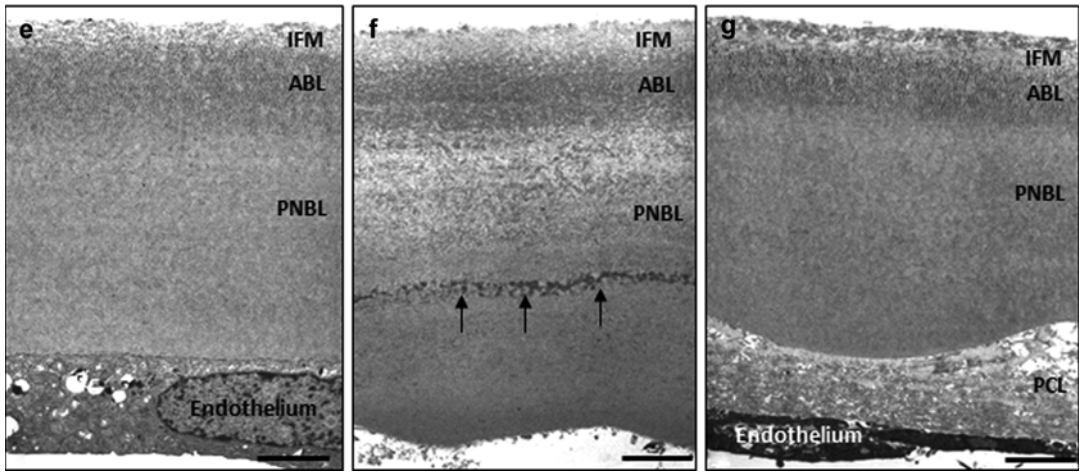

Fig. 2.3 (continued)

transitional zone of amorphous extracellular matrix termed the "interfacial matrix," which contains increased amounts of adhesive glycoproteins such as fibronectin (Fig. 2.1f, g) [64]. Connecting collagen fibers projecting from the "Bowman's-like" stromal layer into this interfacial matrix zone further promote anchorage (Fig. 2.1h). Extracellular matrix complexes formed by keratoepithelin (transforming growth factor β-induced) and collagen type VI are also involved in maintaining adherence at Descemet's membrane–stroma interface. Adhesive forces appear to be slightly stronger in the central than in the peripheral parts of the cornea. Nevertheless, Descemet's membrane can be separated relatively easily from the adjacent stroma, which is utilized during DMEK surgery by a transient splitting of the physiological interface between the interfacial matrix of Descemet's membrane and posterior stroma in both the donor's and recipient's corneas (Fig. 2.3a, b). The high optical and structural quality of this interface remains after reattachment of the donor's Descemet's membrane to the recipient's corneal stroma, allowing for superior functional results after DMEK when compared to other lamellar transplantation techniques producing a stroma–stroma interface [72]. Although Descemet's grafts can be manually prepared from donor corneas with a high level of reproducibility (98 %) using an appropriate technique [40], a small percentage of donor corneas (2 %) reveals individual tissue properties, which may complicate and even prevent proper Descemet's stripping due to exceptionally strong adhesiveness of Descemet's membrane to the posterior stroma [65]. The morphological cause underlying the resistance of Descemet's membrane to proper stripping appears to be ultrastructural or biochemical abnormalities along Descemet's membrane–stroma interface, and any attempts to strip Descemet's membrane result in its lamellar splitting, mostly between anterior banded and posterior nonbanded layers (Fig. 2.3c). Lamellar splitting can also occur during stripping of recipient Descemet's membrane, particularly in patients with Fuchs' dystrophy leaving residual fetal Descemet's membrane retained on the recipient DSAEK or DMEK interface [13, 49]. This phenomenon may be one frequent cause for failure of graft adherence to the recipient posterior corneal surface [76].

DMEK is dependent on the biomechanical elastic properties of Descemet's membrane, which scrolls up with the endothelium on the outside upon removal from the stroma. Age, which is known to correlate with thickness of Descemet's membrane [54], has a significant impact on the degree of scrolling. Thinner grafts from younger donors (<50 years) have a tendency for pronounced curling after stripping making subsequent unfolding in the recipient's

anterior chamber more difficult. Thus, corneas from donors older than 55 years of age are preferably used for DMEK graft preparation [41]. The exact reasons why grafts adhere to the recipient bed are not known. Physical, biochemical, and physiological mechanisms such as endothelial pump function have been proposed. It has been shown that the use of organ-cultured grafts exhibiting modified biochemical properties and a larger removal of Descemet's membrane of the host promote graft adhesion [42, 73].

Although intraoperative manipulation may be a frequent cause of primary graft failure, the majority of failed DMEK grafts revealed ultrastructural signs of preoperative endothelial dysfunction, i.e., inclusions of abnormal collagenous material within Descemet's membrane proper [14]. Due to its continued appositional growth with age [54], Descemet's membrane provides a lifelong record of pathological events and endothelial function [33], and any deposition of abnormal extracellular material is indicative of previous stress or damage to the endothelial cells (Fig. 2.3e, f). Thus, a preexisting subclinical corneal endothelial dysfunction, as indicated by abnormal inclusions within Descemet's membrane, may have contributed to primary DMEK failure [14]. In contrast, a posterior fibrous layer, mainly consisting of collagen types I and IV and fibronectin, may be produced and deposited on the posterior surface of an otherwise normal Descemet's membrane by attenuated endothelial cells that underwent transdifferentiation into (myo)fibroblast-like cells (Fig. 2.3g) [75]. The formation of an abnormal posterior collagenous layer is the result of a final common pathway following endothelial dysfunction and damage, including intra- or postoperative trauma, and has been also reported to contribute to failed lamellar and penetrating grafts [28, 39].

Corneal Endothelium

The innermost layer of the cornea, the corneal endothelium, is a single layer of cuboidal cells, which have a critical role in maintaining corneal hydration and thus transparency (Fig. 2.1f). The cells, which form a hexagonal honeycomb-like mosaic when viewed from the posterior surface (Fig. 2.2f), are 5–6 µm in height and 18–20 µm in diameter. Some cells have apical cilia, which play a role in morphogenesis and repair of the endothelial monolayer [8]. Their lateral surfaces are highly interdigitated and possess apical junctional complexes comprising both gap and tight junctions forming a leaky barrier and allowing paracellular movement of fluid and substances from the aqueous into the cornea. The basal surface of the endothelium contains hemidesmosomes that promote adhesion to Descemet's membrane. The endothelial layer is responsible for dehydration of the cornea and maintenance of corneal transparency by pumping water out of the corneal stroma [24]. The dehydration process is described by the "pump-leak hypothesis," in which leakage of solutes and nutrients from aqueous humor to superficial layers of the cornea is counteracted by pumping water in the opposite direction. This passive bulk fluid movement is fueled by the energy-requiring processes of transporting ions to generate the osmotic gradient. The most important ion transport systems are the membrane-bound Na,K-ATPase and the intracellular carbonic anhydrase, producing a net flux of ions from the stroma to the aqueous humor [9].

The number of endothelial cells decreases with age, trauma, inflammation, surgery, and disease processes such as Fuchs' endothelial dystrophy. Endothelial cell density at birth is approximately 3500–4000 cells/mm^2, decreasing gradually at an average rate of 0.6 % per year to 2500 cells/mm^2 at age 50 and 2000 cells/mm^2 at age 80 [6]. Endothelial cells of the human cornea have a low proliferative capacity and lost cells are replaced by spreading of adjacent cells resulting in an increase in cell size (polymegathism) and an increase in variation of cell shape (pleomorphism). With increasing cell loss, the pump and barrier functions of the endothelium may be compromised. A density lower than 500 cells/mm^2 may lead to endothelial decompensation and corneal edema with concomitant loss of transparency. Endothelial cell loss following penetrating and lamellar keratoplasty has been reported to average about 70 % in PKP, about

50 % in DSEK, and about 40 % in DMEK at 5 years [21]. However, remaining endothelial cells can also migrate along a density gradient and cover denuded areas [30].

In contrast to the in vivo situation, human endothelial cells retain their proliferative capacity in vitro and can proliferate in response to growth stimulation factors [34]. Thus, the use of ex vivo cultured human corneal endothelial cells may represent a potential future alternative to full-thickness or lamellar keratoplasty in the replacement of defective corneal endothelium. Preclinical studies applying corneal endothelial cell therapy are giving promising results [29, 56, 58, 62].

Corneal Innervation

The cornea is densely innervated by unmyelinated sensory nerve fibers derived from the trigeminal nerve, mainly via the long ciliary nerves. About 70 main nerve bundles enter the peripheral cornea in a radial manner and move centrally in the anterior one-third of the stroma. They divide into smaller branches and penetrate Bowman's layer to form the subepithelial or subbasal nerve plexus at the interface between Bowman's layer and the corneal epithelium (Fig. 2.2c). Individual fibers penetrate all epithelial layers and terminate in the superficial layers. It is estimated that there are approx. 7000 nociceptors per mm^2 in the human corneal epithelium [52]. The density of nerve endings per unit area is 400 times higher than in the skin, making the cornea one of the most densely innervated tissues in the body. In conformity with the density of nerve endings, corneal sensitivity increases from the limbus to the central cornea. Corneal nerves release neuropeptides, such as substance P and calcitonin gene-related peptide (CGRP), which have important trophic functions on the corneal epithelium and stimulate epithelial wound healing [22]. Loss of corneal sensory innervation can lead to neurotrophic keratopathy, involving epithelial defects, poor wound healing, and ulcers [10]. Corneal nerves, which can be visualized using confocal microscopy with the Heidelberg retina tomograph (HRT) II and Rostock Cornea Module, show morphological alterations associated with a reduction in central corneal sensation early after DMEK [12]. However, a complete recovery of corneal nerve density and function up to preoperative values occurs within 4–10 months. In contrast, subbasal nerve density does not recover to normal values throughout 30 years after PKP [57].

Corneal Immune Privilege

Since corneal avascularity is an essential factor for corneal transparency, the cornea has developed strategies to maintain avascularity, a phenomenon termed "corneal antiangiogenic privilege" [5, 17]. Several antiangiogenic factors have been shown to contribute to corneal avascularity, including pigment epithelium-derived factor (PEDF), thrombospondins, and receptors binding and inactivating angiogenic growth factors like vascular endothelial growth factor (VEGF). The strong expression of VEGF receptor 3 on the corneal epithelium, which is normally expressed on vascular endothelial cells, seems to be especially potent.

The cornea has also developed strategies to minimize inflammatory reactions, a phenomenon termed "corneal immune privilege." The success of allogeneic corneal transplantation benefits from this property, which is attributed to multiple anatomical, physiological, and immunoregulatory factors [55]. For instance, absence of blood and lymph vessels in the graft bed is essential for graft survival. Thus, the molecular mechanisms of immune privilege are similar to those mediating avascularity, e.g., thrombospondin-1 is involved in both processes. Corneal epithelial and stromal cells secrete soluble factors, including VEGFR-2 and endostatin, which inhibit lymphangiogenesis and hemangiogenesis, thereby maintaining immune privilege [2]. The corneal endothelium also expresses membrane-bound molecules, such as Fas ligand (FasL), which defend against immune effector cells including T cells and components of the complement cascade. Another mechanism contributing to the

success of allogeneic corneal transplantation is "anterior chamber associated immune deviation (ACAID)," which is defined as the systemic downregulation of an immune response against antigens injected into the anterior chamber of the eye [55]. As a consequence, immune reactions against, e.g., donor endothelial antigens are less destructive.

Nevertheless, the central corneal stroma and epithelium are endowed with significant numbers of resident MHC class II-negative inflammatory and antigen-presenting cells, including dendritic cells and epithelial Langerhans cells as well as macrophages. These cells become activated and increase in numbers after contact lens use and inflammation (Fig. 2.2d), causing higher injection rates in inflamed high-risk recipient beds [16].

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Developments in Corneal Banking

3

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Abstract

Eye banks are currently advancing to decrease the unnecessary manipulation of tissues in the operating theatre and reduce the high surgical skill or risk quotient for surgeries like EK or ALK. Development in the tissue storage techniques, surgical devices for advanced and selective surgery, modification and manipulation of the tissues, and designing new methodologies for ocular health care are now becoming a part of the eye bank activities. Apart from the research and development, eye banks are now taking a lead in standardizing and validating new procedures also for the clinics. Precut and preloaded tissues may potentially reduce the overall intervention costs and surgery time and enhance the surgical outcomes in the future. Synthetic media are being evaluated for corneal storage at 31–37 °C (organ culture) to replace serum. Manufacturing of surgical devices using three-dimensional (3D) printing may further enhance the capabilities of the eye banks. Thus, we envision that eye banks are growing not only in the field of procuring the tissues for transplantation but also in the field of research and development.

Keywords

Eye bank • Lamellar keratoplasty • Corneal storage • 3D printing and surgical device

Introduction

Corneal transplantation (penetrating keratoplasty which refers to full thickness transplantation) is performed to replace the central part of a cornea which has lost its physiologic curvature and/or transparency due to related disease or disorder. Lamellar keratoplasty refers to a selective surgery to replace the corneal stroma (anterior

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J. Hjortdal (ed.), *Corneal Transplantation*, DOI 10.1007/978-3-319-24052-7_3

lamellar keratoplasty) or the posterior corneal stroma with the Descemet membrane-endothelium complex (posterior lamellar/endothelial keratoplasty). The field of endothelial keratoplasty (EK) has showed a dramatic impact in current eye banking and corneal transplantation with increasing number of EK procedures every year [1]. The speed of adoption of this new form of selective tissue replacement has been astonishing, and most eye banks are now pre-cutting, pre-bubbling, and pre-stripping tissues for DSAEK, DMEK, and DALK.

Role of Eye Banks

Eye banks recover, evaluate, and preserve donor corneas and other ocular tissues for surgical use. If the next of kin of the donor consents, then the tissues could also be used for research purposes to better understand the fundamentals of the human eye and develop strategies or drugs as potential treatment measures. Tissue removal and tissue processing in an eye bank should be carried out under strict aseptic techniques. The morphologic and functional status of the endothelium is the most important indicator of donor corneal suitability for transplantation. Corneal lenticules can be prepared by eye bank technicians who have demonstrated proficiency in sectioning the cornea. For keratolimbal allografts, the donor limbal epithelium must be protected from trauma and desiccation, and a conjunctival rim of 3–4 mm should be left [2]. Donor sclera is prepared from ocular tissue following excision of the corneoscleral button or from the donor globes.

Recent advances in the fields of eye banking, ophthalmology, and regenerative medicine are challenging the traditional activities of eye banks [3].

Screening of Donors

Transplantation of human cornea involves potential risks of transmission of host disease to recipient. In order to ensure safety, a set of contraindication has been established, after retrospective discoveries of transmission of disease, or on a cautionary basis (theoretical or significant risk of transmission). Making the criteria for donor screening a little more stringent for penetrating than for lamellar grafts.

Diseases with the potential of transmission by corneal transplantation comprise infections (local and/or systemic), hematologic malignancies, prion diseases, and corneal disorders, the latter being more related to quality than to safety issues. Metastatic neoplasia does not exclude from donation and transplantation. The European Eye Bank Association (EEBA) [4] and the Eye Bank Association of America (EBAA) [5] have established Minimum Medical Standards and Medical Standards, respectively (details can be found at www.europeaneyebanks.org or www.restoresight.org). The contraindications comprise a group of systemic disorders (including the death of unknown cause), intrinsic eye diseases, and prior intraocular or anterior segment surgery.

As set by the European Directives, the serological screening for HBV, HCV, HIV, and syphilis must be performed for every tissue that has been donated. Besides the search for antibodies of antigens, some nations require the execution of nucleic acid testing (NAT), a molecular technique developed to shorten the window period (the time between the infection and when a positive antibody/antigen can be revealed). Because of the window period, also the behaviors that may have put the donors at risk, such as intravenous drug use, must be evaluated.

Postmortem blood can be obtained from direct heart puncture or accessible blood vessels, within 24 h from death.

Despite the low incidence, transmissible spongiform encephalopathies, such as Creutzfeldt-Jakob disease, have been transmitted via corneal transplantation. The disease is progressive and fatal. For this reason, any donor who died with neurological symptoms, or degenerative neurological conditions, are excluded from donation.

There has not been any report of a systemic malignancy transmission following keratoplasty. A single case of ocular retinoblastoma transmission has been reported in 1939 and has justified the exclusion of donors with systemic lymphoproliferative disorders, documented ocular malignancies, and retinoblastoma.

It is important to document The source of information which includes pathologist, medical records, attending medical and nursing staff, family members or other relevant persons close to the deceased, family doctor, postmortem report.

Early-stage anterior and posterior membrane dystrophies and keratoconus may escape detection. A thorough tissue evaluation in the eye bank may minimize these events.

Age criteria for cornea donors are not well defined and vary between eye banks and surgeons. The small diameter, thinness, and elasticity of the cornea excised from an infant eye may cause technical problem for the surgeon. In general, the diagnosis of the recipient and the surgeon's experience are more important than donor age in determining the long-term graft clarity.

Ocular tissues should be recovered as soon as possible after death. A short postmortem interval warrants a higher yield of suitable corneas and limits endothelial loss during storage.

The donor's eyelids should be kept wet and closed until the retrieval. Elevating the donor's head prevents pooling of blood in the head and decreases the incidence of bleeding and swelling in the eye region following enucleation.

Ocular Tissue Removal

The removal of ocular tissue for surgical use must minimize the endothelial cell loss and contamination, maximize the number and quality of cells that are ultimately grafted, and should not alter the appearance of the donor.

After the physical inspection of the donor, the enucleator, with the aid of a penlight, should examine the periorbital and orbital tissues, and the anterior segment of the eye, for pathologic findings such as mucopurulent material, congenital or acquired corneal abnormalities, or signs of intraocular surgery.

The donor's head must be kept elevated throughout the procedure and the eyelids must be gently opened to allow excision of the cornea and conjunctival sac of each eye. The eye is washed with sterile phosphate-buffered saline (PBS) prior to the procedure, iodine solution, followed by PBS is used to prevent corneal toxicity. Then, a preparation of the operative area using sterile gauze moistened in the iodine solution should be performed, starting at the medial canthus of the upper closed eyelid and moving out, around and below the lid, over the bridge of the nose, in an ever-widening circular pattern. The donor is then draped to create a sterile field at the operative site.

The upper eyelid of the donor's right eye is gently opened with sterile gauze, and the closed lid speculum is inserted, taking care that the cornea is left untouched. The conjunctiva is grasped with the forceps, near the lateral edge of the cornea at the limbus, and cut using the microsurgery scissors, continuing 360° all the way around the cornea, removing the conjunctiva as far as possible. Closed, straight scissors are inserted under the conjunctiva, and a blunt dissection is performed by gently opening the blades. This will separate any adhesions between the conjunctiva and the anterior globe. The conjunctival remains are carefully scrapped using a scalpel blade from the limbus.

Enucleation

Using a muscle hook, the rectus muscles are exposed and severed where they meet the sclera. The lateral rectus must be severed last, leaving a 5 mm stump on the sclera. The stump is gasped with a hemostat, and the globe is lifted upward with the aid of enucleation scissors. The optic nerve is identified and severed with the enucleation scissors, leaving a 5–10 mm stump. The globe is then lifted from the socket with the hemostat clamped to the lateral rectus muscle, while cutting away any remaining connective tissue. The globe is wrapped in sterile gauze with the cornea facing up, and a small amount of PBS is poured over the cornea to moisten it.

The globe is then placed in the eye jar, carefully inserting at least four rectangular, sterile ophthalmologic tampons between the gauze and the sides of the container. Once moistened, the tampons will swell, keeping the globe in position.

In Situ Corneoscleral Rim Excision

Without perforating the choroid, a scleral incision is performed using a scalpel, approximately 4 mm from, and parallel to, the limbus. The incision is extended 360° with microsurgery scissors, taking care to remain at least 4 mm from the limbus and avoid perforating the underlying uveal tissue.

The removal is completed using one pair of small forceps to hold the scleral rim and a second set of forceps, to push the ciliary body choroid downward and away from the corneoscleral button. The remaining adhesions can now be gently separated from the corneoscleral button, avoiding distortion of the cornea shape with excessive traction. The posterior chamber of the donor eye must be examined to check the presence of the natural crystalline lens.

Donor Reconstruction

After enucleation, a moistened piece of gauze, rolled into a ball of the approximate dimensions of the globe, can be placed in the socket and covered with a plastic eye cap, or a plastic prosthesis can be applied. The conjunctival remains are used for overall covering.

The eyelids will be closed and gently manipulated to restore the donor's appearance. It is recommended to ask the mortuary staff to check the conditions of the donor later.

Tissue Processing

All eye bank manipulations are carried out in a laminar flow cabinet to maintain the aseptic conditions. Prior to any manipulation or evaluation, the ocular tissues and solutions should be allowed to reach normal room temperature, avoiding multiple repeated warming/cooling cycles.

Decontamination of Donor Eyes

The eyes are rinsed with sterile PBS, then immersed in sterile polyvinylpyrrolidone-iodine, sodium thiosulfate in PBS, and rinsed again in PBS, where they are left until the corneal excision is performed. This procedure has been reported to reduce the percentage of contaminated eyes.

Tissue Evaluation

The morphological and functional status of the endothelium is a key factor for the success of corneal grafting and therefore the most important indicator of donor cornea quality. As there is no direct functional test that can be used, the cornea must be evaluated by morphological parameters [5, 6]. A cornea suitable for transplantation is required to display some essential biological characteristics which include, a noninterrupted epithelial layer, a stroma free of opacities, absence of folds of he stroma and a viable and regular endothelium with a cell density above 2000–2200 cells/mm^2 [depending on the eye bank criteria].

Corneas from eligible donors with local eye disease affecting the corneal endothelium, or previous ocular surgery that does not compromise the corneal stroma, can be used for lamellar (anterior, posterior) or patch grafts.

In general, the slit lamp biomicroscopy of the anterior segment (enucleation) or the slit lamp examination of the cornea (*In situ* excision) is combined with specular microscopy (mostly in the USA) or with light microscopy [6].

Slit Lamp Examination

A 10× magnification with a wide slit of light for a general inspection of the corneoscleral rim is performed first. A more in-depth examination allows an evaluation such as epithelial defects, corneal scars/edema/arcus lipodes, infiltrates or foreign bodies, Descemet folds, corneal guttata, defects in the corneal endothelium or adequacy of the scleral rim.

Specular Microscopy

Specular microscopy can be performed on donor eyes or corneoscleral rims by non-contact, com-

puterized microscopes. Endothelial density can be estimated by a calibrated reticule or calculated by built-in software.

Light Microscopy

The endothelial mosaic can be visualized by exposing the cells to a hypotonic solution, which induces an enlargement of the intercellular spaces. The whole surface of the corneal epithelium, stroma, and endothelium can be scanned with an inverted or non-inverted phase contrast or bright field light microscope, at a magnification of 50×, 100× and 200×.

The number of endothelial cells is estimated at about 100× magnification, with the help of a calibrated grid (10 × 10 mm) mounted onto one eyepiece of the microscope. Absent or irregular swelling, associated with a grayish appearance of the cells, has been correlated to metabolic suffering of the corneal endothelium.

The presence of dead cells is studied exposing the endothelium to trypan blue. The trypan blue exclusion assay is a well-established method to test the endothelial cell viability (Fig. 3.1a) or membrane alteration(s), despite the staining not very specific for dead and necrotic cells (Fig. 3.1b). The presence of trypan blue-positive cells (TBPC) in the corneal endothelium is usually related to postmortem degenerative changes or injuries during tissue manipulation. Apart from the intercellular borders and cell count, polymorphism (pleomorphism and polymegathism) are also observed for determining the suitability of the tissues for grafting.

Storage of Corneas

The primary aim of corneal storage is the maintenance of endothelial viability from the time of corneal excision to transplantation. Currently there are two storage practices for the cornea, the hypothermic storage at 2–6 °C, adopted by many eye banks all over the world, and organ culture at 30–37 °C, the current method of choice for most eye banks in Europe [7].

Success came in 1974 with the introduction of the McCarey-Kaufman medium, which allowed the hypothermic storage of donor corneas for 3–4 days. As a consequence, corneal transplantation became a scheduled, rather than emergency procedure. The storage of donor corneas for an extended period allowed extensive donor screening, scheduling of operations, and a more rational dispatching of donor tissue to transplant centers. Other formulations containing chondroitin sulfate in addition to dextran, retarded corneal swelling during storage, and components promoting tissue survival were introduced later.

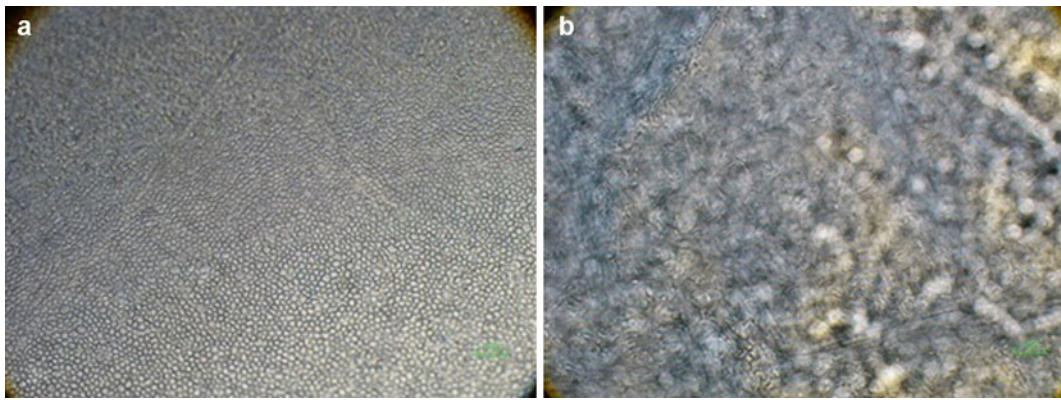

Fig. 3.1 Human corneal endothelium. (**a**) Regular pattern of the corneal endothelium without any trypan blue-positive cells and (**b**) completely damaged corneal endothelium with large area of trypan blue-positive cells determining necrotic cells or total cell loss

Hypothermic Storage

Donor corneas are stored in serum-free tissue culture medium at a temperature of 2–6 °C. At this temperature the metabolic activity of endothelial cells is minimal and pumping function is lost. Corneal swelling may be prevented by the addition of water retentive compounds to the preservation medium. One of the most commonly used is the deturgescent compound dextran either alone or in association with the glucosaminoglycan chondroitin sulfate. Storage liquids also contain antibiotics (gentamicin alone or with streptomycin) that, together with the low temperature, prevent or limit the bacterial growth.

During hypothermia, the cornea shows progressive degeneration of the epithelium and the endothelium, intercellular disruption, decreased adhesion, and, eventually, cell death. Both apoptosis and necrosis occur in cells during hypothermic storage, with apoptosis appearing to predominate. The extent of endothelial loss seems to be related to the biological quality of the tissue, rather than the composition of the medium. Therefore, most corneas are transplanted after 3–5 days of storage, without displaying major alterations.

The hypothermic storage method does not allow time for obtaining preoperative microbiology controls before distribution of the tissue for transplant.

Overall, hypothermic storage seems to offer donor tissues of good quality comparable to that obtained by organ culture, provided that the storage time is kept short. Indeed, according to the literature, the risk of primary graft failure increases significantly after storage longer than 7 days. Furthermore, corneas stored longer than 7 days display epithelial alterations that may hinder the surgical procedure or delay the full recovery of the graft [8].

Organ Culture

The organ culture storage method consists of two phases – a storage period in culture medium at 30–37 °C and a deswelling and transportation phase at 30–37 °C and room temperature in the same medium supplemented with 4–8 % dextran. Organ culture solutions are based on cell culture media. They generally consist of a base of Eagle's MEM or its variant Dulbecco's MEM supplemented by penicillin, streptomycin, and fungicide (amphotericin B or nystatin) to counteract the growth of microbiological contaminants and by 2–10 % fetal calf serum as a source of growth factors. A storage period of 30 days can be achieved without significant loss of endothelial cells. The evaluation of endothelium, which can show reparative phenomena during storage, is usually performed before and after storage.

Cultured corneas have preservation folds caused by swelling of the stroma in the absence of osmotic agents. These folds do not affect the quality of the tissue, provided that they remain covered by viable endothelium. Before transport and surgery, the swelling is reversed by the dextran present in the transport medium. The final thickness is reached after about 24 h and is dependent on the dextran concentration. The dextran also protects the cornea against the lower ambient temperature during transport.

Organ culture offers a longer storage time, corneal endothelium with a better defined quality, and a preoperative sterility control. Organ-cultured corneas always display an epithelium made up of 2–3 layers of viable cells. The 30-day storage period allows an efficient use of valuable donor tissue: planning of operations is easier, allowing sufficient time for the allocation of HLA-matched corneas. The disadvantages of this method are the relative technical complexity and the need for qualified staff to perform tissue culture and selection of the corneas [9].

Samples of the storage medium of cultured corneas are routinely tested for microbiology after 3–7 days in the first phase and after 1 day in the second phase. A gradual change in color of the medium is expected, but any cloudiness or significant color change of the medium is indicative of bacterial or fungal contamination. A contaminated cornea is discarded regardless of whether the microbe is pathogenic or not [10, 11].

It is still a point of debate whether the clinical outcome after grafting corneas stored by hypothermic or organ culture techniques is the same, although few studies comparing the effect of the storage methods on outcome demonstrate similar graft survival and postoperative decline in endothelial cell density [12–15]. Irrespective of the storage method used, inspection of the endothelium after a prolonged storage is essential to prevent transplantation of poor quality corneas.

Tissue Processing for Specific Surgical Purposes

Eye Bank Preparation of Corneal Tissue for Lamellar Keratoplasty

Donor selection criteria for corneas used in lamellar keratoplasty are the same as for penetrating keratoplasty with a few exceptions. Corneas with prior laser photoablation surgery or noninfectious anterior stromal scars may be suitable for posterior keratoplasty, but corneas with previous intraocular surgery scars are not recommended for use since the cornea may rupture under infusion pressure while on the artificial anterior chamber [16–18].

A 3–4 mm scleral rim is needed for corneas used in lamellar keratoplasty procedures to ensure an adequate seal on the artificial chamber of the automated microkeratome.

An automated microkeratome system consists of a control unit, an artificial chamber, microkeratome turbine, and heads. The control unit should be set up in close proximity to the laminar flow cabinet. The cornea is placed using tissue forceps centrally onto the artificial anterior chamber which has been moistened by activating the irrigation system, and the chamber is locked into place. The cornea is pressurized by infusing PBS through the irrigation system. A tonometer lens is placed on the corneal surface to confirm that a minimum of 65 mmHg has been established inside the artificial chamber through the infusion of PBS. In case of anterior lenticules, the graft desired thickness is obtained by the correspondent microkeratome head. For posterior lenticules, a pachymetry reading is obtained after the removal of the epithelium, to determine which microkeratome head to use to obtain a final graft.

Resection of Cornea with a Swinging Microkeratome

The corneal epithelium must be gently removed before preparation, or left in place. In the former case, the subsequent swelling of the stroma during preservation can be limited. Two points are marked on the midperiphery of the cornea using a sterile gentian violet or trypan blue marker to assist with re-aligning the cap back onto the remaining stromal bed after the cut has been made.

The microkeratome head is rotated manually across the cornea. Once the sectioning is completed, the free cap is removed from the microkeratome head and repositioned onto the corneal bed, taking care of re-aligning the marks. A wexel sponge spear is used to smooth out any bubbles between the cap and the graft bed.

Once lamellar keratectomy has been completed, the cornea should be re-evaluated by slit lamp biomicroscopy and specular/light microscopy to confirm that the tissue is suitable for the intended use [19].

Storage of Corneal Lenticules for Lamellar Keratoplasty

Anterior corneal lenticules can be either dehydrated or freeze-dried and stored at 2–6 °C according to the eye bank's validation protocol.

Alternatively, anterior/posterior lenticules can be placed in a cornea viewing chamber filled with preservation media (hypothermic storage) or in the transport medium (organ culture).

The Preparation of Donor Sclera

The donor sclera is used in allografts for a variety of procedures, most commonly to enclose orbital implants for reconstruction of anophthalmic cavities, reconstruct eyelids, cover tubes used in glaucoma surgery, repair scleral thinning, and correct

lid retraction and cicatricial entropion and tumor excision. Selection criteria are the same as cited for penetrating keratoplasty, except that tissue with local eye disease affecting the corneal endothelium is acceptable for use. Being a vascularized tissue, malignancies are applied as additional contraindication. Postmortem interval may be extended.

Donor sclera is prepared from remaining ocular tissue following excision of the corneoscleral button or from donor globes which have been disqualified before corneoscleral rim excision. Since conjunctival tissue is an excellent carrier for microbes, remnants of muscles and conjunctiva must be removed.

The intraocular material is removed by using forceps, iris scissors, sterile gauze, or cotton-tipped applicators. The sclera is finally rinsed in PBS, reshaped to its original spherical form, preserved dehydrated in ethanol (70 % or higher concentration) or glycerol, fixed in formalin, freeze-dried, or frozen.

Future Aspects in Eye Banking

Synthetic Medium for Corneal Preservation

The storage and the final transport medium contain serum of animal origin in it. Apart from serum, other nutrients of animal origin have also been investigated for prolongation of the endothelial metabolic activities, such as chicken feather, ovalbumin, and pig bone amino acids, usually used in combination with other sources of nutrient supplements. Animal viruses, especially retroviruses, could integrate into the human genome and activate human oncogenes or oncosuppressor genes, while prions could lead to human forms of bovine spongiform encephalopathy (BSE). This is why synthetic media have been developed. The potential transmission of BSE primarily comes from donors who have donated their corneas and were at risk of having BSE (e.g., UK donors at the time of mad cow disease). Theoretically, there could be a transmission of animal-derived viruses that could integrate in the genome and activate oncogenes; therefore, technically it would be safer to develop and integrate a totally synthetic-/animal-free media in the routine eye banking procedures [20, 21].

Precut and Preloaded Tissues for Descemet Stripping Automated Endothelial Keratoplasty

Donor tissues for Descemet stripping automated endothelial keratoplasty (DSAEK) can be prepared by the eye banks where the cornea is cut using a microkeratome and the entire tissue is delivered to the surgeon as a precut lenticule. The anterior cap of the cornea can still be left attached to the scleral rim by its peripheral edge for ease of transportation and to lower any potential endothelial cell damage [22–24].

If the tissue is prepared by the surgeon in the operating theater and if it fails due to irregular cut or perforation in some cases, then the surgery has to be postponed or an extra cornea has to be kept ready for replacement which increases the tissue wastage in general. Hence a pre-cut or a preloaded tissue (as described further) may be helpful.

Device Prototyping for Surgical Glides

3D printing technology can be used for initial prototyping of the surgical glides; however, depending on the requirement of the units, it can be custom built, sterilized, and used in a surgical theater. A newly designed ophthalmic device (a surgical glide) is composed of three parts: a) the glide, b) a container for preservation, and c) a penholder to support the glide. The glide is designed to maintain the tissue fixed and without any cell damages during preservation, the lid preventing the tissue from getting out from the glide and the holes on the top of the cap ensuring media exchange. The container is capable to keep the glide completely immersed in the preservation media in a vertical position, thus making the

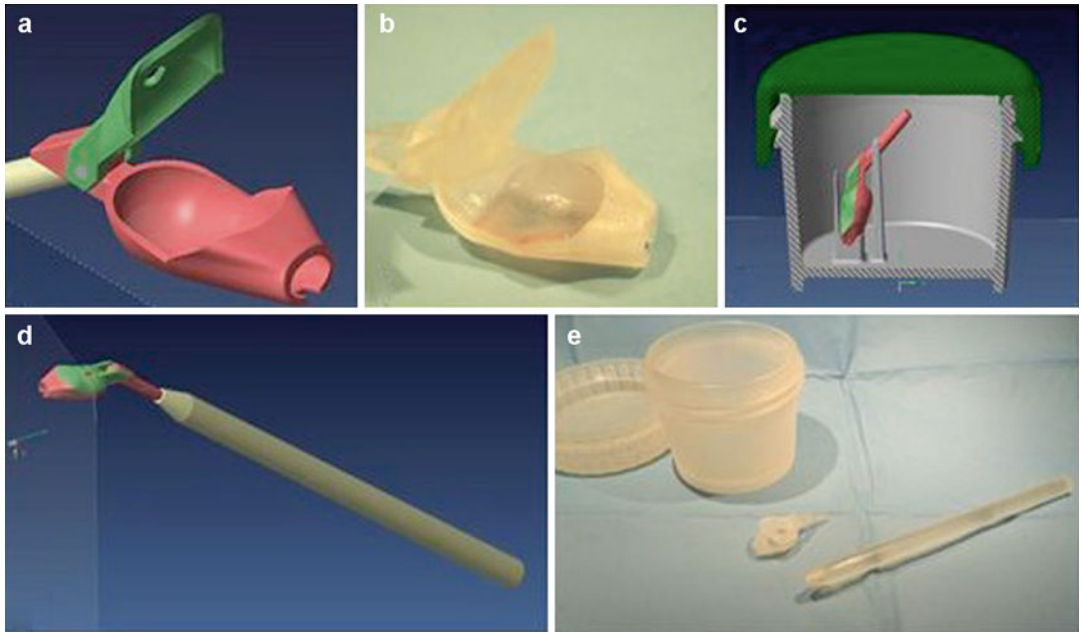

Fig. 3.2 Device layout. (**a**) Computer-generated image of storage glide, (**b**) the working model of the glide printed using a 3D printer with the lenticule, (**c**) the preservation container where the glide is fixed and filled with 50 ml of tissue culture media, (**d**) the computer-aided design (CAD) image of the device with the penholder that was stamped using 3D printer, and (**e**) final working prototype of a 3D printed glide

extraction of the glide easier with the handle during the surgery as shown in Fig. 3.2.

Preparation of the Preloaded Lenticules

In order to keep the procedure easy, especially when ultrathin lenticule is prepared, the posterior lenticules were preserved with a support such as the anterior lenticule of the tissue or a synthetic support such as a contact lens. The tissues can be trephined with a desired diameter (8–9 mm). The posterior lenticule is picked up grasping the support and is inserted into the glide. The device is further filled with 1 mL organ culture medium after removing the air present inside the glide in order to avoid the formation of bubbles that remain in contact with the endothelium during the storage time. The lid of the glide is closed, and the glide is gently fixed in the preservation container. The container is filled with 50 mL of the preservation media with dextran, and all the grafts are ready to be delivered within 4 days from the preparation.

Pre-bubbling the Tissues for Descemet Membrane Endothelial Keratoplasty

Descemet membrane and endothelium can be separated from the overlying stroma with a simple technique using air or liquid dissection. Air injection is usually performed with a high pressure (pneumodissection) to create the separation using a big-bubble technique. However, liquid requires medium to high pressure. The bubble formed using liquid as the medium of separation is shown in Fig. 3.3. The tissues can then be preserved in the transport medium for 7 days. An adequate size of graft tissue can be obtained without the need to manually handle the tissue. The technique allows storage of the tissue in

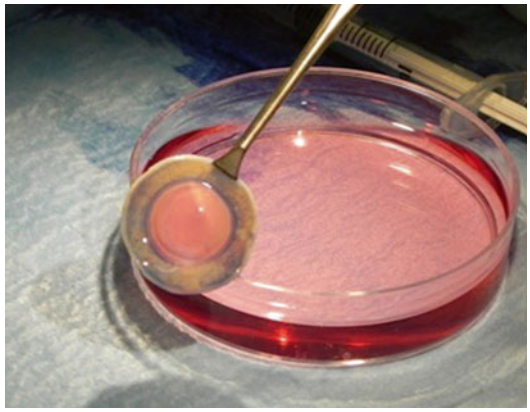

Fig. 3.3 Submerged hydro-separation method to create a liquid bubble in the cornea for separation of Descemet membrane from the stroma for DMEK procedures

organ culture medium with low endothelial cell loss. However, either of the techniques has no significant changes seen in the endothelium apart from that the yield generated using liquid separation was slightly higher than air [25–27].

Pre-stripping the Tissues for Descemet Membrane Endothelial Keratoplasty

Stripping, unlike the bubble separation technique, is performed by peeling the Descemet membrane and the endothelium away from the stroma leaving a hinge at the end of the lenticule. This allows the preservation of pre-separated endothelial grafts in the eye bank further shipped to the surgeons. This technique has showed minimum mortality rate as compared to the other currently performed techniques.

Thus, preloaded, pre-bubbled, or pre-stripped tissues can be prepared in the eye bank and shipped to the surgery to ensure a validated graft by the eye bank for surgery.

Conclusions

Thus, we envision that eye bank is growing not only in the field of procuring the tissues for transplantation but also in the field of research and development. Development in the preservation techniques, surgical devices, modification of tissues, and designing new methodologies for ocular health care are now becoming a part of eye bank world. Serum-free media are being evaluated for corneal storage; autologous serum eyedrops, amniotic membrane transplantation, and ex vivo expanded limbal stem cells are being offered as complementary remedies for ocular surface disorders. Standardizing the posterior lamellar graft preparation methods will reduce unnecessary manipulation of the tissue in the operating theater and reduce the high surgical skill or risk quotient. Precut tissues which would reduce the overall intervention costs and time seem to be the future of eye banking. The efforts by the eye banks on the final quality of the graft would reduce the severe efforts of manipulation by the surgeons, thus providing better quality tissue for patients [28].

Acknowledgements This chapter has been modified from Parekh M, Ferrari S and Ponzin D: Eye Banking: An Overview. In: Eye Banking: Changing Face of Corneal Transplantation (2015) with permission from Nova Science Publishers, Inc.

The chapter was supported by 2014 - Società Oftalmologica Italiana (SOI) grant to Dr. Ponzin.

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Endothelial Keratoplasty

4

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Abstract

Full-thickness corneal transplant was for many years the only surgical option for corneal endothelial diseases. With the advantages of better visual potential, shorter recovery times, and lower rejection risk, endothelial keratoplasty (EK) has now superseded penetrating keratoplasty (PK) for these conditions. This revolutionary change was initiated by the insight of Gerrit Melles that partial-thickness grafts could stick to the back of the cornea without sutures. Progressive surgical refinement and advances in instrumentation by many surgeons have led to widespread adoption. Currently, Descemet's stripping endothelial keratoplasty (DSEK/DSAEK) is the most popular surgical procedure for corneal endothelial dysfunction. Descemet's membrane endothelial keratoplasty (DMEK) has emerged as an alternative to DSEK offering improved vision, shorter recovery time, and reduced rates of immunologic graft rejections. Compared to DSEK, DMEK selectively replaces bare endothelium and Descemet's membrane without a stromal scaffold. This chapter focuses on the evolution of EK, techniques, outcomes, and complications.

Keywords

Endothelial keratoplasty • Penetrating keratoplasty • DLEK • DSEK • DSAEK • DMEK

In comparison to penetrating keratoplasty (PK) where the entire diseased cornea is replaced, endothelial keratoplasty (EK) selectively replaces diseased or dysfunctional corneal endothelium while leaving most of the recipient cornea intact. Although the first EK was performed more than 50 years ago [1], only since 2007 has EK become the standard of care in the US for corneal endothelial dysfunction.

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J. Hjortdal (ed.), *Corneal Transplantation*, DOI 10.1007/978-3-319-24052-7_4

History, Innovations, and Terminology

The concept of selective endothelial replacement was first described in 1956 by Tillet, who named it posterior lamellar keratoplasty [1]. However, his technically challenging technique required the suturing of the donor cornea to the recipient resulting in poor outcomes. A number of other surgeons also tried EK, including Jose Barraquer, but all failed because they used sutures to hold the donor in place, and the sutures disrupted the donor attachment. The success of modern EK is attributed to the pioneering work of Melles. In 1998 he described the successful attachment of a posterior lamellar graft to recipient stroma without the use of sutures [2, 3]. Like Tillet, Melles called this new surgery posterior lamellar keratoplasty. Terry et al. popularized this technique as deep lamellar endothelial keratoplasty (DLEK) in the United States [4]. DLEK required a technically challenging manual posterior stromal lamellar dissection and the use of scissors for excision of posterior stroma to allow placement of the donor tissue, so it was never widely adopted. Moreover, applanation of hand-dissected donor and recipient stromal surfaces led to poor visual results in many patients.

In 2003, Melles et al. proposed a simplified technique involving removal of the Descemet's membrane from the recipient cornea combined with placing the donor endothelial graft onto the back of the recipient posterior stroma [5]. This technique was modified and popularized by Price et al. and termed Descemet's stripping endothelial keratoplasty (DSEK) [6, 7]. In DSEK, the Descemet's membrane and endothelium on a stromal scaffold are transplanted from the donor to the posterior stromal surface exposed on the recipient cornea after successful descemetorrhexis. The lamellar dissection of the donor cornea was further simplified by Gorovoy with the use of a semiautomated microkeratome and called Descemet's stripping automated endothelial keratoplasty (DSAEK or DSEK) [8–10]. By 2005, eye banks in the US were performing lamellar dissections and providing "precut" donor tissue to surgeons, and this greatly facilitated widespread adoption of the surgery.

Compared with PK, which replaced the full corneal thickness, maintaining the recipient anterior corneal surface and implanting only donor Descemet's membrane and endothelium led to improved visual outcomes, lower rejection rates, decreased postoperative complications, and faster rehabilitation. Perhaps, most importantly, it provided a much stronger wound postoperatively and virtually eliminated the risk of losing eyes from intraoperative suprachoroidal hemorrhage. Currently, DSEK is the most popular form of endothelial keratoplasty among corneal surgeons because the procedure is relatively easy to learn and replicate, has good outcomes, and is applicable in eyes with almost any associated complexities in the anterior chamber [11–18].

Because donor posterior stromal tissue is added in DSEK, any irregularity in the dissected surface or the development of folds in the donor tissue as it conforms to the back of the recipient cornea can affect vision. These interlamellar problems lead to delayed visual recovery and suboptimal visual potential in some eyes. These limitations generated interest in eliminating donor stromal tissue and transplanting only bare endothelium and Descemet's membrane. Melles developed a method for doing this called Descemet's membrane endothelial keratoplasty (DMEK) [19, 20]. In DMEK, the Descemet's membrane and endothelium are removed from a donor cornea and implanted in a recipient eye to provide an exact anatomical replacement for dysfunctional endothelium and Descemet's membrane. Despite several advantages over DSEK, the adoption of DMEK has been relatively slow because of its technical challenges including proper graft preparation, insertion, orientation, and positioning while preventing excessive loss of transplanted endothelial cells during the procedure. In addition, there are no corporate champions promoting DMEK because it does not require any expensive equipment, like the microkeratome used with DSAEK, to prepare the donor tissue.

Table 4.1 Indications and contraindications for endothelial keratoplasty

Indications	Contraindications	Consideration
Fuchs' dystrophy	Significant irreversible central corneal scarring	Large iris defects
Bullous keratopathy (aphakic or pseudophakic)	Keratoconus	Aniridia
Posterior polymorphous dystrophy	Hypotony	Glaucoma tubes
Iridocorneal endothelial (ICE) syndrome		Aphakia
Endothelial failure		Anterior chamber intraocular lenses
Failed PK		Peripheral anterior synechiae
Congenital hereditary endothelial dystrophy		

Not all branches in an evolutionary history are successful. Several alternative or hybrid techniques were developed in an attempt to overcome the surgical challenges with DMEK. One approach involved delivering the Descemet's membrane with a small peripheral 360° skirt of posterior stroma, instead of an entire layer of donor stroma. This was termed DMEK with stromal rim (DMEK-S) when performed manually or Descemet's membrane automated endothelial keratoplasty (DMAEK) when a microkeratome was used to perform the lamellar dissection step [21, 22]. Another approach employed stromal support along one side and was called sickle DMEK. These procedures combined the visual advantages of DMEK with the tissue insertion and positioning ease of DSEK, but the donor tissue was prepared by pneumatic (big bubble) dissection, which was more challenging and resulted in a higher rate of tissue loss than the direct peeling method typically used to prepare DMEK tissue (these techniques will be described in greater detail below).

Another approach to improving visual outcomes was to create a thinner donor lenticule with the microkeratome, a technique called ultrathin DSAEK [23, 24]. In many cases, this technique is also associated with increased tissue wastage [25]. Ultrathin DSAEK approaches the advantages of DMEK but may increase the risk of endothelial damage. Also tissue manipulation becomes more challenging as the DSAEK tissue becomes ultrathin. Concomitantly, refinements in the surgical steps of DMEK along with compelling evidence of its excellent visual results and low rejection rates have made it the preferred approach for endothelial diseases at several centers.

The wide range of endothelial disorders can usually be managed with the different EK techniques, and penetrating keratoplasty is rarely required. Table 4.1 summarizes the varied indications that can be treated with EK as well as the important considerations. For example, with the appropriate techniques and sufficient anterior chamber room, EK can be performed in eyes with glaucoma tubes, synechiae, and iris abnormalities.

Surgical Techniques

DLEK

As noted above, DLEK was never widely adopted because of its technical difficulty and unpredictable visual recovery. Although not ideal, DLEK was the first successful EK procedure. It certainly had several advantages over PK because it utilized a smaller incision and maintained the recipient's anterior corneal surface. Visual recovery was similar to PK and the suture-related complications seen with PK were prevented.

DSEK

In DSEK, the endothelium, Descemet's membrane, and deep stromal tissue are delivered to the posterior surface of the cornea after removing the recipient's dysfunctional Descemet's membrane and endothelium. The procedure comprises

three steps: (a) preparation of a posterior lamellar graft, (b) removal of the host Descemet's membrane and dysfunctional endothelium, and (c) insertion of the graft into the anterior chamber and positioning using air tamponade.

(a) Donor preparation: The donor cornea is mounted on an artificial anterior chamber for lamellar dissection. Then it is placed on a tissue-cutting block and trephined to the desired diameter (usually 8–9 mm) from the endothelial side. Methods of lamellar dissection include:

Manual dissection (Fig. 4.1, *first row*) – An initial 4–5-mm curvilinear incision is made at the limbus to a depth of approximately 300–350 μm with a guarded diamond/Bevers' blade. Short and long

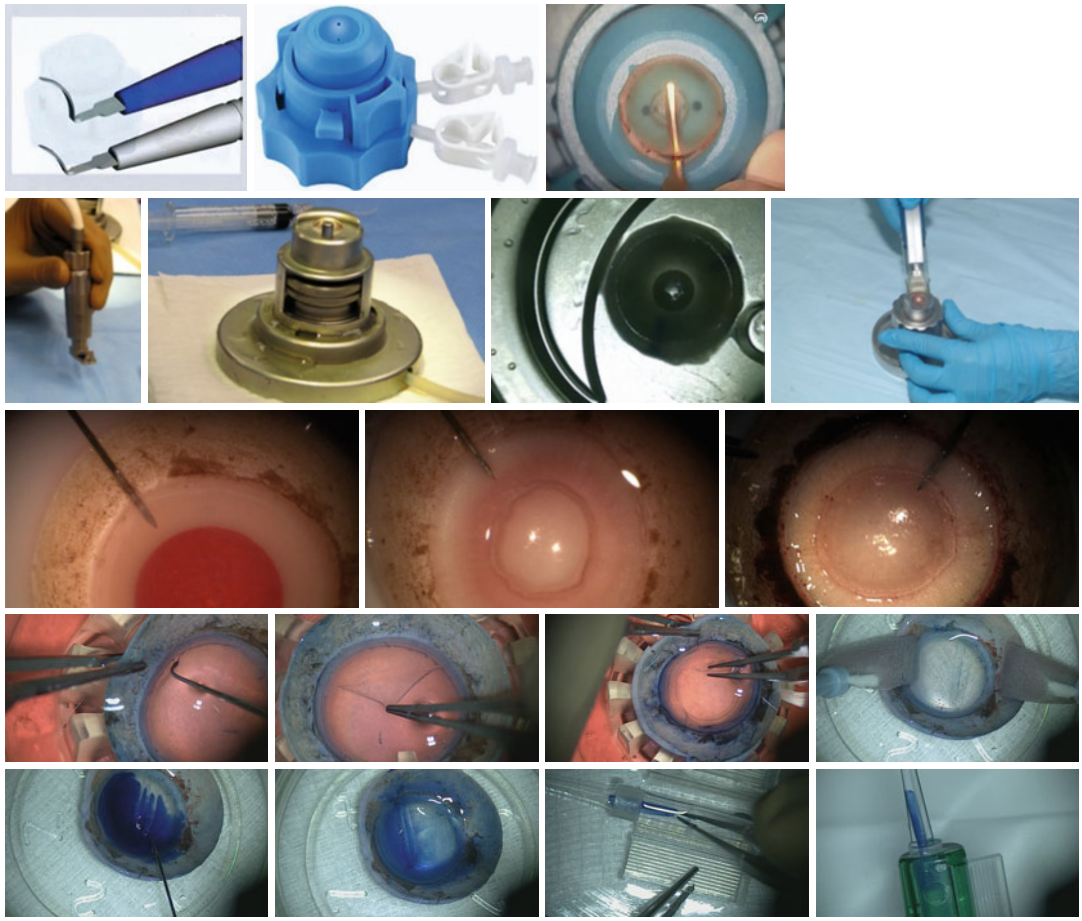

Fig. 4.1 Donor tissue preparation: equipment and methods. *First row (L-R)* Descemet's stripping endothelial keratoplasty (DSEK): manual dissectors (DORC, Netherlands); Barron disposable artificial anterior chamber (Katena Products); manual dissection with donor cornea mounted on artificial anterior chamber. *Second row (L-R)* Descemet's stripping automated endothelial keratoplasty (DSAEK): microkeratome (Moria); reusable artificial anterior chamber (Moria); microkeratome-assisted donor dissection; microkeratome (Gebauer, Germany). *Third row (L-R)* Descemet's membrane automated endothelial keratoplasty (DMAEK) graft preparation: air is injected via a needle inserted through the peripheral scleral rim; this creates a big bubble; the big bubble is enlarged with more air to separate the Descemet's membrane (DM) from the posterior stroma. *Fourth row (L-R)* Descemet's membrane endothelial keratoplasty (DMEK) graft preparation: peripherally scored DM is separated from underlying stromal tissue circumferentially using a microfinger; DM is peeled in four quadrants leaving it attached at the center; final peel to free the center of the tissue; scrolled donor Descemet's membrane and endothelium. *Fifth row (L-R)* DMEK graft insertion: trypan blue is being used to stain the DM scroll to improve visualization; the stained DM scroll; the tissue is being loaded into the cartridge of an intraocular lens inserter; the DM scroll within the inserter

curved dissecting blades are used to extend the lamellar dissection 360° to reach to the limbus.

Microkeratome dissection (Fig. 4.1, *second row*) – The donor dissection plane is created with a microkeratome. The microkeratome head depth can be selected according to the desired plane of lamellar dissection (usually 250–400 μm). Microkeratome dissection produces a smoother and more regular dissection plane compared with manual dissection. Numerous eye banks have purchased microkeratomes and provide pre-dissected tissue. The most commonly used microkeratome (Moria, Antony, France) cuts deeper in the periphery than centrally, and this somewhat compensates for the normally increased thickness of the cornea in the periphery compared with the center, usually resulting in a fairly planar posterior donor button.

Femtosecond (FS) laser dissection (FS-DSEK) – The feasibility of using a femtosecond laser to create lamellar cuts has been assessed in multiple studies. So far all have had suboptimal visual results because the laser does not produce as smooth a dissection plane in the soft posterior stroma as it does when producing a flap in the anterior stroma for laser refractive surgery. In addition, irregularities are induced in the posterior stroma when the donor tissue is applanated against a solid laser interface. Finally, the anterior corneal surface is usually used as a reference surface for the cut causing the peripheral graft to be variably thicker than the center, depending upon the thickness gradient in the donor cornea. The latter limitation could potentially be addressed with appropriately sophisticated imaging technology and laser software [26, 27]. So far, no one has been able to demonstrate that this much more expensive approach results in any tangible benefits.

(b) Stripping of the host Descemet's membrane (Fig. 4.2, *first row – first*): The host DM is removed within an area corresponding to the graft diameter or slightly smaller. DM stripping is necessary in Fuchs endothelial dystrophy to remove the guttae; however, this step may be optional in conditions where the DM is optically clear and devoid of any structural alterations, such as in failed PK and pseudophakic corneal edema [28–30].

(c) Graft insertion and positioning: The graft was initially inserted through a 5-mm incision using forceps; however, a number of glides and inserters have been developed to facilitate this process and help minimize damage to the tissue during insertion. The incision size has also been decreased in many cases down to 3.4 mm with curled donors. Currently used donor insertion instruments include:

Forceps – The donor tissue is folded into a 60/40 configuration (with the endothelium facing inward and protected with a small amount of viscoelastic) and inserted into the anterior chamber using atraumatic non-coapting forceps (Ex. Charlie II, Goosey, Kelman forceps) [3, 6, 7].

Sheets glide – This method can be helpful in eyes with a shallow anterior chamber and predisposed to iris prolapse. The anterior chamber is maintained using an anterior chamber (AC) maintainer. A Sheets intraocular lens (IOL) glide is inserted halfway into the chamber, which serves to keep the iris from prolapsing out of the wound. The donor graft is placed onto the glide with the endothelial side facing downward and protected with a generous amount of cohesive viscoelastic. An intraocular forceps is inserted through the site opposite to the main incision. The donor edge is grasped with the forceps and pulled inside the anterior chamber (pull-through method) [31]. Alternatively, the graft may be inserted through the main incision using a Sinskey hook or small-gauge needle (push-in method) without the use of an anterior chamber maintainer.

Busin glide (Fig. 4.2, *first row – second*) – This reusable funnel glide (Moria, Inc., Antony, France) curls the graft into a cylindrical shape as it is pulled through the

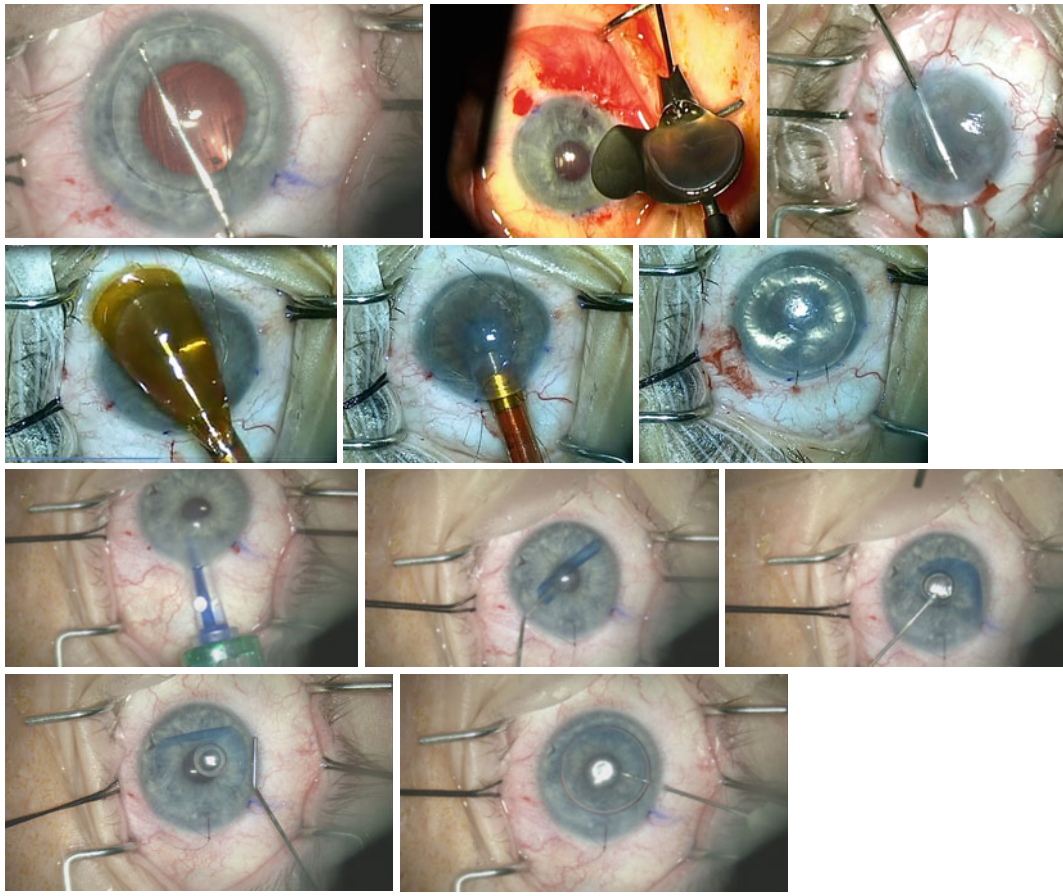

Fig. 4.2 Recipient preparation, graft insertion, and positioning. *First row (L-R)*: Descemet's membrane scoring; loading a DSEK graft into a Busin glide (Moria); pull-through method of graft insertion using a Busin glide and intraocular forceps. *Second row (L-R)*: loading a DSEK graft into an EndoSerter (Ocular Systems, Winston-Salem, NC); graft insertion with EndoSerter; air tamponade. *Third row (L-R)*: DMEK graft insertion; short bursts of balanced salt solution unfold the scrolled tissue; partial unfold achieved. *Fourth row (L-R)*: unwrapping the scrolled DMEK donor tissue over a posterior air bubble (the air bubble helps anchor the graft in the correct position); final air injection to press the donor tissue against the recipient cornea

glide to minimize endothelial trauma during insertion [32]. The leading edge of the graft is grasped and pulled into the anterior chamber with an intraocular forceps introduced through an incision opposite to the main incision (Fig 4.2 first row - third).

Suture pull-through – In this method [33], a 10-0 prolene suture is passed through a 5-mm main incision and across the anterior chamber to exit through the cornea approximately 1 mm beyond the edge of stripped DM. The donor endothelium is coated with viscoelastic, and the second arm of the suture is passed through the periphery of the donor lenticule, entering from the endothelial side and exiting from the stromal side. Both sutures are then passed through the incision, across the anterior chamber, and out through the cornea 1 mm peripheral to the edge of stripped DM. The donor lenticule is gently folded in half with the suture at the leading edge, and the anterior lip of the incision is lifted as both ends of the suture are pulled to guide the graft into the eye. The graft unfolds as the AC is filled with

air, and it is secured by tying off the suture, which helps minimize the risk of graft dislocation.

Injectors/Inserters (Fig. 4.2, *second row – first and second*) – Several single-use devices have been designed to deliver the graft with minimal endothelial trauma [34, 35]. Adoption has been limited by the cost and the good results surgeons have obtained with the other methods described above.

After the graft is inserted, it is positioned and attached to the host posterior stroma using an air bubble (Fig. 4.2, *second row – third*). Complete air fill in the anterior chamber is maintained for 10–12 min, followed by a partial air-fluid exchange to avoid risk of raised intraocular pressure (IOP) secondary to pupillary block. Alternatively, a prophylactic inferior iridectomy may be made prior to graft insertion to prevent pupillary block because of air, and a 90 % air fill can be left in the eye as long as the iridectomy is not covered by air. Postoperatively, supine positioning is maintained for 15–30 min or longer.

DMEK

DMEK involves harvesting of donor DM and endothelium followed by insertion, unfolding, and positioning in the proper orientation. Just as in DSEK, the central host DM is removed before inserting the donor tissue. However while the DSEK grafts will easily stick to retained areas of the host Descemet's membrane and endothelium, DMEK grafts stick much better to bare stroma.

(a) Donor preparation (Fig. 4.1, *fourth row*): Donor DM can be isolated by direct peeling [36] or by injection of air to create a big bubble [37]. Direct peeling has a higher success rate with less endothelial cell loss [38]. Giebel and Price described a direct peeling method called submerged cornea using backgrounds away (SCUBA) that has a success rate of almost 99 % [39, 40]. Earlier reports had lower success rates, but with experience and refined technique modifications like totally freeing up the scored peripheral Descemet's membrane before stripping, results have greatly improved. The donor corneoscleral rim is submerged with the endothelial side up in a viewing chamber filled with corneal storage solution, or it can be placed on a cutting block. The DM is lightly scored 1–2 mm inside the trabecular meshwork using a Y-hook, because peripheral DM is the area most likely to tear during edge lift due to adhesions. Trypan blue staining improves visualization of the scored edge, which is then lifted circumferentially with a microfinger (Moria, Inc.). Radial tears are identified and removed prior to edge lift with the microfinger because these tears can extend centrally. The edge of the DM is grasped with a Tubingen forceps (Ambler Surgical), while fixation of the limbus is achieved with 0.5-mm forceps. DM is partially peeled in four quadrants, leaving the center part attached ("corridor method") – a technique which decreases tension during the peel by decreasing the width of the peel zone [41]. The membrane is floated back into position and the donor is trephined lightly into stroma. The donor is placed back in the viewing chamber for a final peel to detach DM centrally. The detached DM spontaneously forms a scroll with the endothelium on the outside. The DM scroll is placed either in the storage medium or used immediately for transplantation. The DM thickens with age; thus grafts from older donors generally scroll less tightly, which makes graft unwrapping easier during surgery. Therefore, donor tissue over 40 years of age is preferable for DMEK. As with DSEK, DMEK donor tissue can be prepared several days before the surgical procedure [42]. Other variations of donor stripping have been reported by Kruse [43] and Jardine [44], with the latter technique leaving one side of the donor attached so that the tissue can be laid back in place for endothelial cell density assessment by the eye bank.

A newer method for detaching the Descemet's membrane with air has been

termed PDEK [45]. The developer claims that this method detaches Dua's [46] layer along with the Descemet's membrane and endothelium, allowing use of younger donors and reportedly easier unfolding of the tissue [45]. However, donor diameters are limited to about 7–7.5 mm because of the diameter of Dua's layer.

(b) Graft insertion, unfolding, and positioning: The DM scroll can be loaded into a glass pipette or IOL cartridge and injector and inserted through a 2.4–3-mm wide corneal incision. Various types of IOL cartridges are suitable for graft delivery including Carl Zeiss inserters (Jena, Germany) and Viscoject (Medicel AG, Wolfhalden, Switzerland) (Fig. 4.1, *fifth row*). A variety of new glass tubes have also been used including the Straiko modified Jones tube for DMEK (Gunther Weiss, Portland, Oregon). Graft adhesion is reported to be better with use of a closed system without any addition of viscoelastic [47].

Several graft unfolding maneuvers are available (Fig. 4.2, *third and fourth row*). Dapena et al. [48] described a standardized "no-touch" technique for DMEK transplants. A glass injector is used to deliver the DMEK roll into the anterior chamber with the endothelium facing the cornea. A small air bubble is delivered between the double rolls to unfold the graft. After unfolding, the air bubble is removed, and an air bubble underneath the graft (between iris and graft) is injected for graft fixation [48]. Liarakos et al. [49] described 4 standard (standardized no-touch DMEK, Dirisamer technique, Dapena maneuver, and single sliding cannula maneuver) and 3 auxiliary techniques (flushing, manual centration, and bubble bumping) for unfolding the graft in the anterior chamber depending upon the orientation and how tightly the DM is curled. Essentially, the DM scroll in the anterior chamber is opened using short quick bursts of BSS. A portable slit beam or optical coherence tomography attachment on the operating microscope can be used to confirm the graft orientation [50]. After the scroll is partially unwrapped, a small air bubble is injected under the donor to secure the orientation [40]. The recipient corneal surface is stroked to center and unfold the graft completely, followed by air fill in the anterior chamber. The patients are advised to keep supine position for 60 min to allow for donor adherence.

Hybrid Techniques

Hybrid techniques have been developed to combine the optical outcomes of DMEK with the easier handling of DSEK. Studeny described transplantation of a posterior corneal lamella consisting of endothelium and DM centrally with a stromal supporting rim (DMEK-S), and McCauley et al. described a partially automated variation (DMAEK) [21, 22]. The bare central endothelium and DM provide excellent optical outcomes, comparable with those of successful DMEK patients, while the stromal rim provides support to the fragile and thin central portion of the donor. This helps to maintain donor shape and orientation while preventing scrolling, allowing for easier delivery into the anterior chamber while maintaining correct orientation. The donor tissue is dissected as in DSEK using either hand dissection or a microkeratome. The Descemet's membrane is then detached from the posterior stroma using a big bubble technique (Fig. 4.1, *third row*). Rapid big bubble formation can cause rupture of DM. More importantly, the bubbles can sometimes develop in the periphery of the cornea, instead of the center. Peripheral bubbles are thinner and tend to rupture and break easily. If a peripheral bubble forms, the donor tissue can no longer be used for the hybrid technique, but an attempt can be made to convert the tissue for use with DMEK. The DMEK-S or DMAEK donor tissue is inserted into the eye using the pull-through technique with the aid of a Busin glide, and air is injected to attach the donor to the recipient stroma. Donor insertion and positioning is easier than DMEK because the tissue unfolds spontaneously because of the added rigidity afforded by the skirt of stromal tissue. Both centers developing the hybrid techniques have discontinued their use because of increased donor

loss relative to DMEK and increased need for reinjection of air to promote donor adherence. Nevertheless, hybrid donor tissue can still be ordered from eye banks as precut tissue options.

Ultrathin DSAEK

Some surgeons have reported better visual acuity and faster visual recovery with thinner endothelial grafts [24]. Busin et al. described a microkeratome-assisted double-pass method for obtaining ultrathin posterior lamellar grafts (<100 μ) [23]. The first pass is done with a 300- or 350-μm microkeratome head to debulk the cornea. The thickness of the residual bed determines the selection of the microkeratome head for the second pass, which is necessarily made going the opposite direction to help avoid perforation, because the blade penetrates deepest at the beginning of the pass. Nomograms which take into account donor thickness, corneal storage medium, pressurization of the artificial anterior chamber, and cutting speed have been devised to help select the appropriate microkeratome head size to obtain thin EK donor tissue with either single- or double-pass techniques [24]. Some eye banks reportedly prepare ultrathin DSAEK by varying the pressure inside the artificial anterior chamber to control the depth of cut. The risk of tissue loss from perforation is higher with ultrathin grafts compared with standard DSEK grafts.

Surgical Considerations with Ocular Comorbidities

EK is most straightforward in an eye with a normal anterior segment and a stable posterior chamber IOL. Because of the well-known advantages of DSEK over PK, the indications for EK have expanded to eyes with anterior segment complexity such as those with pupillary abnormalities, peripheral anterior synechiae, glaucoma-filtering procedures, glaucoma tube shunts, prior PK, or anterior chamber IOL. While DSEK can be attempted successfully in all mentioned conditions, the decision for DMEK is more reserved because the DM graft is delicate and requires more manipulation to position the graft in comparison to DSEK. When deciding about the type of EK, the potential advantages of a given procedure need to be weighed against the technical ease in an individual case-based scenario.

Aphakic Eyes with Complete or Partial Aniridia

In eyes with aphakia with complete or partial aniridia, a potential concern is intraoperative or postoperative graft detachment, which may result in graft dislocation into the posterior segment. Several options are available to manage this situation depending upon the extent of iris abnormality [17]. A DSEK pull-through technique with or without a suture is typically used. If forceps are used to pull the tissue in, a fixation suture can be used to secure the donor once air fills the anterior chamber and the donor is in position [33]. A posterior chamber IOL (sulcus/scleral fixated) with or without iris reconstruction/pupilloplasty can be planned simultaneously or a few weeks before an EK procedure. In eyes with large iris defects, the host DM stripping should be avoided or performed cautiously to prevent the fragments from falling into the posterior segment. Furthermore, in aphakic eyes, the air bubble used to promote graft adhesion may migrate to the posterior segment intra- or postoperatively, leading to shallow anterior chambers and iridocorneal touch. Prolonged air tamponade and proper head positioning may be additional measures to promote graft adhesion.

In aniridic eyes with an artificial iris implant, the graft can slide between the edge of the implant and the wall of the eye and fall into the posterior segment. To prevent this from happening, air should be injected under the graft before it is released from the insertion forceps, and a temporary fixation suture should be used to hold the graft in place [17]. Alternatively, the graft can be inserted with a suture pull-through technique, and the pulling sutures can be used to affix the graft until adherence is confirmed in the postoperative period [33].

Phakic Eyes

In eyes with significant cataract, a triple procedure (cataract surgery with DSEK or DMEK) is the preferred approach. This approach is also advantageous with DSEK because cataract extraction deepens the anterior chamber and facilitates unfolding of the graft. In patients with endothelial disease and clear crystalline lenses, one may contemplate endothelial keratoplasty alone [51]. It is prudent to avoid iatrogenic damage to the clear lens and the endothelial graft by avoiding anterior chamber fluctuations. As with PK, following EK the rate of cataractogenesis is accelerated as a result of intraocular manipulations during surgery and the postoperative use of steroids. The probability of cataract progression requiring extraction is significantly associated with the age of the patient [52]. Subsequent phacoemulsification utilizing a soft-shell technique and dual ophthalmic viscoelastic devices (OVDs) in patients who develop visually significant cataracts after DMEK has been found to result in minimal endothelial cell loss and no graft detachment [53].

Prior Glaucoma-Filtering/Tube Surgery

In an eye with prior glaucoma-filtering or aqueous shunt surgery, it may be difficult to achieve an air fill in the anterior chamber as the injected air finds its way into the subconjunctival space through the ostium. Therefore several attempts at achieving air fill may be required. Also, after obtaining adequate air tamponade, the intraocular pressure (IOP) needs to be strictly monitored to avoid extremes of high pressure for prolonged periods, which can be detrimental to the already compromised optic nerve. In the rare cases where the air just escapes easily and the pressure cannot be increased enough to firm the eye, a few drops of viscoelastic can be placed over the end of the tube or ostium of the filter to block the flow. Viscoelastic should not be used until the graft is in place to prevent it from coating the graft interface. Once the patient sits up, the viscoelastic will fall away from superior tubes and ostia, so postoperative IOP spikes should not be an issue.

In the eyes with glaucoma drainage devices, it is important to ensure that mechanical contact between the graft and tube is avoided by properly trimming and repositioning the tube, as required. In eyes with a trabeculectomy, once the patient sits up, the air may fill the trabeculectomy bleb leading to high intraocular pressures. Thus, checking patients a few hours after surgery is important.

Vitrectomized Eyes

Eyes with prior vitrectomy and associated iris/zonular defects may have difficulties in graft adhesion, because air may escape into the vitreous cavity increasing the risk of appositional angle closure/graft detachment. Similar to other situations, prolonged air tamponade may help avoid these problems.

Failed Prior PK

EK under a failed graft can successfully restore the graft clarity and avoid repeat PK. However, in situations where the refractive result of the prior PK was unsatisfactory, it may be better to consider a repeat PK, rather than EK. As mentioned earlier in the DSEK technique section, the DM may be left intact in a failed graft if it does not show any abnormalities [13, 28]. This prevents the weakening of the graft-host junction that may occur inadvertently during the stripping maneuver. In eyes with prior therapeutic PK, the DM may be hazy and require removal for optimal results [54]. If stripping is planned, it should be made internal to the graft-host junction or even in a small area overlying the pupil to avoid disrupting the incision. If a DMEK is planned under a failed PK, then DM needs to be stripped inside the PK graft-host wound because a DMEK graft does not adhere well to intact host DM [55].

The graft can be over-, under-, or same-sized. Oversizing provides the advantage of a larger endothelial cell reserve, while undersizing avoids the need for the EK graft to conform

to the irregularity at the graft-host junction that may interfere with the graft attachment process. Particularly with DMEK, an uneven posterior profile of a previous failed PK can make positioning of the donor DM and endothelium more difficult, and reinjection of air to promote graft attachment is required more often than it is in virgin eyes.

One of the most remarkable findings in a series of 60 eyes with DSEK after failed PK was that neither neovascularization nor the number of previous graft failures increased the risk of graft failure. The only preoperative characteristic associated with increased risk of graft failure was previous filtration surgery, either trabeculectomy or aqueous shunt [56].

Iridocorneal Endothelial (ICE) Syndrome

These eyes may have a very shallow anterior chamber because of broad peripheral synechiae [14]. In addition they may have undergone a prior glaucoma-filtering surgery/drainage surgery for IOP control. Extensive synechiolysis may be required for deepening the anterior chamber. Postoperatively, frequent follow-up and aggressive control of IOP are needed for graft survival [57].

Pediatric Endothelial Keratoplasty

DSEK can be more challenging in pediatric eyes as compared with adults. The main reasons for performing EK in pediatric eyes are for failed graft, congenital hereditary endothelial dystrophy (CHED), and pseudophakic corneal edema. Surgical challenges involve insertion and unfolding of the donor tissue in the small anterior chamber of a child, avoiding trauma to the crystalline lens, postoperative positioning requirements, and anesthesia issues [58]. CHED eyes in particular are difficult because of poor visibility. These young eyes have very thin DM, which can be difficult to strip. Pediatric eyes can have a positive vitreous pressure, which can make the surgical maneuvers difficult. Discussing with the anesthetist the need for hypotensive anesthesia during graft insertion and the use of an anterior chamber maintainer during the surgery helps in maintaining the anterior chamber during the DSEK surgery.

Surgical Outcomes

Visual Acuity

When compared with PK, the visual recovery is remarkably rapid, occurring within a few weeks of EK, and mean visual outcomes continue to improve for up to several years afterward, although delayed improvement is more common in DSEK than DMEK. The average Snellen corrected distance visual acuity (CDVA) reported after DSEK has ranged from 20/30 to 20/60 in different studies, with variable follow-up periods [18, 59]. Several factors may interfere with complete visual recovery: graft folds, thickness irregularity, centration, interface haze, and residual anterior abnormalities in the host cornea [60].

DMEK virtually eliminates any thickness variation or folding of tissue to conform to the back surface of the recipient cornea, thereby resulting in better and faster visual recovery with fewer higher order aberrations from the posterior surface of the cornea [61]. Most patients achieve 20/25 or better vision within several weeks with DMEK [39, 40]. Like DMEK, DMAEK also provides superior visual recovery with high rates of 20/25 or better vision [24]. The relationship between DSEK graft thicknesses and visual acuity has been debated [62, 63]. While some believe that thinner grafts are associated with better vision, others have failed to establish this association. Thinner, well-centered, and planar grafts may induce fewer higher order optical aberrations and contribute to superior visual results. Busin et al. have reported excellent visual outcomes with ultrathin DSAEK [23].

Refractive Results

DSEK does not significantly alter anterior corneal topography, but tends to cause a mean hyperopic shift of 0.75–1.5 D through changes in the posterior corneal curvature [64]. Because of the

nonplanar configuration of the DSEK donor lenticule, which is typically thinnest in the middle, a minus lens is introduced on the posterior corneal surface. Also, the increase in the thickness of the cornea caused by implanting additional stroma leads to a decrease in the radius of curvature of the posterior surface. The resulting hyperopic shift should be taken into consideration when planning a triple procedure to better achieve the target refraction. Although DMEK does not increase the corneal thickness or introduce a minus lens effect, it also results in a mean hyperopic shift of 0.25–0.50 D that is attributed to the resolution of the corneal edema after restoration of the endothelial function [65, 66].

Endothelial Cell Loss

The endothelial cell loss reported after DSEK is 18–35 % at 6 months, 31–36 % at 1 year, 31–41 % at 2 years, 44 % at 3 years, and 54 % at 5 years [34, 67–72]. Compared with the 5-year cell loss experienced with PK procedures performed in the Cornea Donor Study for similar indications, the cell loss at 5 years may be lower with DSEK [71]. Hence, despite the higher initial endothelial cell loss experienced with DSEK compared with PK, the rate of subsequent cell loss appears to be less with DSEK for reasons that have not been fully elucidated.

There are few reports so far on long-term endothelial cell loss after DMEK because the technique is relatively new. In a report by Tourtas et al., the mean endothelial cell loss at 6 months after DMEK and DSAEK was comparable [73]. In another comparative study between DMEK and DSAEK, there was no difference in the endothelial cell loss at 1 year [74]. Baydoun et al. [75] reported endothelial cell density (ECD) for eyes that underwent DMEK. They report decreases in ECD by 35 % at 6 months, 38 % at 12 months, 43 % at 24 months, 47 % at 36 months, 52 % at 48 months, and 55 % at 60 months compared to preoperative values [75]. Feng et al. compared the 5-year endothelial cell loss after DMEK to reported rates for DSEK and PKP [76]. The median 5-year cell loss was 39 % with DMEK compared with 53 % for DSEK and 70 % for PKP. They also found that a single air reinjection, which is sometimes required in DMEK to promote graft adherence, did not greatly affect endothelial cell density [76].

Graft Survival

The reported graft survival rates through 1 year with DSEK range from 55 to 100 % in various studies [18, 71, 77]. This wide range reflects differences in sample size, indications for endothelial keratoplasty, associated comorbid conditions, and varying rates of iatrogenic graft failure due to the surgeon's initial learning curve. Price et al. reported a 5-year survival rate of 95 % for Fuchs endothelial dystrophy and 76 % for pseudophakic and aphakic corneal edema [70]. Prior glaucoma surgery was the most significant risk factor for early graft failure [71]. With this risk factor taken into account, the 5-year DSEK survival rate was comparable to 5-year PK survival rates at the same center [70]. The reports on 1-year survival rates after DMEK are encouraging, and longer term follow-up is awaited.

Complications

Early Postoperative Intraocular Pressure Elevation

Elevated IOP can occur as a result of pupillary block by the injected air required to promote graft attachment. The pupillary block may be relieved with pupillary dilation or partial anterior chamber decompression. Air may migrate to the posterior chamber in an eye with a floppy/abnormal iris or tone or even to the posterior segment in an eye with an open posterior lens capsule leading to an appositional angle closure, iridocorneal touch, and raised IOP. This may be managed by removing the air from the anterior segment and allowing the iris to drop back into place or by having the patient properly positioned with head facing down. Eyes with large residual air bubbles should always be checked a few hours after surgery.

Graft Detachment (Fig. 4.3, *First Row*)

The reported graft detachment rates after DSEK vary from 0 to 82 % [18, 77, 78]. Although the precise mechanism of graft adhesion is unknown, it is probably an interplay of three factors: mechanical, biochemical, and physiological. Achieving a complete air fill in the anterior chamber helps in the initial mechanical apposition of the graft to the stroma, followed by the physiological effect of the endothelial pump [78]. Graft detachments are more common in situations where it is either difficult to achieve an air fill for a required time interval or to maintain a firm eye, such as eyes with vitrectomy, aphakia, glaucoma-filtering surgery, or repeated squeezing or eye rubbing on the part of the patient. Strategies described to reduce the risk of graft detachments include: scraping of the peripheral host stromal bed, mid-peripheral venting incisions, good wound integrity, prolonged air tamponade, and supine positioning, especially in high-risk situations. Partial DSEK graft detachments will often seal down spontaneously without any intervention. Total DSEK graft detachments can be managed by reinjecting air (called rebubbling). Spontaneous reattachments of totally detached DSEK grafts have been reported but are unpredictable and may be decentered.

As with DSEK, graft detachment is one of the most frequent complications with DMEK. Partial graft detachments are less likely to spontaneously resolve with DMEK than DSEK. In contrast to DSEK, where residual DM may not affect graft adhesion, remnants of host DM in the stripped bed can definitely interfere with the adhesion of a DMEK graft. Also, the elastic forces of the DM scroll need to be overcome for a firm adhesion of the DMEK graft, whereas the donor posterior stromal tissue helps keep the graft uncurled with DSEK. In an early report by Guerra et al., the rebubbling rate was 62 % in a prospective series of eyes undergoing DMEK [40]. In this study, the graft insertions were done

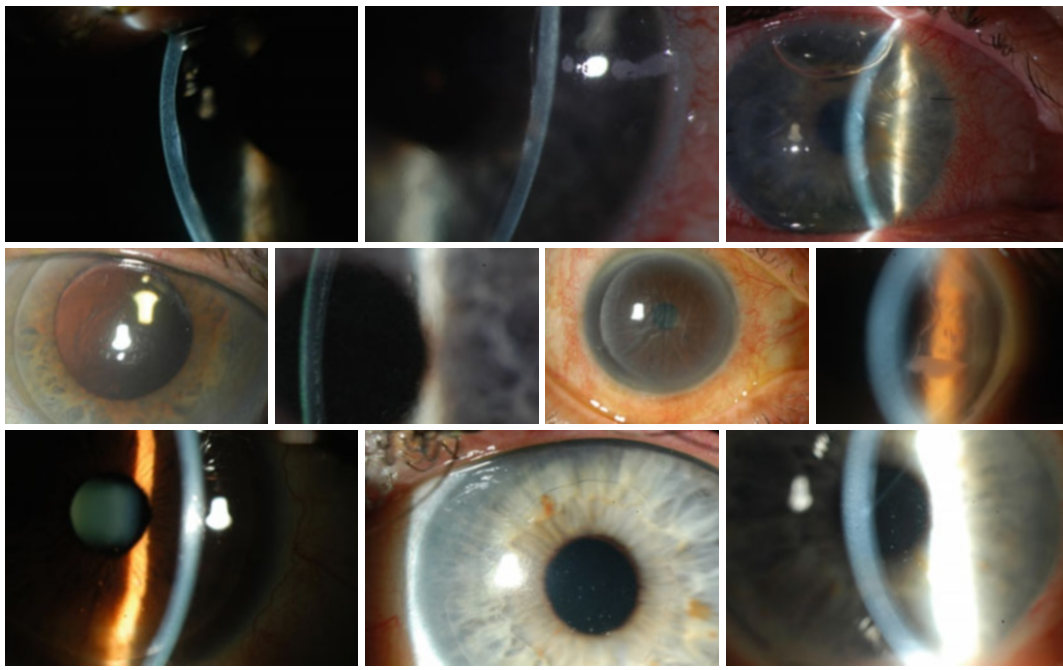

Fig. 4.3 Postoperative complications. *First row (L-R)* first and second panels: DMEK partial detachments; third panel: complete detachment (DM scroll shown in the anterior chamber). *Second row (L-R)* folds in DSEK graft; interface haze with DSEK; late DSEK graft failure; epithelial downgrowth at the interface originating from the edge of the DSEK graft. *Third row (L-R)* rejection episodes in DSEK, DMAEK, and DMEK, each showing fresh keratic precipitates without noticeable corneal edema

with an injector that required a viscoelastic plug. With the modifications in the insertion techniques that avoided the viscoelastic use, the rebubbling rates dropped to 15 % [79]. Some surgeons are less likely to intervene with partial DMEK graft detachments and thus may have relatively low rebubbling rates.

Primary Graft Failure

Primary graft failure is a potential complication following any type of keratoplasty procedure. The incidence of primary graft failure after PK ranges from 0.3 to 2 % [78]. The reported rates of primary graft failure after DSEK have ranged from 0 to 29 %, suggesting that iatrogenic endothelial trauma may be a factor [18, 80–83]. The rate of primary graft failure after DMEK was 8–9 % in early studies that included the surgeon's initial learning curve [40, 74]. With modifications and refinements in some of the surgical steps and increased surgeon experience, both DSEK and DMEK have become more predictable with more consistent results [80].

Immunologic Rejection (Fig. 4.3, *Third Row*)

The rate of rejection after DSEK has varied widely in small series from 0 to 46 % with the mean rate of approximately 10 % [18, 54, 77]. Hjortdal et al. [84] reported rejection rates of 5 % of patients during the first 2 years after DSAEK for Fuchs. Rejection rates are lower in EK compared with PK because the use of corticosteroids can be continued without much concern about healing of the relatively small incision [85]. Additionally, less donor tissue is implanted in EK versus PK and the reduced antigenic load could be another favorable factor. A study by Anshu et al. reported that DMEK eyes had 15 times lower risk of having an immunologic rejection episode within the first 2 years than DSEK eyes and 20 times lower risk than PK eyes [86]. Allograft rejection in EK may present with keratic precipitates, Khodadoust line, redness, and anterior uveitis.

Glaucoma

Because of the required air bubble for graft adherence, acute pupillary block glaucoma after EK is a relevant complication affecting long-term visual rehabilitation. The reported rates of acute glaucoma after DSEK range from 0 to 54 % [77, 87]. A previous history of glaucoma or ocular hypertension is a significant risk factor for development of raised IOP after DSEK [87]. In DMEK, the longer air bubble time and higher rate of air reinjection pose an increased risk of acute glaucoma. However, Melles et al. found that, compared with PK and DSEK/DSAEK, DMEK was not associated with an increased risk of uncontrolled glaucoma [88].

Topical corticosteroids are used for the prevention and treatment of corneal graft rejection. However, long-term topical corticosteroids lead to elevated IOP and cataracts. Since immunologic rejection rates are lower in DMEK due to the donor graft only consisting of DM and bare endothelium, Price et al. studied different corticosteroid strengths after DMEK [89]. They found that the difference between the rejection rates between the prednisolone acetate 1 % and fluorometholone 0.1 % arms was statistically insignificant. They conclude that decreasing postoperative topical corticosteroid strength significantly reduces the risk of IOP elevation without substantially increasing the risk of immunologic graft rejection episodes [89].

Epithelial Downgrowth (Fig. 4.3, *Second Row: Fourth*)

Decentration during trephination can lead to incorporation of donor epithelium with a DSEK donor lenticule, which may lead to epithelial downgrowth. Also, recipient epithelium can be introduced during the donor insertion or introduced through surface venting incisions if proper

technique is not employed. Epithelial ingrowth can be associated with graft failure.

Interface Abnormalities

Donor graft interface or thickness irregularities can occur with manual dissection or irregular microkeratome cuts. No attempt is made to match donor and recipient curvature, and a significant mismatch can result in folds and wrinkles in an EK graft that may be visually significant (Fig. 4.3, *second row – first*) [60]. Another cause of interface abnormalities is incomplete removal of viscoelastic after stripping DM (Fig. 4.3, *second row – second*) [90]. This haze or reticulated-looking interface may take months to clear if the viscoelastic is not removed. Removing it leads to immediate resolution of the interface haze.

Infections

EK techniques create an interface between the donor tissue and the recipient stroma in which infectious agents can be introduced and get trapped during the surgical intervention. There have been reports of both bacterial and fungal infections deep in the interface after DSEK [91, 92]. Bacterial infections usually develop within a few days of the procedure. In contrast, fungal contamination may present more of an insidious course over weeks to several months. If both the endothelial lenticule and the recipient cornea show infiltration, a PK is required to ensure complete eradication of the infection. If the infection is limited to the endothelial graft alone, replacement of the EK graft may be attempted.

Future Prospects

Current research on endothelial diseases is directed toward culturing endothelial cells, pharmacological agents to stimulate endothelial stem cell proliferation, and inhibition of apoptosis of the endothelial cells [93, 94]. The results of these investigations have been promising. Other research is directed toward the identification of genetic mutations involved in specific endothelial cell diseases such as Fuchs endothelial dystrophy and customizing treatment [95]. It is possible that many new medical modalities may emerge for the management of some of the endothelial diseases.

Conclusion

DMEK, at this time, is the ideal selective transplant procedure for endothelial disorders providing perfect anatomical replacement of the diseased endothelium. However, DSEK continues to remain the surgery of choice for endothelial diseases associated with more complex anterior segment pathologies. Pharmacological modalities of treating endothelial diseases are an exciting breakthrough, but at this point it is uncertain whether these have potential to completely replace endothelial keratoplasty or serve as an adjunctive treatment modality in the management of endothelial dysfunctions.

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Anterior Lamellar Surgery

5

Naoyuki Maeda

Abstract

Anterior lamellar keratoplasty (ALK) is classified into two categories: superficial anterior lamellar keratoplasty (SALK) and deep anterior lamellar keratoplasty (DALK).

Superficial anterior lamellar keratoplasty consists of an automated lamellar therapeutic keratoplasty (ALTK) as an optical procedure for the superficial stromal disorders and lamellar grafting as a therapeutic procedure for corneal perforation, corneal thinning, or ocular surface diseases.

Recently, DALK has been actively performed for the stromal pathologies of the cornea with healthy corneal endothelium as a selective lamellar keratoplasty for optical purposes. DALK is also performed as a therapeutic procedure for serious corneal infection that does not respond to pharmacological therapy.

The advantages of DALK over penetrating keratoplasty are the elimination of endothelial rejection and better ocular integrity. As a trade-off, a steep learning curve, intraoperative complications such as corneal perforation and postoperative complications including double chamber, persistent stromal folds, and insufficient visual recovery due to residual stromal opacity may be found. To solve these problems in DALK, novel procedures and new instruments are being developed.

Keywords

Deep anterior lamellar keratoplasty • Automated lamellar therapeutic keratoplasty • Rejection reaction • Selective lamellar keratoplasty • Descemet's membrane • Femtosecond laser • Big bubble technique

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J. Hjortdal (ed.), *Corneal Transplantation*, DOI 10.1007/978-3-319-24052-7_5

Classification of Anterior Lamellar Keratoplasty

For a long time, penetrating keratoplasty had been conducted for the majority of corneal transplantation cases. However, the advances in techniques and technologies in eye surgery have enabled us to perform the selective lamellar keratoplasty on the eyes where corneal pathology is limited to the anterior or posterior part [2, 4, 32, 37, 44, 45].

Anterior lamellar keratoplasty has been performed for the tectonic purpose or optical purpose as shown in Fig. 5.1. For the optical purpose, automated lamellar therapeutic keratoplasty (ALTK) or deep anterior lamellar keratoplasty (DALK) is indicated for the corneal stromal disorders. For the tectonic purpose, central, peripheral, or ectopic lamellar keratoplasty is indicated for corneal perforation, corneal stromal thinning, immunologic corneal diseases, and ocular surface disorder.

Superficial Anterior Lamellar Keratoplasty

Automated Lamellar Therapeutic Keratoplasty (ALTK)

Purpose and Indication

The purpose of the automated lamellar therapeutic keratoplasty (ALTK) is visual recovery from the corneal disorders that have stromal opacity from superficial to mid-stroma. Good indications are the superficial stromal disorders such as lattice corneal dystrophy, stromal scar after corneal infection, refractive surgery, or trauma [9, 42, 48]. If the depth of the stromal opacities is deeper than 100 μm from the surface, ALTK rather than PTK is indicated.

ALTK is less invasive than the conventional anterior lamellar keratoplasty. The procedure can be sutureless with topical anesthesia. The wound quality by microkeratome [16] or femtosecond laser [5, 51] is less irregular than that by the manual cut, and the femtosecond laser can minimize the disparity of the size between host and graft. The shape of the donor by the microkeratome basically resembles the free-cap flap in LASIK. The thickness profile of the graft is meniscus shaped in the center, and the edge is tapered with an acute angle. Therefore, one needs to pay careful attention to wrinkles and dislocation of the graft. In contrast, the graft made by femtosecond laser has a planar shape in the center, and the edge has a side cut with an obtuse angle. Therefore, less dislocation of the graft and better wound adaptation is expected.

On the other hand, femtosecond-assisted ALTK cannot be performed on eyes with dense corneal opacity. In addition, one should recognize that the shape of the stromal bed would be irregular if ALTK was performed on eyes with an irregular anterior surface.

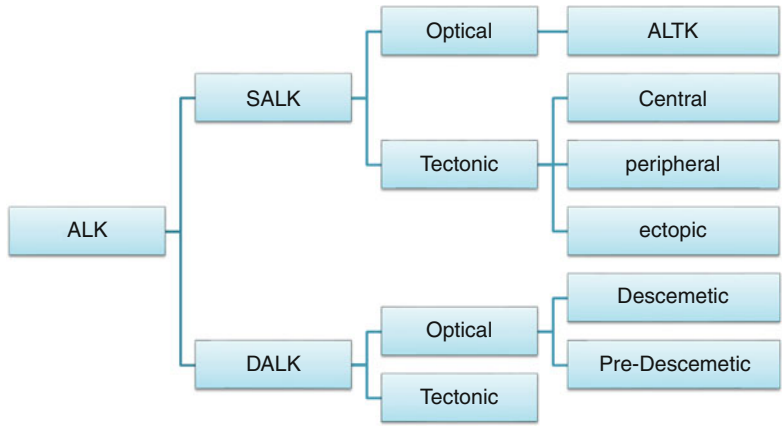

Fig. 5.1 Classification of anterior lamellar keratoplasty

Preoperative Examination

To evaluate the surgical indication for ALTK, the depth and density of the stromal opacity and the smoothness of the anterior corneal surface are critical. Slit lamp examination and corneal topography are useful for identifying the irregularity of the anterior corneal surface. Also, it will be difficult to use femtosecond laser if the iris under the lesion is invisible with slit lamp examination. The anterior segment OCT is helpful for checking the depth of the stromal opacity and determining the depth of cut. To avoid keratectasia, the stromal bed should be more than 250 μm in ALTK.

Procedure and Postoperative Management

The graft is prepared first using microkeratome or femtosecond laser with the use of the artificial anterior chamber for the corneoscleral donor. The thickness is generally set to 250 μm for microkeratome and 200–280 μm for femtosecond laser with the epithelium off. The graft is removed under the surgical microscope and stored. With the topical anesthesia, the stromal cut is made using microkeratome or femtosecond laser for the recipient. After the removal of the pathological tissue, the donor tissue will be placed on the stromal bed for a waiting period of more than 3 min.

When the interface between host and graft is irregular or there is a disparity in size between the host and graft, several sutures will be placed. Finally, a bandage soft contact lens is put in place to avoid the dislocation of the graft.

Adjunctive surgery might be conducted to enhance the outcome in the femtosecond-assisted ALTK. PTK is sometimes done in order to remove the residual scars or smooth the stromal bed simultaneously or separately [38].

The postoperative treatment is basically similar to LASIK or PTK. After surgery, the contact lens is removed, and epithelial healing is confirmed. The topical steroid and antibiotics are prescribed and tapered.

The main complications associated with ALTK are residual stromal opacity at the stromal bed and irregular astigmatism. Epithelial ingrowth rarely occurs at the interface.

Central SALK

Purpose and Indication

Currently, conventional ALK is being replaced by penetrating keratoplasty (PK) or DALK in most cases. This is because the visual improvement by conventional ALK is suboptimal as a result of the irregular astigmatism or scattering associated with the irregular thickness profile in the lamellar donor and stromal bed in the recipient cornea in addition to the residual opacity at the stromal bed.

However, there are a couple of exceptions in which conventional ALK is still being performed. One is for ocular surface disease, and another is for the tectonic purpose such as corneal perforation or Descemetocele.

For the ocular surface diseases associated with limbal stem cell deficiency (LSCD), PK was considered to be contraindicated because of the high incidence of endothelial rejection. Although DALK can be performed for ocular surface disease, the procedure is usually more difficult than for keratoconus or corneal stromal dystrophy, and there are higher risks of perforation which might require the conversion to PK. To avoid the corneal perforation, central SALK combined with LT or cultured cell sheet is preferred in such cases, especially for allograft transplantation. In addition to improving the transparency of the stroma, SALK is useful for providing the healthy stroma as the base for harvesting the implanted epithelium migrating from the LT [19, 47] or cultured cell sheet [27, 29].

PK has been performed on eyes with corneal perforation and Descemetocele for a long time. As the ocular inflammation and anterior synechia associated with perforation can be a risk factor for the rejection reaction of the endothelium, lamellar grafting has advantages over PK in such conditions. Mini-lamellar graft is preferred for sealing the corneal perforation due to stromal melting associated with rheumatoid arthritis and other autoimmune diseases. This can be done with a cryopreserved donor as the emergency surgery.

Preoperative Examination

It is not easy to observe the condition of the corneal thickness and the anterior chamber with slit lamp examination in patients with severe ocular surface diseases such as Stevens-Johnson syndrome/toxic epidermal necrosis and ocular cicatricial pemphigoid. Anterior segment OCT is useful for evaluating the thickness profile of the cornea for these conditions. More surgical skill will be required if the eye has extreme corneal thinning. Measurement of corneal thickness at the site of partial trephination can be performed preoperatively and/or intraoperatively with OCT and pachymetry to determine the depth of cut.

Procedure

In the central ALK for ocular surface diseases, the partial trephination up to about half depth of the thinnest pachymetric reading at the site followed by the manual dissection of host cornea is performed first using a knife or a spatula. The use of spatulas and lamellar dissectors exclusive to lamellar keratoplasty is recommended. The very fine slit illuminator that can be attached to the operation microscope is commercially available and is very useful during delamination in terms of the prevention of perforation and for maintaining uniform thickness of the stromal bed. The handheld slit lamp can be used instead.

After completing the lamellar dissection of the host cornea, the lamellar donor will be prepared. The donor cornea will be mounted on the artificial anterior chamber and well pressurized. The donor was trephined partially with the same diameter and dissected using spatulas. Then, the donor cornea will be placed and sutured.

After surgery, watch carefully for possible infection at the interface between host and graft, persistent epithelial defect in limbal stem cell deficiency, and stromal rejection in case of vascularized cornea.

Peripheral SALK

Purpose and Indication

Peripheral SALK is considered for the tectonic purpose when there is a perforation or a remarkable stromal thinning at the peripheral cornea. This procedure is performed to increase the ocular integrity by treating or preventing corneal perforation and also by reinforcing the peripheral cornea and inhibiting the protrusion of the thinning area. Terrien marginal corneal degeneration, Mooren ulcer, and marginal ulcer associated with autoimmune diseases are the main indications for the peripheral SALK [6, 30].

Preoperative Examination

It is important for surgeons to evaluate the area of thinning at the peripheral cornea. In addition to the slit lamp examination, anterior segment OCT and Scheimpflug camera are useful for mapping the thickness profile of the cornea. Based on the information, one can determine the shape and size of the graft.

The inflammation of the ocular surface should be checked and reduced as much as possible before surgery. The loss of palisades of Vogt (POV) and delayed fluorescein staining of the corneal surface are important signs indicating limbal stem cell deficiency.

For Mooren ulcer, the lesion of corneal infiltrate and area of inflammation at the conjunctiva should be checked carefully, and Brown's conjunctival excision and keratoepithelioplasty (KEP) should be considered for the active inflammatory stage with the general and topical use of steroid and immunosuppressant [20, 46].

Procedure

If only localized corneal perforation was found with minimum localized area of corneal thinning, mini-lamellar graft can be considered. However, if the corneal thinning is extended circumferentially for most of the eye, peripheral SALK is indicated. In such cases, the use of the usual round graft does not fit the shape of the corneal thinning, and the host-graft junction may cross the area on the entrance pupil, resulting in irregular corneal astigmatism. Therefore, an annular, sector annular, or crescent-shaped lamellar graft is made. In the extremely advanced cases, total lamellar graft may be used. To create the lamellar graft, the marking of the thinning zone of the host cornea is critical, and in addition a paper pattern may be useful for measuring the area with a caliper.

For the annular or sector annular graft, marking with large and small trephine blades is useful for creating an identical incision at both host and graft. Incision is made manually with a knife on the mark. Then, the stroma inside the incisions is carefully dissected with lamellar spatulas. The donor is mounted at the artificial chamber or sutured to the base for improving handling during the preparation. The same marking is made on the donor, and identical incisions are made followed by the dissection in the same fashion. For advanced cases, free-hand corneoscleral graft may be used.

After aligning the graft on the host, the graft is sutured from the proximal side followed by the suture at the distal side alternately. The deep and short bite is preferred especially at the proximal side. The soft contact lens will be placed for a while to facilitate wound healing and to prevent the persistent epithelial defect. The appropriate use of general and topical steroid and immunosuppressant is critical for inflammatory disorders.

Ectopic SALK

Purpose and Indication

Ectopic SALK is sometimes considered for limbal dermoid, recurrent pterygium, conjunctival defect associated with tube shunt, necrotizing scleritis, or scleromalacia [35, 39]. This is mainly for the tectonic lamellar patching, to prevent the recurrence of the original disease, conjunctival epithelization, and reinforcing the scleral tissue. Although scleral tissue can be used for the condition, corneal tissue has advantages over scleral tissue, such as better epithelization or prevention of the original disease, because of the intact Bowman's layer and for the cosmetic reason that the graft is close to the peripheral cornea or the lesion extending to the peripheral cornea.

Preoperative Examination

Ocular infection and limbal stem cell deficiency should be ruled out. Diplopia and the limitation of eye movement should be checked carefully for recurrent pterygium. For limbal dermoid during childhood, the prevention of amblyopia is the key for the procedure. Examination and treatment for amblyopia should be conducted not only after surgery but also before surgery.

Procedure

For limbal dermoid, the conjunctiva is removed from the surrounding area of the dermoid. The cautery of feeding vessels is helpful for avoiding bleeding during excision of the tissue. The partial cut with trephine followed by dissection under the dermoid is conducted. The slit illumination during excision is useful for avoiding the corneal perforation. Full-thickness graft can be used in most of cases. If necessary, trimming at the posterior edge of the graft is effective for good adaptation of the wound.

In general, the recurrence of pterygium can be treated with the combination of mitomycin C, amniotic membrane, and conjunctival autograft. However, massive proliferation of the conjunctiva or thinning at the peripheral cornea and/or sclera may require the additional use of a tectonic lamellar patch.

Deep Anterior Lamellar Keratoplasty (DALK)

Purpose and Indication

For a long time, penetrating keratoplasty (PK) had been the gold standard of surgery for loss of vision associated with corneal opacities or severe irregular astigmatism. PK is a straightforward procedure, and the results are generally excellent without the serious general complications that are sometimes inevitable in allogenic transplantation, including the kidney, heart, liver, or other organ transplantations.

However, there are some problems in PK to be solved. Intraoperatively, surgeons have to worry about the risk of suprachoroidal hemorrhage as part of the nature of the open sky procedure. Postoperatively, endothelial rejection and endothelial decompensation can be the main cause of graft failure. Topical steroid that is used for the prevention of rejection reaction might induce glaucoma in steroid responders and also increase the risk of infection and cataract. The ocular integrity following PK is not as good as that in

normal eyes, and there is a risk of wound dehiscence associated with trauma or suture removal [18, 22].

In addition, there is a trend toward minimally invasive ophthalmic surgery nowadays. If patients had stromal pathologies of the cornea with healthy corneal endothelium, DALK, which replaces only pathological stroma and preserves the corneal endothelium and Descemet's membrane, will be less invasive and can be performed as a closed surgery. Because corneal endothelium is auto-tissue in DALK, endothelial rejection and prolonged used of topical steroid can be avoided, and a stronger wound is advantageous for earlier suture removal and less risk of wound dehiscence.

Although the concept of selective lamellar keratoplasty has been considered for a long time, sufficient dissection of stroma in DALK was technically very difficult and time consuming. Lamellar keratoplasty, which is currently termed anterior lamellar keratoplasty (ALK), had been mainly performed not for optical purposes but for tectonic purposes. This is because manual dissection of the stroma has to be conducted for both host and donor in ALK. As the lamellar graft and stromal bed of the host tended to be irregular by the manual dissection, visual recovery following ALK was suboptimal and inferior to that following PK. Therefore, PK has been the primary procedure even for eyes with healthy corneal endothelium.

The advances in surgical microscopes and instruments for stromal dissection made it possible to perform layer-by-layer removal of pathological stroma. In addition, the visualization and handling of the very thin corneal stroma were remarkably improved by the air injection to the stroma [3] or hydration of stroma [40]. With these techniques, surgeons can expose the Descemet's membrane or reach the pre-Descemetic layer and implant the full-thickness graft. This procedure was previously called deep lamellar keratoplasty (DLK). As DLK also stands for diffuse lamellar keratitis after LASIK, the acronym DALK took the place of DLK.

These layer-by-layer techniques required specific skills on the part of the surgeon and longer surgical time with unique intra- or postoperative complications such as the rupture of Descemet's membrane and double chamber.

The big bubble technique developed by Anwar made DALK more popular [2]. This is because one can expose the Descemet's membrane more easily in less time than with layer-by-layer techniques. When the big bubble was not shown, the visibility of corneal stroma was deteriorated, and the layer-by-layer procedure became more difficult. For avoiding perforation with the needle and increasing the probability of big bubble formation, modified techniques such as the use of blunt cannula and lamellar dissection before air injection were devised. As alternative techniques for exposing the Descemet's membrane, many variations including hooking technique and viscodelamination technique were introduced.

Currently, DALK can be classified into two categories: Descemetic DALK and pre-Descemetic DALK (Fig. 5.2). In pre-Descemetic DALK, the thin stroma layer still remains on the Descemet's membrane, so its surface is irregular. On the other hand, in Descemetic DALK the Descemet's membrane or pre-Descemetic layer is exposed, and the surface is shiny and smooth.

Indications for optical DALK are irreversible stromal opacity with intact corneal endothelium and Descemet's membrane. Therefore, keratoconus, stromal corneal dystrophy, necrotizing keratitis in herpetic keratitis, old interstitial keratitis, stromal scar after trauma, or corneal infection are good indications for DALK. Also, keratectasia following LASIK and extremely irregular cornea following radial keratotomy or other refractive surgeries can be indications for DALK. On the other hand, if there is a history of acute hydrops in keratoconic eye or damage to Descemet's membrane for any reason, there will be a higher chance of rupture in Descemet's membrane during big bubble technique.

Recently, therapeutic DALK for severe corneal infection has been attempted [1, 41].

Preoperative Evaluation

As DALK is basically the procedure that replaces the pathological corneal stroma with a healthy one, all the other components, i.e., corneal

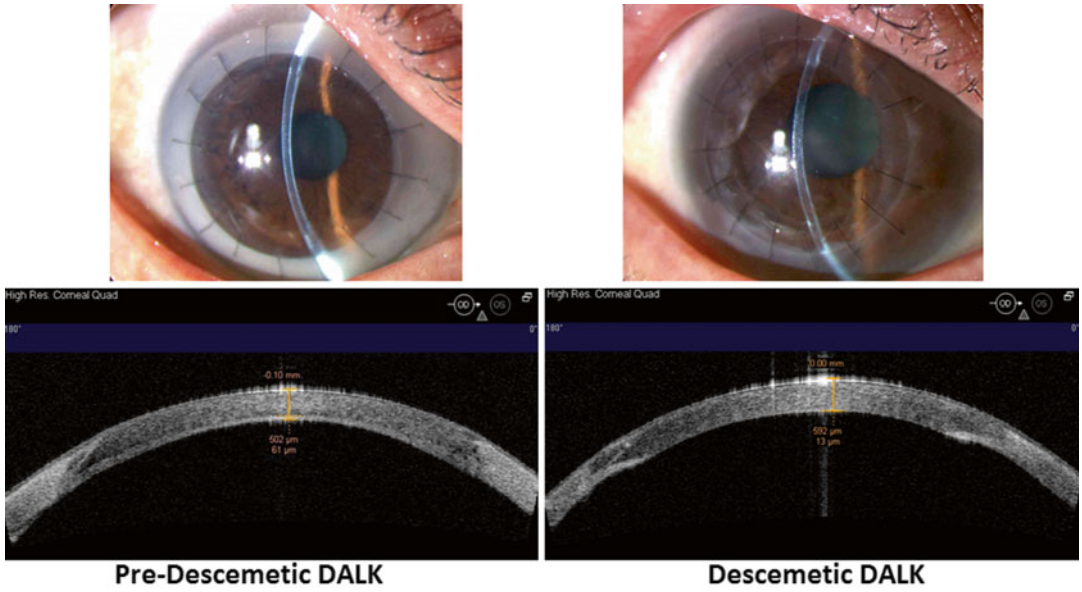

Fig. 5.2 Pre-Descemetic DALK and Descemetic DALK

endothelium, Descemet's membrane, and corneal epithelium should be determined as being in good condition before surgery.

Endothelial function can be estimated by the combination of slit lamp examination, specular microscope, pachymetry, and OCT. The slit lamp findings such as stromal edema, subepithelial edema, and folds in Descemet's membrane are signs of endothelial dysfunction. The endothelial cell density at the clear part of the cornea is useful for estimating the corneal endothelial count when the corneal pathology is in an inactive stage. Pachymetry with ultrasound or OCT is useful for quantitating the stromal edema. The cross-sectional images of the cornea with the anterior segment OCT can show us the information at the invisible area with slit lamp because infrared light penetrates more deeply than visible light. The folds in Descemet's membrane or retro-corneal fibrosis may be identified with OCT in the invisible area with the slit lamp. The observation of Descemet's membrane with the slit lamp examination and OCT is also very important for excluding its damage due to acute corneal hydrops or perforating corneal injury.

For working out a surgical strategy, confirming the thickness profile of the stroma, especially the location and degree of the thinnest point and the thickness at the site of partial trephination, is helpful.

Similarly to PK, the assessment of cornel epithelium for ruling out limbal stem cell deficiency is critical, and also the evaluation of the condition of the lid, lacrimal function, and function of the nasolacrimal duct is necessary. Glaucoma, ocular infection, and inflammation of the anterior segment should be treated and stabilized before the surgery. Ocular comorbidities including cataract, vitreoretinal diseases, and neuro-ophthalmological disorders should be evaluated.

Procedure

DALK can be performed under local or general anesthesia while soft eye is maintained during surgery. It is important to ready for the conversion to PK in all cases, even if the risk is low. For considering the risk of conversion to PK, the pupil is usually constricted with miotic drug before surgery. Partial thickness trephination is performed on half to two-thirds of the corneal thickness at the area. Then, stromal dissection is started with one of the following techniques.

Big Bubble Technique

The original technique introduced by Anwar uses the fine needle with the bevel side down and inserts the tip of the needle to the deep stroma near the center from the partially trephined area followed by the injection of the air to the stroma [2]. Although the emphysema at the stroma is usually made of fine bubbles, the whitish ring (big bubble) that expands from the site of needle to the periphery can be seen when the tip of the needle is close enough to the posterior corneal surface. This phenomenon is called the formation of big bubble, and it represents the separation of Descemet's membrane from the stroma inside the whitish ring.

There are two types of big bubble [12]. The Type 1 bubble starts from the center of the cornea and expands to the periphery in a concentric fashion. It usually stops when the diameter reaches about 8.5 mm diameters. The Type 1 bubble has a well-circumscribed central dome shape. The dissection in Type 1 is conserved between the stroma and pre-Descemetic layer [12, 15]. This pre-Descemetic layer is acellular and strong, and its thickness is about 10 μm. The Type 2 bubble is sometimes eccentric, and it starts anywhere and easily expands up to angle. The Type 2 bubble is thin walled and can extend up to 10.5 mm in diameter. The dissection is considered between the pre-Descemetic layer and Descemet's membrane. The Type 2 bubble is easy to rupture even with the mild touch of blunt instruments.

After the formation of the big bubble, a side port is created to reduce the intraocular pressure, and a little air will be injected inside the anterior chamber. This small bubble in the anterior chamber is used for the "small-bubble technique" or "bubble test" [28]. If Descemet's membrane is separated from the corneal stroma, there will be a protrusion of the posterior corneal surface to the anterior chamber. In such a case, small bubbles will stay at periphery and cannot pass across the center. On the other hand, the small bubbles will locate at the center while no big bubble formation is made.

When the big bubble formation is confirmed, lamellar dissection of the pathologic stroma inside the partial trephination is performed. Then, the big bubble is opened by a slash with a knife, and viscoelastic material is injected inside. The margin of the separation of Descemet's membrane should be more periphery than the partial trephination. If not, viscoelastic material or a spatula is used to enlarge the separation. Next, the cross incisions are created, and the stromal roof of the big bubble is removed. After washing the viscoelastic material on Descemet's membrane, the full-thickness graft from which the endothelium and Descemet' membrane was peeled out is put on Descemet's membrane and will be sutured.

When the big bubble is not formed, a layer-by-layer technique is employed. In such a case, the transparency of the stroma is lost through the emphysema in the stroma, making it difficult to expose Descemet's membrane. Also, there are some risks of perforation during the reinsertion of sharp needle. The air-visco bubble technique is the method to create the big bubble with viscoelastic material in the case of a failed big bubble. After partial lamellar dissection, viscoelastic material is injected to the deep stroma using a 27G sharp needle after the small bubbles in the anterior chamber [26].

Currently, there are many modifications of the big bubble technique that avoid the incomplete big bubble or improve the visualization of the stroma (Fig. 5.3).

One is the use of blunt cannula instead of sharp needle. By using the cannula with the hole to the inferior side, the risk of perforation is less, and the chance of the big bubble formation is more. The stromal dissection prior to big bubble creation is helpful for controlling the depth of the cannula or the needle and makes it easier to slash the big bubble or to cut the residual stroma [28]. The use of viscoelastic material instead of air (visco-delamination) is another alternative [25], it can create the big bubble slowly, and the size of the big bubble is well manageable. Although the visibility of the stromal bed is better, the border of the big bubble is difficult to recognize. Therefore, the bubble test or the use of slit illumination is still necessary.

The hooking technique is the method for reaching the tip of the cannula to the Descemet's membrane by hooking the stroma with a fine forceps [50].

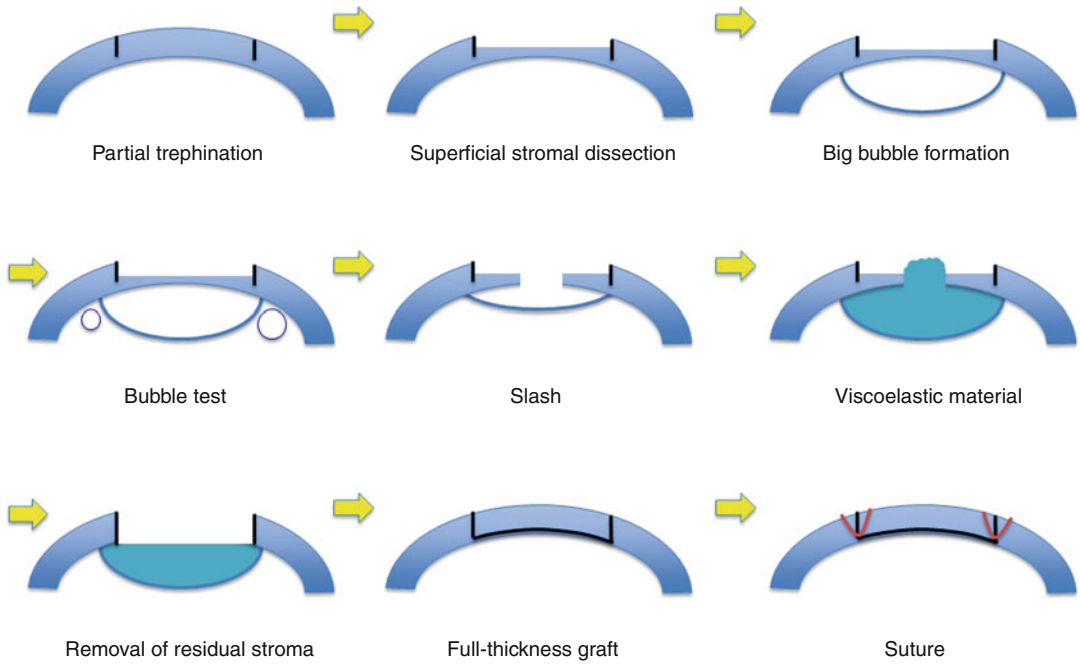

Fig. 5.3 Example of modified big bubble technique

Layer-by-Layer Dissection

The stromal dissection by the layer-by-layer method is performed using the forceps and dissecting knife or spatula. When the tissue is lifted with a forceps, the edge of the incision shows the whitening line along the lamellae as a result of the air penetration. Then the dissection can be performed with the knife along the whitened line. However, it is sometimes very difficult to grasp the thin stroma because of the risk of perforation.

Intrastromal air injection is a method for stromal dissection [3]. The air distends the corneal lamellae and facilitates the stromal dissection. Another method for facilitating the layer-by-layer technique is the hydration of the stroma by BSS (hydrodelamination) and delamination with a spatula [40].

When the spatula is reached to Descemet's membrane, the resistance of the stroma will suddenly be reduced, and the spatula can be inserted in the space between the stroma and Descemet's membrane. The eye should be softened by releasing the aqueous humor from the side port in order to avoid the bulge of Descemet's membrane. Then, residual stroma above Descemet's membrane can be peeled off with a knife or scissors. As the risk of perforation will be higher when Descemet's membrane is exposed until the peripheral cornea, exposure of Descemet's membrane is usually limited to central 5 mm diameter or so in the layer-by-layer technique.

Limbal Approach

The deep lamellar pocket can be created through the limbal approach [24]. From the side port, the aqueous is aspirated, and the anterior chamber is filled with air. The scleral incision is created, and stromal dissection is started with the special blade for corneal lamellar dissection. The tip of the blade can be placed very close to Descemet's membrane with the aid of specular reflex from the posterior corneal surface as described later (Melles technique). Then, the blade is positioned parallel to the posterior surface and creation of the stromal pocket across the cornea is started. The air in the anterior chamber is removed, and the stroma pocket is filled with the viscoelastic material followed by trephination of the overlying anterior stroma. After removing the residual anterior stroma with scissors and irrigating the

viscoelastic material, the donor will be placed on the recipient bed and sutured.

Identification of Stromal Thickness or Instrument Position

One of the most difficult points in DALK is the inability to visualize the residual stromal depth or the distance between the instrument and Descemet's membrane under the operating microscope. Coaxial illumination and oblique illumination are not useful for the purpose.

Intraoperative findings are sometimes useful for estimating the depth. During the stroma dissection, the lamellar structure at the superficial stroma is compact and firm. On the other hand, the posterior stroma is rough and soft. During the delamination with spatula, the radial folds from the tip of the spatula will appear if the tip is close enough to Descemet's membrane.

The Melles technique utilizes the specular light reflex at the posterior corneal surface [24]. To enhance the specular light reflex, the aqueous in the anterior chamber is replaced with air. Between the blade tip and light reflex, the dark band that indicates the unincised posterior corneal tissue can be seen. Therefore, surgeons can reach just anterior to the Descemet's membrane when the dark band disappears.

A more direct method is the visualization of the cross section of the cornea with a slit illuminator as we do in the office with a slit lamp. Slit illuminators for vitreoretinal surgery (Zeiss) and the slit illuminator utilizing a very fine LED light for selective lamellar corneal surgery (MS-SI01, Topcon, Japan) are commercially available and can be mounted on the operating microscope. With slit illumination, the surgeon can recognize the residual stromal thickness or the distance between the instrument and Descemet's membrane continuously during the maneuver. Also, a handheld slit (510 L, Eidolon Optical LLC) can be used not only for DALK but also for Descemet's membrane endothelial keratoplasty (DMEK) [8].

Currently, anterior segment OCT is available for evaluating the information about the depth. One is the OCT exclusive for surgery by Zeiss, which is mounted in the operating microscope and can measure cross-sectional images in real time. Another OCT is the portable-type OCT (iVue 100–2: Optovue, USA) mounted on the arm [11]. In such cases, observation with the operating microscope and measurement by OCT are performed alternatively.

Application of Femtosecond Laser

Recently, the femtosecond laser has been applied not only for the LASIK but also penetrating keratoplasty, DSAEK, and astigmatic keratotomy [5]. This trend is also true in DALK. Instead of partial straight-edge trephination performed manually, zigzag or mushroom configuration by femtosecond laser is performed [31, 36]. The deep incision minimizes air escape into the peripheral cornea during the big bubble technique and also is useful as the reference for manual stromal dissection. In addition, non-straight wound configuration can facilitate the matching of anterior surface between host and graft [31].

Clinical Outcome

Results

The clinical outcomes after DALK from the experts showed similar results. Visual outcome in DALK using the big bubble technique is comparable to that in penetrating keratoplasty in patients with keratoconus [13]. There were no relevant differences between Descemetic DALK and pre-Descemetic DALK in patients with keratoconus except for the faster visual recovery in Descemetic DALK [33]. The comparison among Descemetic DALK, pre-Descemetic DALK, and PK indicated that visual acuity in Descemetic DALK is significantly better than that in pre-Descemetic DALK or PK [43]. Also, there were no significant clinical outcomes between successful big bubble and failed big bubble followed by manual dissection [7].

A report by the American Academy of Ophthalmology concluded that DALK is equivalent to PK for the outcome measure of BSCVA, particularly if the surgical technique yields minimal residual host stromal thickness on the basis of level II evidence in 1 study and level III

evidence in 10 studies. DALK has important theoretic safety advantages for no endothelial rejection and an extraocular procedure [32].

Long-term graft survival in DALK for various corneal disorders is very good and does not vary significantly over time with stable endothelial cell density [34]. Although donor cornea with a total tissue age of more than 100 years is still clear, keratoconus patients had keratoplasty while they are relatively young and might have cataract surgery in the future [49]. DALK might have potential advantages over PK after couple of decades.

In terms of the effects of femtosecond laser-assisted trephination, the comparison between straight-edge trephination by manual trephine and mushroom configuration by femtosecond laser showed comparable results except for the earlier visual recovery by the femtosecond laser [36].

A therapeutic success rate of 84.6 % was achieved in the DALK group, and 88 % in the PK group ($P=0.74$) A BCVA of > or = 6/9 was achieved in 50 % of patients in the TDALK group and 20.2 % in the TPK group ($P=0.01$). Kaplan-Meier survival analysis at 1 year showed better graft survival for TDALK (90 %) compared with TPK (78.4 %) [1].

On the other hand, the reports from the multicenter study indicated different aspects. A large study by the national registry of corneal transplantation in Australia indicated that DALK is being performed more than ever before. Survival of DALK is worse than the survival of penetrating grafts performed for the same indications over the same timeframe [10]. The study from the United Kingdom revealed that DALK for keratoconus had a higher overall failure rate than PK, mainly in the form of early failure related to the surgeon's experience [17].

Intraoperative Complications

The major advantage of DALK over PK during the surgery is that choroidal hemorrhage is not reported.

On the other hand, there is a unique intraoperative complication inherent in DALK, which is perforation of Descemet's membrane. This complication is subdivided into micro-perforation and macro-perforation. In micro-perforation, DALK can still be performed, and air is injected into the anterior chamber at the end of procedure to avoid the postoperative double chamber. When macro-perforation occurs during DALK, conversion to PK is necessary, from 4 to 39 %, and conversion rate to PK is from 0 to 14 % [44].

Postoperative Complications

The most important advantage of DALK over PK is that the late corneal failure due to endothelial cell rejection is not anticipated [32]. The decreased dependency on the topical steroid will be beneficial for reducing the incidence of steroid-induced glaucoma. As DALK is basically not intraocular surgery, endophthalmitis is rare compared with PK, and also no cystoid macular edema or retinal detachment is likely to be found. In addition, wound dehiscence is less compared with PK because the posterior corneal surface is maintained from limbus to limbus.

In terms of irregular and regular astigmatism, there was no difference between the two procedures [21].

On the other hand, there are some complications unique to DALK. The double chamber can be found in eyes with and without perforation. The double chamber in eyes without perforation tends to be self-limiting and will disappear in a couple of weeks. Air injection is necessary for double chamber with perforation. Pupillary block, gas-induced cataract, or persistent mydriasis (called Urrets-Zavalia syndrome) are associated with the air tamponade [23].

Similarly to the other corneal lamellar surgeries, bacterial or fungal infection might be found at the interface between donor and host [14]. The epithelial rejection reaction may be found in the form of a linear white lesion with indirect illumination and irregular linear fluorescein staining. It usually happens without being noticed, and the epithelium of the donor will be replaced by that of the host. No additional treatment will be required. The stromal rejection reaction is shown as the stromal edema limited in the donor with conjunctival injection. If treatment was not started earlier, neovascularization at the interface and inside the stroma can be recognized.

The disparity in size between donor and host sometimes induces wrinkles at the posterior corneal surface, especially in advanced keratoconus, because the arc length in keratoconus is longer than that in normal subjects even in the same diameter.

The residual opacity at the stromal bed or irregular posterior surfaces can be the origin of insufficient recovery of vision in pre-Descemetic DALK.

Postoperative Management

Although postoperative management following DALK is basically similar to that following PK, topical steroid can be tapered earlier in DALK than in PK, and suture removal can be performed earlier in DALK than in PK.

When air injection is required for the double chamber, the appropriate amount of air and mydriasis must be employed in order to prevent extreme IOP rise and pupillary block.

For cataract surgery after DALK, incisions should be created carefully, and the stress to the wound by the instruments should be minimized in order to avoid double chamber. Therefore, the sclerocorneal incision may be better than corneal incision.

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The Penetrating Keratoplasty (PKP): A Century of Success

6

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Abstract

Besides routine postoperative follow-up, the prophylaxis of complications in penetrating keratoplasty (PKP) includes special preoperative and intraoperative aspects. Preoperative prophylaxis consists of the therapy of systemic diseases and eyelid abnormalities, determining individual optimal graft size, avoiding PKP in cases of uncontrolled intraocular pressure, avoiding PKP in cases of acute corneal hydrops, pretreatment of vascularized cornea, amniotic membrane transplantation before PKP in cases of ulcerative keratitis, quality-controlled organ-cultured transplants, and preoperative counseling by the microsurgeon to ensure patient compliance. Intraoperative prophylaxis consists of controlled arterial hypotension and complete relaxation during general anesthesia and application of a Flieringa ring in aphakic vitrectomized eyes. Precautions for intraoperative prophylaxis of astigmatism must be followed. A measurable improvement seems to be possible using the technique of nonmechanical trephination of patient and donor from the epithelial side using the excimer laser but not the femtosecond laser. Graft size should be adjusted individually ("as large as possible, as small as necessary"). Limbal centration should be preferred over pupil centration (especially in keratoconus). In addition to the situation-specific diagnosis and preoperative planning, the critical selection of the donor tissue, and the minimally invasive

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J. Hjortdal (ed.), *Corneal Transplantation*, DOI 10.1007/978-3-319-24052-7_6

microsurgical technique, it is especially the indication-dependent close-meshed follow-up which plays an important role in the long-term success of penetrating keratoplasty. In the follow-up process, the repeated emphatic sensitization of the patient to alarming subjective symptoms and the informed involvement of the ophthalmologist in private practice providing the follow-up treatment must be considered of crucial importance. "Treat them and street them" is certainly not the motto to follow!

Keywords
Corneal transplantation • Penetrating keratoplasty • Trephination technique • Suture technique • Astigmatism • Immune reactions • Complications • Excimer laser • Femtosecond laser • Prophylaxis

Summary

Besides routine postoperative follow-up, the prophylaxis of complications in penetrating keratoplasty (PKP) includes special preoperative and intraoperative aspects.

Preoperative Prophylaxis Preoperative prophylaxis consists of the therapy of systemic diseases and eyelid abnormalities, determining individual optimal graft size, avoiding PKP in cases of uncontrolled intraocular pressure, avoiding PKP in cases of acute corneal hydrops, pretreatment of vascularized cornea, amniotic membrane transplantation before PKP in cases of ulcerative keratitis, quality-controlled organ-cultured transplants, and preoperative counseling by the surgeon to ensure patient compliance.

Intraoperative Prophylaxis Intraoperative prophylaxis consists of controlled arterial hypotension and complete relaxation during general anesthesia and application of a Flieringa ring in aphakic vitrectomized eyes. Ten precautions for intraoperative prophylaxis of astigmatism include:

1. An attempt should be made to determine donor topography for exclusion of previous refractive surgery and keratoconus/high astigmatism and to allow for "harmonization" of donor and recipient topography.
2. Donor and recipient trephination should be performed from the epithelial side with the same system, which is the prerequisite for congruent cut surfaces and angles in donor and recipient. For this purpose an artificial anterior chamber is used for donor trephination.
3. Horizontal positioning of the head and limbal plane are indispensable for state-of-the-art PKP surgery in order to avoid decentration, vertical tilt, and horizontal torsion.
4. Orientation structures in donor and host facilitate the correct placement of the first four or eight cardinal sutures to avoid horizontal torsion.
5. A measurable improvement seems to be possible, using the Krumeich guided trephine system (GTS), the second-generation Hanna trephine, and the Erlangen/Homburg technique of nonmechanical trephination with the excimer laser. Since 1989 more than 4,000 penetrating keratoplasty operations (PKP) have been performed successfully with the Zeiss Meditec MEL60® and, recently, with the Schwind Amaris® excimer laser in Erlangen and Homburg/Saar.
6. Graft size should be adjusted individually ("as large as possible, as small as necessary").
7. Limbal centration should be preferred over pupil centration (especially in keratoconus).
8. Excessive graft over- or undersize should be avoided to prevent stretching or compression of peripheral donor tissue.
9. As long as Bowman's layer is intact, a double-running cross-stitch suture (according to Hoffmann) is preferred since it results

in higher topographic regularity, earlier visual rehabilitation, and less suture loosening requiring only rarely suture replacement.
10. Intraoperative keratoscopy should be applied after removal of lid specula and fixation sutures.

Postoperatively Postoperatively, periodical control examinations using fluorescein and blue light are indispensable. All loose sutures have to be removed as early as possible. In cases of herpetic eye disease, 2 × 400 mg of oral acyclovir should be administered for at least 1 year. In cases of therapy-resistant epithelial defects, 100 % autologous serum eye drops or amniotic membrane transplantation (patch technique) is a valid option. Immune reactions must be diagnosed and treated immediately with high doses of corticosteroids (topically, intracamerally, systemically).

Results Prospective clinical studies have shown that the technique of non-contact excimer laser PKP improves donor and recipient decentration and reduces vertical tilt and horizontal torsion of the graft in the recipient bed, thus resulting in significantly less all-sutures-out keratometric/topographic astigmatism, higher regularity of the topography, and better visual acuity. Besides less blood-aqueous barrier breakdown during the early postoperative course after PKP, excimer laser trephination does not induce cataract formation and does not impair the graft endothelium. Likewise, the rate of immunological graft rejections is not adversely affected by the excimer laser but by femtosecond laser trephination. In addition, trephination of an instable cornea is facilitated using the non-contact excimer laser.

Conclusions Because of undisputed clinical advantages, especially in eyes with keratoconus, excimer laser trephination with orientation teeth/notches is still favored in Homburg/Saar in daily practice. The femtosecond laser-assisted keratoplasty technique has been very exciting but due to major lack of all-suture-out data after introduction of the technique 10 years ago – the superiority of this high-price and difficult-to-maintain option has not been proven, yet!

Surgical Techniques

The first successful total penetrating keratoplasty (PKP) was performed by Eduard Zirm on December 9, 1905, in Olmütz, which today is located in the Czech Republic. This means that corneal transplantation is the oldest, most common, and most successful transplantation in humans overall [85]. In the USA approximately 45,000 keratoplasties are performed per year, with the equivalent figure being more than 5.200 in Germany; in Homburg/Saar we performed around 300 in 2014. In the year 2013, 43.5 % of all corneal transplants performed in Germany were of the posterior lamellar type, with only 4.4 % being anterior lamellar grafts and 52.1 % still being carried out as PKP. This survey is based on the German Keratoplasty Register, which since 2002 has been maintained by the DOG-Sektion Kornea.

With a better understanding of immunological transplant reactions and "secondary glaucomas" after PKP, the demands placed on microsurgeons with regard to corneal transplantation have increased. Today, a crystal-clear cornea after PKP with high and/or irregular astigmatism, especially in combination with high anisometropia, can no longer be considered successful in normal-risk keratoplasty. With the increasing experience of the microsurgeon, the *technique of keratoplasty* goes far beyond the replacement of two collagen disks and is crucial for the functional postoperative outcome.

Astigmatism and Keratoplasty

Definition of Astigmatism after Keratoplasty

The cornea provides approximately two thirds of the refracting power of the human eye. Surgical interventions on the cornea can therefore significantly affect the refractive power. Astigmatism

after PKP is often irregular, i.e., two or more meridians are separated from one another by an angle which is not equal to 90°. Two or more steep hemi-meridians are not located opposite one another. The same applies to the flat hemi-meridians. In addition, the refracting power in corresponding hemi-meridians may be different [52]. Especially in the case of irregular astigmatism, patients accept only a smaller subjective cylinder than the objective cylinder measured by keratometry or topography analysis [58]. With high irregular astigmatism, it is only possible to achieve good visual acuity with hard contact lenses.

In addition to the keratometry, topography analysis today is essential in order to determine the refracting power distribution over the entire graft. The refracting power and the individual axes of the four hemi-meridians are supplemented by system-specific indices (e.g., SRI "surface regularity index" or SAI "surface asymmetry index" of the TMS topography system) [30].

Causes of Astigmatism after Keratoplasty

Each individual step, from the selection of the donor, intraoperative trephination, and the suture technique to the quality of the postoperative follow-up treatment, can be decisive not only for corneal transparency but also for the final refractive outcome [20, 40, 45, 81, 82].

In addition to tissue-intrinsic factors in the donor and recipient, early astigmatism *with sutures in place* appears to depend strongly on the suture placement technique and the approaches used for intra- and postoperative suture adjustments (the "signature" of the microsurgeon) [42]. After suture removal the corneal curvature normally becomes more regular [58], although the "net astigmatism" can significantly increase [15, 36, 39, 46].

We have to distinguish between the *early postoperative astigmatism with sutures in place* and the *late persisting astigmatism after suture removal* (Table 6.1). Concerning the *pathomechanism of the increase in astigmatism* after suture removal, the following suggestions are made:

Table 6.1 Classification of post-PKP astigmatism

Early postoperatively with sutures in place:
Symmetry of suture positions
Homogeneity of suture tension
Late postoperatively persisting without sutures:
Cut quality
Wound configuration (horizontal/vertical)
Symmetry of graft placement

The low quality of the trephination wound and geometric incongruities (horizontal and vertical) require higher suture tension in order to guarantee a watertight wound closure and pseudo-optimal topography during the early postoperative phase. Asymmetrical regional forces between the donor and recipient can lead to inhomogeneous wound healing processes. The removal of the sutures results in the release of forces due to geometric incongruities and inhomogeneous wound healing. For this reason, horizontal, vertical, and topographic discrepancies between the donor and recipient *intraoperatively* appear to be responsible for the increase in astigmatism *after* removal of the sutures. Thus, it is reasonable to conclude that in addition to wound healing, factors associated directly or indirectly with the quality of the wound geometry (quality of the cut, wound configuration (horizontal/vertical), symmetry of the graft fit) have a strong influence on long-term astigmatism *after removal of the sutures* [10, 11, 58].

The main intraoperative determinants (Table 6.2, Fig. 6.1) for high and/or irregular astigmatism *after suture removal* are [42, 61, 63]:

- Decentration (donor and/or recipient trephination)
- "Vertical tilt" (incongruent cut angle between donor and recipient)
- "Horizontal torsion" (horizontal discrepancy between the donor and recipient form and/or asymmetrical graft fit – *"The second cardinal suture is crucial!"*)

Table 6.2 *Intraoperative* determinants of high and/or irregular astigmatism after penetrating keratoplasty

Decentration of donor and/or recipient trephination
"Vertical tilt" due to incongruent wound configuration [42] Application of different trephines for the donor and recipient Trephine tilt (i.e., not parallel to the optical axis) Limbus level not supported horizontally Slipping of the trephine in the stroma during the incision process Intraocular pressure too high/too low
"Horizontal torsion" [42] Asymmetrical placement of the second cardinal suture (angle unequal 180°) Incorrect fit of the donor and recipient due to incongruity
Focal overlap or dehiscence of the donor disk in the recipient bed
Excessively over/undersized donor disk
Distortion or compression of the cornea
Trauma to the cornea caused by instruments
Properties of the suture Suture material Suture technique (interrupted suture, single running suture, double running suture, combinations) Length of the stitch Depth of the stitch Angle of the stitch to the donor-recipient junction "Depth disparity" Tightness of the suture
Simultaneous intraocular surgery (e.g., triple procedure, artificial lens replacement, etc.)
Flieringa ring and lid speculum
Personal experience of the microsurgeon

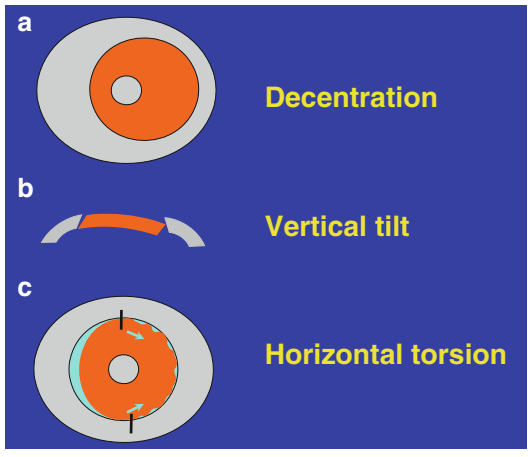

Fig. 6.1 Main reasons for high astigmatism after keratoplasty. (**a**) Decentering of the donor and/or recipient trephination, (**b**) "vertical tilt" due to uneven cutting angle, (**c**) "horizontal torsion" due to asymmetrical suture placement

Trephination Technique

In principle, keratoplasty may be indicated for optical, curative, and tectonic reasons. In practice there can be overlaps between different categories. However, corneal grafts can also be classified according to the type of donor tissue, the vertical/horizontal shape of the graft, and the location of the graft within the recipient [40, 42].

An *"optimal trephination"* requires*:*

- Full visual control
- No contact
- Optimal donor and recipient centration
- Identical shape of donor and recipient (typically circular round)
- Congruent incision angles
- 360° symmetrical donor-recipient alignment
- No completion of the trephination using scissors required
- No damage to intraocular structures (iris, lens)
- *In the future:* self-sealing donor-recipient apposition ("keylock principle")

Donor Trephination

From a 16 mm corneoscleral disk, as provided by the cornea bank, the graft can be produced in two ways:

In the past it was punched *from the endothelial side* against a firm surface, e.g., paraffin or Teflon block, with the help of special trephines (e.g., "punch trephine"). Here, particular attention must be paid to ensuring that the excision is performed in a centered position and that the trephine is not tilted, as this would result in an elliptical disk with slanted edges [11]. This risk can be reduced through the use of "guided donor trephine systems" (e.g., guillotines). In the histological assessment, the cut surfaces without consideration of the cut angles appear to be almost "perfect." However, the deviation in the direction of the cut toward the exterior results in a convergent cut angle due to the smaller diameter on the level of Descemet's membrane and a greater diameter on the level of Bowman's layer ("undercut," Fig. 6.2) [82].

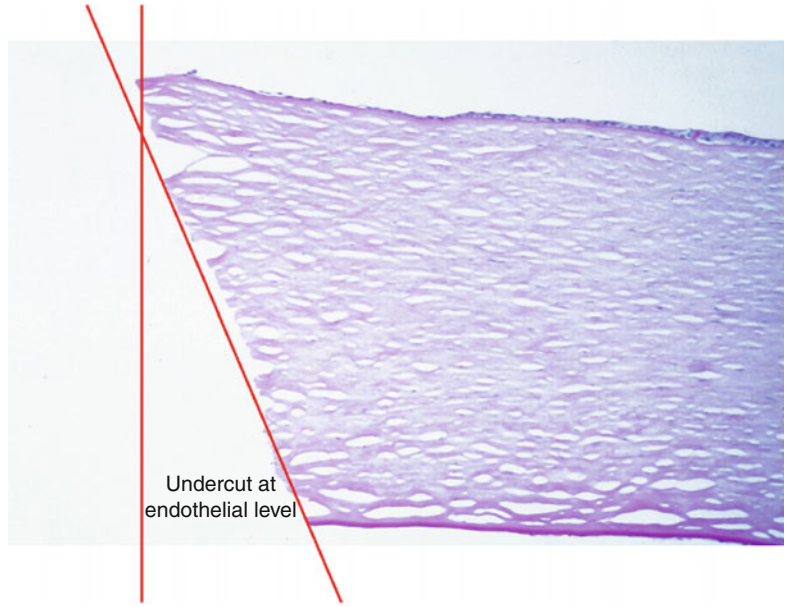

Fig. 6.2 Donor trephination from the endothelial side: smooth cutting surface, but "undercut" at the level of Descemet's membrane

Since the introduction of "artificial anterior chambers," microsurgeons have been able to carry out donor trephination *from the epithelial side*, i.e., in the same direction as in the patient. If the pressure in the artificial anterior chamber is kept at its normal level (e.g., 22 mmHg), the advantages with respect to the congruence of the cutting angles are self-evident [48].

Recipient Trephination

In order to increase the overview and reduce vis à tergo, a Liebermann lid speculum can be used. Almost any viscoelastic agent is suitable for stabilizing the anterior chamber during trephination and suture placement.

A Flieringa ring is not necessary for keratoplasty only or a triple procedure, however, for aphakic and/or vitrectomized eyes, especially if a secondary sclera-fixated or retroiridal artificial lens implantation is planned.

Investigations by van Rij and Waring have shown that in recipient trephination, the use of all trephine systems results in openings which are larger than the nominal trephine size. Furthermore, the diameter at the level of Descemet's membrane is greater, resulting in divergent cut angles [49, 82]. This can be explained at least in part by bulging ("ballooning") of the cornea in the trephine opening due to the pressure exerted.

The higher the intraocular pressure, the more divergent angles can be expected in recipient trephination [48].

This phenomenon of "ballooning" is one of the main disadvantages of the mechanical trephine and can at least partially be prevented by the use of a so-called obturator (exception: keratoconus).

The combination of a donor disk that has been punched from the endothelial side and therefore has convergent cut angles, with a recipient opening with divergent cut angles, leads to a triangular-shaped tissue defect at the level of Descemet's membrane. This has to be compensated for intraoperatively by means of increased suture tension, which can result in flattening, vertical tilt, and irregular astigmatism (Fig. 6.3).

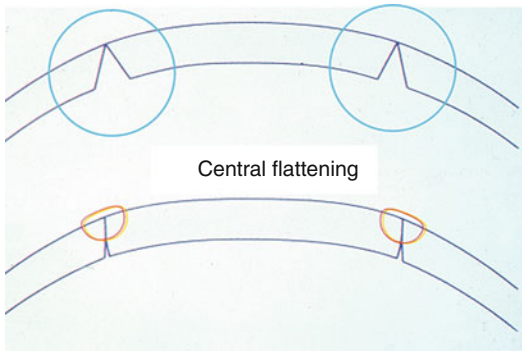

Fig. 6.3 The combination of the donor cornea (convergent cutting angle) trephined from the endothelial side and the mechanically trephined recipient cornea (divergent cutting angle) causes a triangular deficit at the level of Descemet's membrane, which has to be compensated for by suture tension. This can lead to central flattening, vertical tilt, and irregular astigmatism

Technical Details of PKP

- *General anesthesia* has clear safety advantages over local anesthesia, especially in young keratoconus patients. The arterial blood pressure should be kept as low as possible when the eye is open *("controlled arterial hypotension").*
- Typically, the *pupil is constricted with pilocarpine* in order to protect the lens of the phakic eye.
- *Paracentesis at the limbus* is recommended before trephination.
- The head and *limbus level* must be *horizontal* during trephination.
- A peripheral iridotomy at 12 o'clock prevents pupillary block and therefore an acute glaucoma attack. In case of keratoconus after the administration of atropine, this may appear as a so-called Urrets-Zavalia syndrome with a persistent maximally dilated pupil due to an iris sphincter necrosis [80].
- The correct position of the *second cardinal suture* is absolutely crucial for a correct graft alignment.
- *Intraoperative keratoscopy* should be applied *after* removal of the lid speculum and fixation sutures [6, 40, 42, 63].

Graft Size and Oversize

Graft Size

In a quantitative study we were able to show that the corneal diameter in keratoconus patients is significantly greater than the diameter in Fuchs' patients (mean horizontal diameter of 11.8 mm in keratoconus compared to 11.3 mm in patients with Fuchs' dystrophy [54]). In general, larger graft dimensions have a favorable effect on the optical qualities and endothelial cell count, while a low rate of immunological rejection and lower risk of postoperative ocular hypertension are affected by a small graft.

> The graft size should be determined on an individual basis: "as large as possible, but as small as necessary."

For many eyes with keratoconus, an 8.0 mm diameter has proven to be a good preset for communication with the cornea bank (arcus lipoides!), while for many eyes with Fuchs' dystrophy not suitable for posterior lamellar keratoplasty, a 7.5 mm diameter is appropriate. Today, diameters from 5.5 to 7.0 mm are only very rarely needed and are usually reserved for small eyes with special immunological implications.

It has been assumed that a smaller graft is associated with higher astigmatism after keratoplasty. In a more recent study, in which we compared 8.0, 7.5, and 7.0 mm grafts with each other, we were able to show the following [58]:

- Smaller grafts are associated with a flatter curvature.
- Smaller grafts are associated with higher topographic irregularity.
- Smaller grafts result in a higher proportion of non-measurable keratometry images.
- Suture removal is associated with a positive tendency toward regularization of the topography.
- It has not been possible to show a difference with respect to the amount of net astigmatism

between smaller and larger grafts, neither for grafts with nor without suture.

Recent studies show that the rate of *chronic endothelial cell loss* after PKP depends on the initial diagnosis [34, 47]. Endothelial migration along a density gradient from the donor to the recipient ring is probably the main reason for this phenomenon in pseudophakic bullous keratopathy. For this reason, eyes with bullous keratopathy are probably better treated with a larger graft, not only in order to improve the optical quality but also to transplant as many endothelial cells as possible. Nevertheless, the graft size must be determined individually by the microsurgeon for each individual patient before recipient trephination in order to find the best compromise between immunological purposes and optical quality. A slit lamp with a measuring device, such as the Haag-Streit slit lamp, can be helpful. Furthermore, the removal of a vascularized pannus is recommended (in contrast to vascularized stromal corneal scars) before trephination in order to achieve a greater "individually ideal" graft diameter [52].

In repeat PKP an attempt should be made to excise the old graft completely and recenter the trephination if the cornea is large enough and a host rim of about 1.5 mm is left [17, 73]. This is especially of importance in eyes with high and/or irregular astigmatism as the reason for repeat grafting.

Graft Oversize

In mechanical trephination the diameter of the recipient bed tends to be larger than the trephine diameter. In contrast, the diameter of the donor disk, which is punched from the endothelial side, tends to be smaller than the trephine diameter, which has a corresponding effect on the spherical equivalent [21, 82]. For this reason, "donor oversizing" of 0.25–0.50 mm is usually carried out in this situation. This is performed on the one hand to prevent the flattening of the graft and on the other hand to prevent narrowing of the iridocorneal angle where there is a predisposition to secondary glaucoma [24, 46]. In contrast, no oversizing is required with the use of guided trephine systems or laser trephination in which the donor is cut from the epithelial side. In the case of keratoconus, it has been recommended not to carry out donor oversizing.

> Undersizing of the graft for the purpose of simultaneous correction of keratoconus-intrinsic axial myopia will result in irregular astigmatism and is *not* recommended. The difficulty of achieving a watertight wound closure requires excessive suture tension, with the consequence of irregular astigmatism and a relative cornea plana.

Pupil or Limbal Centration?

Centration is essential, both in terms of the immunological graft reaction and the astigmatism after keratoplasty [31, 32, 53, 81]. Typically, an attempt is made to reach a compromise between limbus and pupil centration in nontraumatized pupils. However, limbus centration is preferred especially in the case of keratoconus, scars after trauma, or irregular astigmatism due to other causes. In such eyes, the center of the entrance pupil is in fact optically displaced from the position of the actual anatomical pupil [33]. For example, the pupil in the typical keratoconus eye tends to be optically displaced superonasally due to the inferotemporal location of the cone.

We use a radial keratotomy marker with eight lines in order to ensure limbal centration (Fig. 6.4). Additional central punctate marking can be helpful for certain trephine systems (e.g., Hessburg-Barron trephine, GTS after Krumeich).

Keratoconus

In keratoconus, a large (typically 8.0 mm) central circular keratoplasty is indicated as soon as hard oxygen-permeable contact lenses are no longer tolerated. If nonmechanical excimer laser trephination is used, corneas which are extremely steep before keratoplasty do not have worse prognosis than those which are less deformed [84].

A larger graft diameter in keratoconus contributes toward obtaining a sufficiently thick cornea on the trephination edge, since as a result the cone can usually be completely excised. We advise against centering the trephination at the center of the cone, as this typically necessitates decentering of the trephination with regard to the limbus. This would have unfavorable impacts on the astigmatism [81]. Cauterization of the cone has been suggested in order to avoid divergent cutting angles, but the achieved effect is not reproducible. For this reason we do not recommend cauterization of the cone. An obturator should *not* be used with keratoconus in order not to produce undesirable irregularly elliptical or even pear-shaped host openings (Fig. 6.5). In this context, "non-contact" excimer laser trephination is preferred over the mechanical method in order to avoid noncircular recipient openings.

In keratoconus, the inhomogeneous corneal thickness typically results in premature perforation at the thinnest point of the cornea, which has to be considered when using conventional trephines in order not to inadvertently traumatize the iris or even the lens.

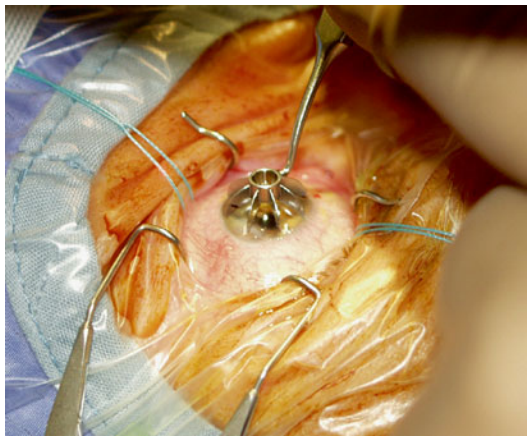

Fig. 6.4 Radial keratotomy marker for recipient centration with respect to the limbus

Suture Technique

The type of trephination has a major impact on the correct placement of the first four or eight cardinal sutures [45, 63]. The main purposes of these cardinal sutures include:

- The symmetrical horizontal distribution of donor tissue in the recipient bed
- Good adaptation of the donor and recipient wound edge on the level of the Bowman's layer
- Stabilization of the anterior chamber to ensure that further suturing is uniform.

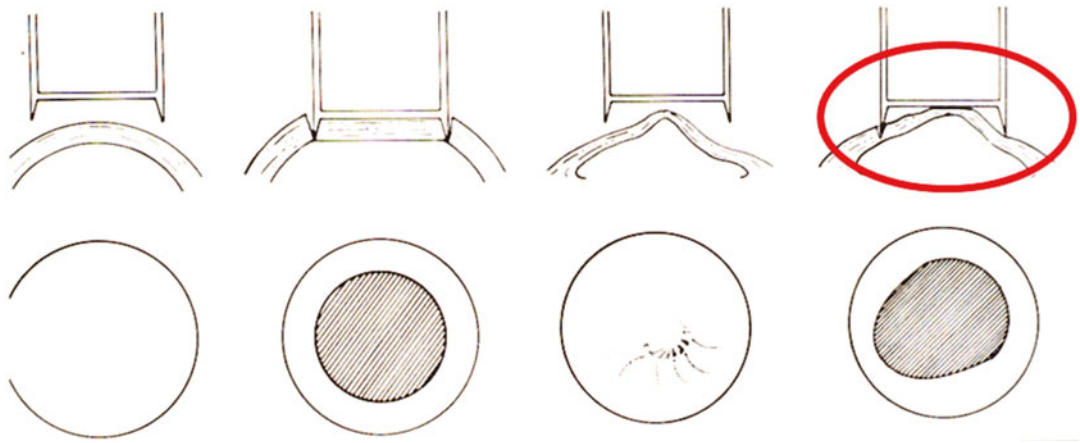

Fig. 6.5 In case of keratoconus it has been recommended not to use an obturator in order to avoid elliptical or pear-shaped excision shapes. The same principle of applanation during trephination applies in femtosecond laser application (Courtesy of Professor Herbert Kaufman)

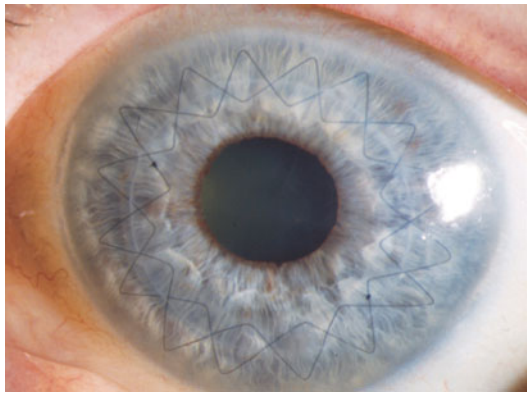

Fig. 6.6 Excimer laser keratoplasty (8.0/8.1 mm) with typical double-running 10-0 nylon cross-stitch suture, each with 8 stitches (after Hoffmann [18]) in keratoconus

Concerning donor-host alignment, external steps must be avoided, although internal steps sometimes have to be tolerated in the case of thin recipient corneas, for example, in pellucid marginal degeneration or herpetic scars.

As far as the correct placement of the second cardinal suture is concerned, unintentional deviations from circular recipient openings can represent a challenge even for the experienced keratoplasty surgeon. After removal of the cardinal sutures, the quality of the trephination and the correct positioning of the graft are the main determinants for a watertight wound closure. The better the trephination, the lower the final suture tension which is necessary to ensure a watertight wound closure after removal of the cardinal sutures. The lower the final suture tension is, the more quickly an improvement in visual acuity can be expected. In the case of an intact Bowman's layer, a 16-stitch double-running diagonal cross-stitch suture (10-0 nylon) after Hoffmann is typically preferred in Germany [18] (Fig. 6.6). The faster visual rehabilitation with running sutures – in contrast to multiple interrupted sutures and combined suture techniques – is attributable to the regular topography of the cornea and the avoidance of a relative cornea plana. In addition, with this double-running suture, the risk of suture loosening is reduced [22].

The better the trephination, the more easily a watertight wound closure is achieved.If excessive suture tension is required in order to achieve a watertight wound closure, the regularity of the topography and, therefore, the visual acuity after keratoplasty are generally impaired.

Conventional Mechanical Trephines

Unfortunately, conventional mechanical trephination is always associated to some extent with the deformation of corneal tissue, including distortion of the cut edges, with irregular cutting surfaces as a consequence of the axial and radial forces which are induced by the use of these trephines [42, 63]. The cut angles deviate from the perpendicular and are often different in the donor and recipient, especially when the donor trephination is performed from the endothelial side [11, 21, 45, 46]. The fitting of the donor tissue into an unstable recipient bed is sometimes very difficult to achieve in a perfectly symmetrical manner. After the suturing in of incongruent cut edges and the resulting induction of a vertical tilt [31, 32], the healing of the wound can result in pronounced distortion of the graft topography, especially after suture removal [10, 15, 36, 39]. Moreover, the asymmetrical placement of the cardinal sutures can lead to the uneven distribution of donor tissue in the recipient bed, in particular if the second cardinal suture is not positioned exactly 180° opposite the first stay suture ("horizontal torsion" [42]).

If conventional trephines are used, it is recommended that systems should be applied which in the case of *donor trephination from the epithelial side* provide for the use of an artificial anterior chamber for fixation of the corneoscleral disk. The trephines should always be as sharp as possible in order to keep inappropriate squeezing and shearing forces as small as possible. Disposable items may be advantageous

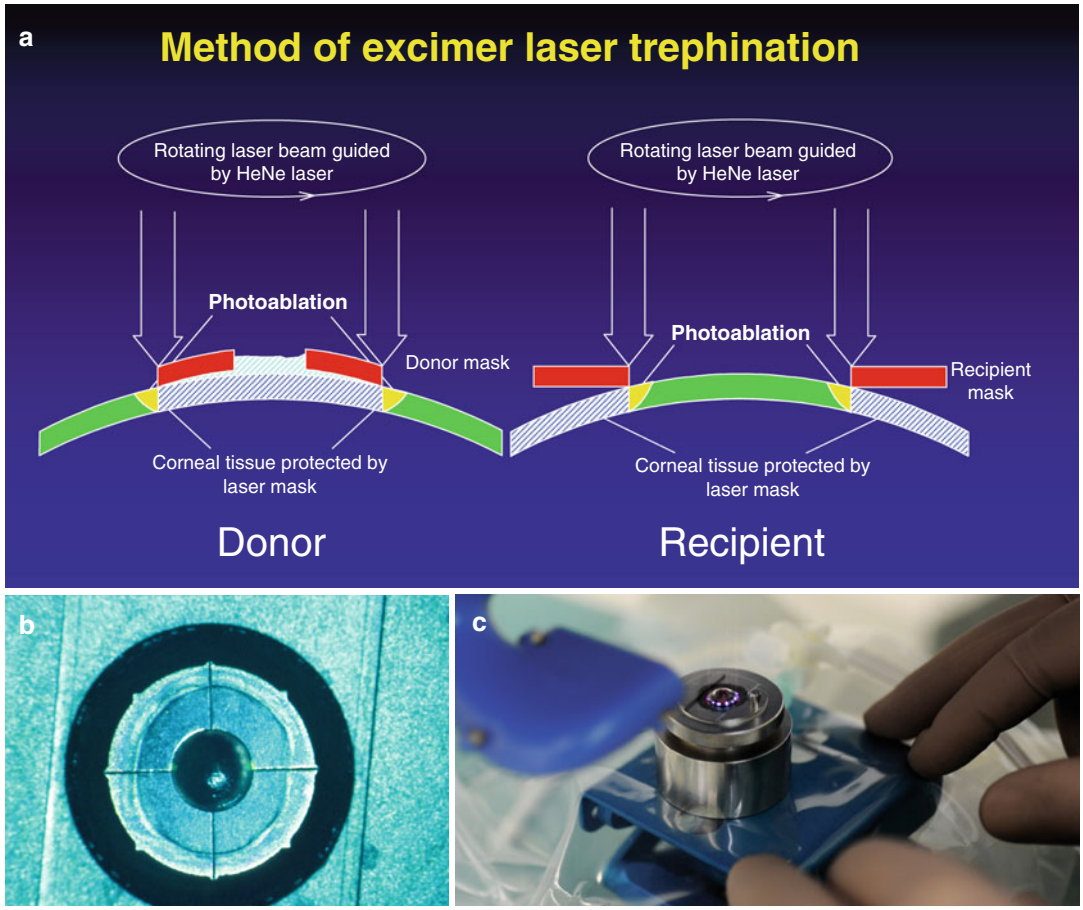

Fig. 6.7 (**a**) Principle of excimer laser trephination in the donor and recipient (schematic sketch, sagittal view). (**b**) Donor mask (8.1 mm in diameter) with eight "orientation teeth" on the outside lying directly on the corneoscleral disk in the artificial anterior chamber. The laser is guided along the outer edge. (**c**) Pseudo-ring-shaped automated Schwind AMARIS excimer laser ablation profile along the outer edge of a donor mask on a corneoscleral disk in an artificial anterior chamber. (**d**) Donor trephination immediately before penetration with smooth cut edges and orientation teeth (*arrows*; macroscopy). (**e**) Histology of straight, almost perpendicular incision edges immediately before donor trephination with the excimer laser. (**f**) Side view of a very prominent keratoconus immediately before trephination. (**g**) During host trephination with the excimer laser, the metal recipient mask (8.0 mm in diameter) is well centered around the cone without deformation. The laser is guided along the inner edge of the mask. (**h**) Schematic sagittal view of the cone protruding through the central hole of the metal recipient mask allowing a trephination without deformation. (**i**) Exact positioning of the second cardinal suture in penetrating excimer keratoplasty through the use of a small tooth and a corresponding notch to prevent "horizontal torsion" (intraoperatively)

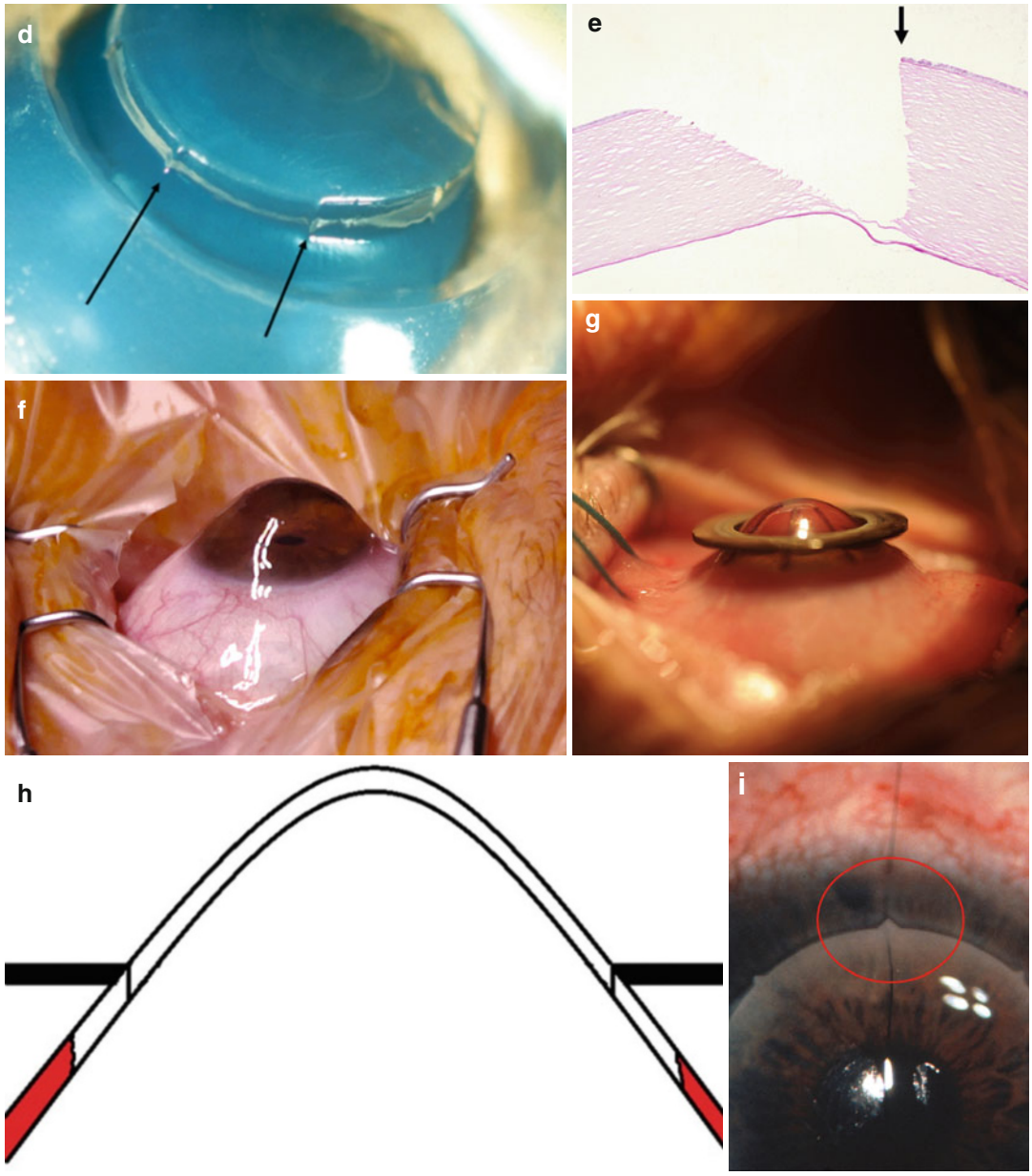

Fig. 6.7 (continued)

here – also with regard to prion-caused contagious diseases.

Nonmechanical Excimer Laser Trephination (Fig. 6.7a, b)

Under the hypothesis that the characteristics of the wound bed are considerably more important for the astigmatism after suture removal and the optical quality of the graft than various suture techniques or methods of subsequent suture adjustments, the technique of nonmechanical corneal trephination has been developed and optimized in Erlangen since 1986 [41]. Originally, the elliptical shape was proposed on the basis of the idea that an elliptical graft could best be fitted to the natural elliptical human

cornea, both from the optical and the immunological perspective [28]. Prof. G.K. Lang published details of the first two patients after elliptical keratoplasty in 1990 [29]. A total of 42 elliptical keratoplasties were performed in humans from 1989 to 1991 [75]. Subsequently, this method was abandoned for optical reasons, because the need for simple interrupted sutures to prevent rotation of the graft in the recipient bed and the need for asymmetrical suture tension in these multiple interrupted sutures had ultimately not resulted in improved curvature, neither with nor without sutures [76]. Today, we still use elliptical excimer laser keratoplasty for elliptical ulcers with descemetoceles or penetration for the purposes of keratoplasty à chaud (a typical example of elliptical ulceration would be acanthamoeba keratitis) or pellucid marginal degeneration with eccentric thinning of the cornea at the bottom close to the limbus [27].

Since July 01, 1989, more than 4,000 eyes have been successfully operated in Erlangen and Homburg/Saar with the MEL70 excimer laser made by Zeiss Meditec and, recently, with the AMARIS excimer laser made by Schwind (Fig. 6.7c).

With a share of approximately one third, keratoconus has always been by far the most common indication for PKP with this "non-contact" excimer laser technique.

Technique Before trephination, the limbus is centered along the vertical helium-neon target beam in the donor and patient in order to ensure a reproducible position to the laser beam and therefore symmetrical cutting angles in the entire circumference.

For *donor trephination* from the epithelial side, a round open metal mask (diameter 5.6–8.6 mm, central opening 3.0 mm for centering, thickness 0.5 mm, weight 0.2 g, 8 "orientation teeth") is placed on a corneoscleral disk (16 mm) which is fixed in an artificial anterior chamber under microscopic control (Fig. 6.7b–e). The pressure within the artificial anterior chamber is adjusted to approximately 22 mmHg using Maklakoff tonometer [2].

For *recipient trephination* which is performed clinically with the manually or automated guided laser beam, a corresponding recipient mask is used (diameter 12.9 mm, central opening 5.5–8.5 mm, 8 "orientation notches"). Before the start of trephination, centering relative to the limbus is achieved through the association of the eight notches in the mask with the eight linear marks of a blue-stained radial keratotomy marker which has been previously applied under microscopic control (Fig. 6.7f–h).

Advantages of Nonmechanical Trephination The main advantage of this excimer laser cutting method, which is performed from the epithelial side in donor and recipient, is the avoidance of mechanical distortions during trephination (Table 6.3). This results in smooth cut edges which are congruent in both the donor and recipient, so that the "vertical tilt" is reduced [32]. "Orientation teeth" on the edge of the graft [4]

Table 6.3 Advantages of nonmechanical trephination with the 193 nm excimer laser along metal masks with "orientation teeth"

1. No trauma to intraocular tissues
2. Prevention of deformation and compression of the tissue during trephination
3. Reduction of "horizontal torsion" ("orientation teeth")
4. Reduction of "vertical tilt" (almost perfect congruent incision edges)
5. Improvement of recipient and donor centration
6. Possibility of "harmonization" of donor and recipient topography
7. Reduction of anterior chamber inflammation after keratoplasty
8. Reduction of astigmatism after suture removal
9. Increase in the regularity of the topography of the cornea
10. Significantly better spectacle-corrected visual acuity
11. *Feasibility of trephination of an instable cornea* (e.g., "open eye," descemetocele, status post-radial keratotomy, iatrogenic keratectasia after LASIK). Any shape possible (e.g., elliptical)

and corresponding notches in the edge of the recipient for undoubted symmetrical positioning of the first eight cardinal sutures reduce the "horizontal torsion" (Fig. 6.7i). In this way it is possible to improve the optical quality after transplantation. Furthermore, donor and recipient centration is improved [31, 53]. These beneficial influences on the main intraoperative determinants of astigmatism after keratoplasty (Table 6.2) result in lower keratometric net astigmatism, higher topographic regularity, and improved spectacle-corrected visual acuity after suture removal [50, 51, 74].

In addition to less disruption to the blood-aqueous barrier in the early phase after keratoplasty [26], the laser trephination does not result either in increased cataract formation [5] or higher endothelial cell loss of the graft [56]. In addition, the frequencies of the immunological graft reaction [55] and secondary ocular hypertension were comparable in both techniques [57]. The use of metal masks allows an arbitrary trephination technique [75, 76]. Moreover, the use of the laser allows the trephination of an instable cornea, such as in a perforated corneal ulcers (or descemetoceles) or after radial keratotomy or in iatrogenic keratectasia after laser in situ keratomileusis LASIK [27, 59, 60].

Practical Considerations for the Microsurgeon

The somewhat longer trephination time (around 90 s with the Schwind laser) is largely compensated for by the practical advantages for the microsurgeon during the subsequent course of the surgery [41, 42, 51, 60, 66, 74]:

- Injury to intraocular structures is impossible with the laser, as tissue ablation ceases as soon as the aqueous humor fills the trephination canal after focal perforation.
- The need to complete the cut using scissors is reduced to a minimum.
- The location of the first eight cardinal sutures is unequivocally specified by the eight "orientation teeth/notches."
- Crescent-shaped tissue deficits in the region of the donor-recipient junction (such as in the case of noncircular recipient openings, e.g., in keratoconus) are avoided, so that a latent watertight closure is often achieved after just four cardinal sutures.
- During the subsequent suturing procedure, the anterior chamber remains largely stable as a rule.
- The final double-continuous suture only has to be tightened to a very slight extent in order to maintain an anterior-step-free wound adjustment and watertight wound closure – even after the removal of the eight cardinal sutures.
- For this reason, additional interrupted sutures with an unfavorable effect on the graft topography are needed only very rarely at the end of surgery.
- Furthermore, the so-called barrel-top formation at the proximal ends of the sutures, which result in a relative cornea plana and, therefore, delayed optical rehabilitation, is largely avoided.
- After removal of the eyelid speculum and the fixation sutures, the use of a Placido disk after intraoperative suture adjustment often provides a round projection image.

Nonmechanical trephination using the 193 nm excimer laser along metal masks has significantly improved the results of penetrating keratoplasty after suture removal. The use of the excimer laser also allows controlled trephination of instable corneas, such as in perforated ulcers or iatrogenic keratectasia after LASIK.

Excimer Laser-Assisted Deep Lamellar Keratoplasty DALK

It is well-known that deep lamellar keratoplasty (DALK) only results in good visual acuity when Descemet's membrane was exposed intraoperatively [69, 71]. When Descemet's membrane is perforated, this usually results in a "conversion" to PKP. In order to ensure that the typically young keratoconus patient does not experience any disadvantages as a result of the planned DALK, we prepare the donor and recipient trephination with the excimer laser in a typical manner. However,

we do not perforate the patient's cornea. If the "big bubble" is successfully achieved and we can bare Descemet's membrane – without perforating – we terminate the operation as DALK. If this does not succeed to our satisfaction, the operation can be completed as excimer laser PKP with all of the advantages described above without any disadvantage for the patient. Primum nil nocere….

Femtosecond Laser Trephination for PKP

The femtosecond laser (FSL) operates at a wavelength of about 1 µm (infrared) and the excimer laser at 193 nm (UV). The cornea is transparent to the FSL. The excimer laser is absorbed by the cornea. The pulse duration of the excimer laser is a few nanoseconds, whereas that of the FSL is a few 100 femtoseconds. The repetition rate of the excimer laser today reaches up to 1000 Hz and in the FSL within the range of several kHertz. The energy density of the excimer laser fluctuates between 150 and 400 mJ/cm^2, and that of the FSL between 1 and 10 J/cm^2. The pulse size of the excimer laser fluctuates between 0.6 and 6 mm, whereas in the FSL it is a few micrometers. The tissue interaction of the excimer laser is based on direct photoablation, while the tissue interaction of the FSL is plasma mediated.

> The principal advantages of the femtosecond laser use are that no masks are needed and that no tissue loss and no thermal effects occur.

In contrast to the excimer laser, which only allows surface ablation, with the *femtosecond laser* (a femtosecond corresponds to 10^{-15} s), it is also possible to cut the cornea within the stroma, so that actual three-dimensional cuts without opening the eye and without thermal damage are possible. With real 3-D sections, it may be possible to achieve self-sealing wounds. Based on the publication by Massimo Busin in 2003, we proposed the "inverse mushroom" (now commonly referred to as the "top hat" configuration) in 2005 in order to achieve a watertight wound closure [8, 62].

The Fundamental Problem of Femtosecond Laser Trephination

Over the last 10 years, femtosecond laser keratoplasty has caused a good deal of excitement. The advantages of femtosecond laser keratoplasty are the arbitrary horizontal and vertical shapes, including the "top-hat," "mushroom," "zigzag," "Christmas tree," "octagon," "decagon," "dovetail," etc. [3, 16]. The fundamental problem of every femtosecond laser trephination is that – even with a curved interface – a certain amount of flattening of the cornea is necessary, which is associated with deformation. In advanced keratoconus in particular, this results in "noncircular" excisions in the patient's cornea and, therefore, "horizontal torsion" as *the* main intraoperative determinant of high/irregular astigmatism after PKP [52].

In "regular trephination" during keratoplasty, maximum intraocular pressure values of 135 mmHg are measured with the IntraLase, 65 mmHg with the VisuMAX, 205 mmHg with the Femtec and 184 mmHg with the Femto LDV in experimental use [83]. Furthermore, in advanced keratoconus in particular, applanation results in "noncircular" (often oval or pear-shaped) apertures in the patient's cornea and therefore horizontal torsion as *the* main intraoperative determinant of high/irregular astigmatism after PKP. The eight lines which are applied, for example, for the IntraLase femtosecond laser, in the donor and recipient cannot be brought into alignment sometimes intraoperatively in the treatment of keratoconus.

Some authors claimed that femtosecond laser PKP has advantages in the short-term follow-up concerning refractive cylinder and visual acuity [3, 14, 23, 35]. However, there is a large amount of missing data with respect to the potential advantages of femtosecond laser keratoplasty *after complete suture removal*. Only few groups have published results pertaining to the situation *after complete suture removal* [7, 9]. After a mean follow-up of 14 ± 5 months, the topographic astigmatism without sutures in the mushroom

profile was 6.4 ± 3.0 dpt, and in the top hat profile 5.8 ± 4.6 dpt [7]. The degree of the astigmatism after femtosecond PKP is therefore comparable with that after motor trephination (now withdrawn from the market [51, 74]). Moreover, in the mushroom profile, the rate of the postoperative immune reactions is significantly increased [79].

FSL keratoplasty has been very interesting, but no prospective randomized study has so far been carried out in which both trephination procedures (FSL and excimer laser) for PKP in keratoconus and Fuchs' dystrophy have been compared to each other. Such a study has just been finished in Homburg/Saar [13]. With FSL-PKP in keratoconus using a double-running suture, we found more decentration, more vis à tergo, and more often the need of single sutures to achieve donor-host apposition without steps and gaps. After suture removal, topographic astigmatism after FSL trephination in keratoconus (6.8 ± 3.1 D) was significantly larger that after excimer laser trephination (2.5 ± 1.4 D). In addition, the surface regularity index (SRI) of the TMS-5 system in keratoconus was significantly unfavorable after FSL trephination (0.8 ± 0.3) than after excimer laser trephination (0.5 ± 0.4). Best spectacle corrected visual acuity after suture removal in keratoconus was 0.8 ± 0.2 after excimer laser and 0.7 ± 0.2 after FSL laser trephination [13].

Certainly "manifest cylinder" is not appropriate to compare the outcome of different trephination procedures for PKP [35]. In case of a highly irregular surface, the manifest cylinder will be zero although the benefit for the patient is nil [58]. True benefits of excimer laser versus femtosecond laser trephination for PKP are summarized in Table 6.4.

On principle, the minimal requirements for comparative studies on various trephination techniques in PKP are:

- Visual acuity with spectacle correction (not contact lens acuity!) and central refracting power
- Keratometric or topographic astigmatism (*not only refractive manifest cylinder!*)
- Measure of the topographic regularity (e.g., SRI (surface regularity index) or SAI (surface asymmetry index) of the TMS system and ISV (index of surface variance) or IVA (index of vertical asymmetry) of the Pentacam), in each case before and after suture removal

Summary

Donor and recipient trephination should be performed with the same system from the epithelial side. The horizontal position of the limbus plane is essential. The graft size should be adapted individually to the cornea size ("as large as possible, as small as necessary") and limbal centration preferred to pupil centration in cases of doubt (especially with keratoconus). Furthermore, excessive graft over- or undersizing should be avoided. At

Table 6.4 True benefits comparing excimer laser versus femtosecond laser trephination – practical considerations (+ + + = very favorable, – – – = very unfavorable)

"Cumbersome procedure"	+	– –
Centration	+ + +	+
Avoid deformation and compression of tissue during trephination	+ + +	– – –
High IOP during laser action	+ + +	–
Minimizing amount of completion of incision by scissors	(+)	+ +
Location of first 8 cardinal sutures unequivocally given	+ + +	+ +
Stable anterior chamber during suturing	+ +	+ + +
Feasibility of double-running suture	+ + +	+ + +
No need for additional single sutures	+ + +	+
Feasibility of trephination with instable cornea	+ + +	– – –
Feasibility of trephination in repeat keratoplasty	+ + +	–
Helpful for DALK	+ +	+ +
Potential for DSAEK (*donor/recipient*)	– – –	+
(But: "suboptimal" stromal surface quality!)		
Immune reactions	+	– –

the end of the operation, adjustment of the continuous cross-stitch suture should be carried out using a Placido disk. Nonmechanical excimer laser trephination results in lower astigmatism, higher topographic regularity, and better visual acuity (especially in younger patients with keratoconus). In the case of an unstable cornea (e.g., after RK, iatrogenic keratectasia after LASIK, descemetocele, perforated ulcer), trephination by laser application is possible. New "keylock" variants for the possible self-sealing fit of the donor disk in the recipient bed were looming on the horizon 10 years ago (future "no-stitch keratoplasty") after introduction of femtosecond laser application. However, recent *all-suture-out data* demonstrate that the potential superiority of this high price and difficult to maintain option cannot be proven! Thus, today the femtosecond laser application for PKP must be called *"the excitement of yesterday."*

Conclusions

Today, the expectations with regard to the results after PKP are limited not only to the achievement of a clear graft. The only criterion that matters to the patient is good visual acuity, preferably without contact lenses, but with a well-tolerated pair of spectacles. For this reason, transplant microsurgeons should not only respect all options for preventing high or irregular astigmatism after keratoplasty. Due to the fact that it is never possible to foresee the refractive outcome in an individual patient after keratoplasty, surgeons should also be familiar with the surgical procedures for correcting refraction errors after PKP (especially in high astigmatism) in order to achieve the best individual result for the patient.

Prophylaxis and Management of Complications

Complications in keratoplasty can be divided up into immunological and optical.

This chapter is structured as follows [67]:

- Preoperative prevention of complications
- Prevention of intraoperative complications
- Prevention of early postoperative complications
- Prevention of late complications after keratoplasty

Besides these aspects the adequate preoperative preparation and selection of donor tissue are of utmost importance for the outcome the PKP.

Preoperative Prevention of Complications

Assessing Phototherapeutic Keratectomy or Lamellar Techniques as an Alternative

At all events, an examination should be carried out to determine whether superficial avascular corneal opacities, e.g., in granular dystrophy or Salzmann's nodular degeneration, cannot be treated by means of excimer laser phototherapeutic keratectomy (PTK), so that corneal transplantation can be avoided [12].

Furthermore, consideration should also be given in all cases today to whether anterior (DALK) or posterior lamellar keratoplasty (DSAEK or DMEK) is feasible in order to minimize the risk of expulsive hemorrhage during the "open-sky" period of PKP [69].

Recognizing and Treating Underlying System Diseases and Eyelid Abnormalities

As a matter of principle, systemic underlying diseases, in which problems with the surface of the eye are very common, must be identified and consistently treated before PKP. These include, among others, neurodermitis, rosacea, primary chronic polyarthritis, alcoholism, liver diseases, and diabetes mellitus.

In cases of very severe neurodermitis, consideration should be given as to whether cyclosporine A oral can be administered at a dosage of 150 mg twice a day for 4 weeks before PKP. Conventional eyelid margin hygiene and a dermatological consultation are indispensable *before* PKP. Both drug therapy for existing blepharitis and the surgical correction of eyelid malpositions (e.g., entropion with trichiasis) must be carried out *before* PKP.

In cases of severe limbal stem cell insufficiency (such as in congenital aniridia), a limbal transplant might better be carried out *before* PKP [70].

No Keratoplasty if the Intraocular Pressure Is Not Controlled

General rule: Keratoplasty must not be performed if the intraocular pressure is not controlled.

A pressure of 20 mmHg under 3 topical antiglaucoma agents can*not* be considered as controlled! Here it should be borne in mind that the validity of the indirect methods (including Goldmann applanation tonometry) is doubtful. Despite a thickened cornea, the intraocular pressure is often measured as being too low in bullous keratopathy. Here, direct intracameral needle pressure measurement can be an alternative approach [37]. Predisposing factors for secondary ocular hypertension after keratoplasty are preexisting "glaucoma," pseudoexfoliation syndrome, aphakia, scars after a penetrating injury, persistent anterior synechiae, and simultaneous artificial lens replacement, especially in the case of anterior chamber lens removal and secondary scleral-fixated posterior chamber lens implantation and simultaneous vitrectomy [24, 57].

Pretreatment of Vascularized Corneas

In principle, anti-VEGF drugs can be applied topically as drops or as a subconjunctival injection prior to keratoplasty in order to reduce corneal neovascularizations. With a sizeable singular vessel, which typically occurs with a vascularized disciform corneal scar of herpetic origin, fine-needle diathermy – as first proposed by the working group under Dua – may be successful at limiting intraoperative hemorrhaging [25].

Amniotic Membrane Transplantation AMT Before Penetrating Keratoplasty in Ulcerative Keratitis

If possible, emergency keratoplasty (à chaud) should not be carried out today in the case of ulcerative keratitis. It is well known that the risk of immune reactions, epithelial healing disorders, and the rate of suture loosening are increased after emergency keratoplasty. Here we prefer an amniotic membrane transplantation AMT (typically referred to as "multi-graft sandwich") [64] in order to achieve a reduction in the symptoms of inflammation and the acceleration of epithelial healing. Instead of emergency keratoplasty in the highly inflamed eye, we plan elective keratoplasty in the non-inflamed eye after 3–6 months. This improves the graft prognosis, not least because of the possibility of selecting an optimum donor cornea [19].

Quality-Assured Grafts from Organ Culture

Quality-assured donor corneas from organ culture are widely used in Europe today. This includes not only microbiological and serological analyses of the donor blood and culture medium but also an examination of the corneas using the slit lamp to detect scars, endothelial damage, or other abnormalities. In accordance with the guidelines of the European Eye Bank Association EEBA, only corneas with an endothelial cell density of at least 2,000 cells/mm^2 as assessed by phase contrast microscopy are transplanted. Anterior-segment OCT methods are currently being developed which can ensure during the organ culture stage that the cornea concerned has not undergone any refractive surgical intervention or suffers from keratoconus.

Individually Optimized Graft Size

As a matter of principle, an individually optimized graft size should be selected for each keratoplasty. The graft size is determined preoperatively for each individual, e.g., using a slit lamp with a measuring device. Each graft should be as large as possible (for optical reasons) and as small as necessary (for immunological reasons). In keratoconus, grafts of 8.0–8.5 mm are ideal, whereas in the case of Fuchs' dystrophy with typically smaller and more elliptical corneal dimensions, a 7.5 mm graft is often suitable if this eye is not eligible for DMEK or DSAEK [54, 58, 63].

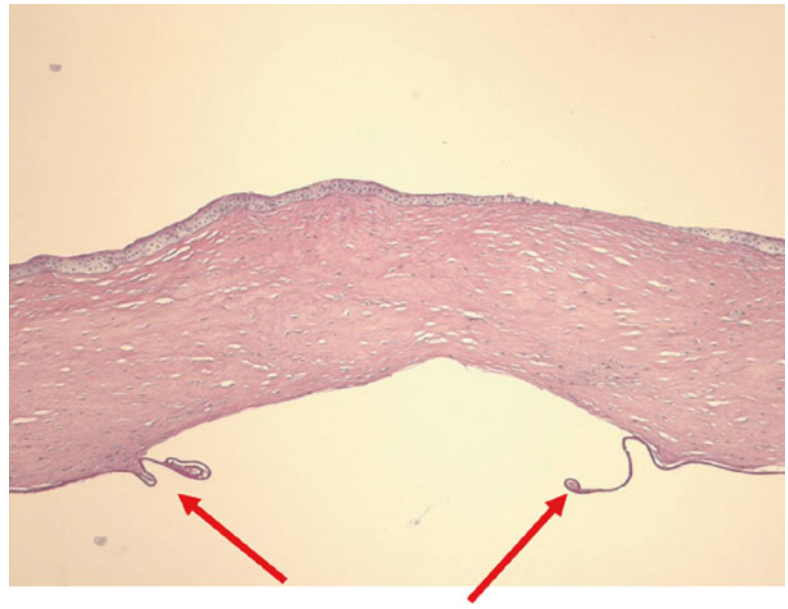

Fig. 6.8 Histology of an excised cornea after corneal hydrops due to rupture of Descemet's membrane. The retracted ends of Descemet's membrane are curled. It is advised against DALK using the big-bubble technique

No Keratoplasty in the Acute Stage of Keratoconus

PKP in the acute phase of keratoconus (the so-called corneal hydrops) should be avoided because postoperatively this often results in suture loosening with corresponding adverse consequences such as infectious infiltration and neovascularization. The fear of the doctor and patient of perforation is largely unjustified in acute keratoconus! Smoothing and hyperosmolar drops are administered, with the PKP then being performed successfully between 3 and 6 months after wound healing and scar formation is complete [60]. Certainly, in these eyes with ruptured Descemet's membranes, DALK is *not* advisable (Fig. 6.8)!

Preoperative Patient Information Provided by the Microsurgeon to Ensure Compliance

The prophylaxis of complications includes a patient briefing before surgery by the microsurgeon. This includes:

- The operative risk, including loss of the eye
- The slow increase in visual acuity over weeks and months
- The possibility of immunological graft rejection, even after several years
- The risk and symptoms of suture loosening
- The risk of epithelial defects with a risk of infection
- Hypesthesia of the graft over several years

For this reason, glasses with side protection should be worn postoperatively for several months. The briefing before the operation includes the instruction that if the patient experiences "red eye," tears, pain, or blurred vision, he or she should *immediately* seek medical attention. This personal briefing by the surgeon on the evening before surgery and also before dismission contributes toward ensuring patient compliance and the long-term success of the operation!

> The following principle applies: "If you are in doubt, avoid to wait 3 days and hope for spontaneous improvement!"

Intraoperative Complication Prophylaxis

The technique of keratoplasty, which goes far beyond the replacement of two collagen disks with the increasing experience of the microsurgeon, is crucial for the postoperative functional

result. General anesthesia has safety advantages over local anesthesia, especially in young keratoconus patients. The arterial blood pressure should be kept as low as possible when the eye is open ("controlled arterial hypotension" with maximum relaxation). In children, consideration should be given to the preoperative intravenous administration of acetazolamide and mannitol. In every case the anesthetist should have been trained in the specific aspects of penetrating keratoplasty *before* a large opening is made in the eye ball – especially in children [68].

Typically, the pupil is constricted with pilocarpine in order to protect the lens of the phakic eye.

Horizontal positioning of the head and limbal plane is an indispensable precondition for the avoidance of decentration, "vertical tilt," and "horizontal torsion." Paracentesis at the limbus is recommended before trephination. In aphakic vitrectomized eyes, the transconjunctival attachment by suturing (e.g., with 8-0 Vicryl sutures) of a Flieringa ring to stabilize the open globe is recommended [43]. In cases of doubt, limbal centration should be preferred over pupil centration (the optical displacement of the pupil must be taken into consideration, especially in keratoconus). A peripheral iridotomy at 12 clock serves as prophylaxis of a so-called Urrets-Zavalia syndrome [80] (Fig. 6.9).

As long as Bowman's layer is intact, a double-running cross-stitch suture according to Hoffmann is preferred, since it results in higher topographic regularity, earlier visual rehabilitation, and a lower rate of suture loosening [22]. All knots are buried in the stroma to avoid mechanical irritation and the attraction of neovascularization. We aim to produce deep lamellar "pre-descemetal" stitches. Typically, Descemet's membrane should be pushed forward as a triangle in front of the tip of the needle ("wave of Descemet's"). In all diseases with defects in the Bowman's layer or where there is a risk of melting, we use multiple interrupted sutures (typically 24 in number), in order to avoid the need for the postoperative replacement of sutures if some become loose.

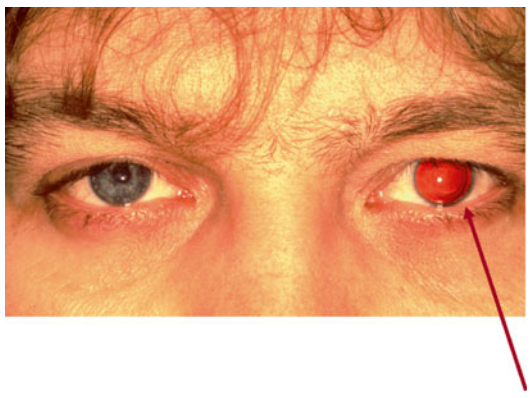

Fig. 6.9 Urrets-Zavalia syndrome (persistent dilated pupil with intraocular pressure rise) after keratoplasty with keratoconus – without peripheral iridotomy

Intraoperative keratoscopy using a handheld Placido disk with adjustment of the continuous sutures or replacement of too tight interrupted sutures should be performed *after* the lid speculum and cardinal sutures have been removed [6].

Special Aspects in Case of Acanthamoeba Keratitis

Approximately 1 week before PKP (in the subacute stage), photodynamic therapy (PDT) is a potential method that is available today for an attempt to reduce the load of acanthamoeba. Typically, the clinical application of PDT is performed today as riboflavin/UVA cross-linking [77]. Simultaneously with excimer laser PKP, corneal cryocoagulation ("freezing-thawing-freezing") is always performed intraoperatively (before the opening of the globe!) [78]. In cases of elliptical corneal ulcers, we use elliptical excimer laser trephination with the aid of a metal mask [27, 75]. After "acanthamoeba keratoplasty," we currently carry out treatment in the form of dual therapy with Brolene and Lavasept, tapering off for approximately 1 year.

Early Postoperative Complication Prophylaxis

As part of postoperative follow-up, we dismiss patients with side protection glasses. The ophthalmologist performing the follow-up should see the patient in the first 6 weeks at least once a week. The follow-up of the Department of

Ophthalmology should be carried out in a specialized "cornea/keratoplasty outpatient service," every 3 months if possible, until the removal of the last sutures. Standard aspects of keratoplasty follow-up include:

- History
- Slit lamp biomicroscopy with fluorescein/blue light
- Subjective/objective refractometry
- Sc/cc visual acuity
- Keratometry
- Topography analysis
- Endothelial cell analysis (quantitative and qualitative)
- Pachymetry
- Intraocular pressure

Using fluorescein/blue light it is possible to accurately determine whether the suture is tight, whether leakage is occurring ("Seidel positive"), and whether erosion or an infiltrate is present. Furthermore, using the slit lamp at maximum magnification, an examination is carried out with respect to retrocorneal precipitates, cells/Tyndall in the anterior chamber, and the presence of a focal epithelial or stromal edema of the graft as an early sign of an immunological graft reaction. After 3 months we routinely carry out gonioscopy in order to be sure that no anterior synechiae are present.

Typically, topical steroids (e.g., prednisolone acetate 1 % AT) are initially tapered 5 times a day for 6–9 months. In aphakic or pseudophakic eyes, we recommend one drop of prednisolone acetate "lifelong" [44].

We remove the first of the two running sutures after 1 year and the second running suture after 18 months. After epithelial closure following suture removal, we resume use of the steroids over 6 weeks, tapering them from 5 times a day in order to prevent immune reactions. Earlier suture removal is carried out for every (!) loose suture, infiltrate, and progressive neovascularization along a suture, but not necessarily in subepithelial fibrosis or intra-epithelial pseudocyst formation at the proximal suture ends [67]. Experience has shown that a loose, continuous suture can be removed in the case of a double-running cross-stitch after 6 weeks without having to replace sutures.

If ocular hypertension is present during the subsequent course after keratoplasty [57], we initially consider the steroid response, which occurs in about 15 % of patients. Postoperative pressure increases must be treated aggressively with medication (including carbachol in the case of pseudophakic eyes) or preferably with cyclodestructive methods (e.g., cyclophotocoagulation) because long-term hypotension often leads to an immune reaction after a filtering operation. In terms of drugs, we avoid prostaglandin analogues in underlying herpetic disease and topical acetazolamide if the endothelium is borderline.

For the treatment of "surface problems," the options available include not only unpreserved artificial tears and soothing gels or ointments (with/without a pressure bandage), vitamin A, and hyaluronic acid (without phosphate!) but also the application of 100 % autologous serum drops. In persistent epithelial defects, single or multilayer amniotic membrane transplantation, temporary (lateral) tarsorrhaphy, or botulinum toxin injection may be indicated for the temporary induction of ptosis ("natural bandage").

In cases of primary graft insufficiency (i.e., the graft is not clear at any time after PKP), the aim should be to replace the graft at an early stage, i.e., after not more than 6 weeks. Here, if the donor tissue has been documented as good at the cornea bank and the surgical technique is uncomplicated, it is always important to consider a latent herpes simplex virus infection of the graft as the cause [72].

In underlying herpetic disease, pretreatment is carried out with topical/systemic acyclovir and steroids. Postoperatively, systemic acyclovir is prescribed for at least 1 year at a dosage of 400 mg twice a day (initially 5 times 400 mg for 6 weeks), in zoster 800 mg twice a day for the prevention of relapse [65, 66]. In vascularized herpetic scars, a combination therapy with 1 g of mycophenolate mofetil twice a day for one year should be considered [38]. After keratoplasty, no steroids should be administered without acyclovir protection. Long-term therapy with acyclovir

ointment once a day at night – immediately before bedtime – is considered. We always treat supposed graft reactions, in which the differential diagnosis of herpes recurrence can hardly ever be confirmed clinically, with a combination of topical/systemic steroids *and* acyclovir [65, 66].

Late Postoperative Complications

Predisposing factors for late suture loosening are defects in the Bowman's layer, stromal vascularization, underlying rheumatic disease, a single continuous suture, keratoplasty in children (26–34 %) [68], and acute keratoconus (the so-called corneal hydrops).

> Each loose corneal suture must be removed as soon as possible.

If a suture infiltrate is present, the suture is removed immediately and typically treated topically with antibiotics and systemically with steroids.

Predisposing factors for step formation – in addition to trauma – are premature suture removal (especially in elderly female patients with bullous keratopathy). Here, the first suture should never be removed before 1 year has passed. In trauma, the steps typically appear to occur inferonasally and after suture removal inferotemporally.

Experience has shown that preexisting corneal neovascularizations tend to regress on the host cornea in the case of underlying herpetic disease with appropriate therapy after PKP. In contrast, new vessels typically grow again on the graft in the host tissue in the case of limbal stem cell insufficiency [1].

The so-called idiopathic endothelial cell loss after PKP in keratoconus is significantly lower in keratoconus than in Fuchs' dystrophy and again lower than in corneal endothelium epithelial decompensation (the so-called bullous keratopathy). We attribute this to endothelial cell migration along a density gradient from the graft to the host cornea [34, 47].

Even after several years, an immunological graft reaction can occur [44, 79]. This may be epithelial, stromal, or endothelial. Typical of the so-called chronic stromal immune reaction are nummuli-like, fine subepithelial infiltrates such as in epidemic keratoconjunctivitis. However, in the immune reaction these are restricted to the transplant. The stromal immune reaction may also occur in a peracute manner in the form of a graft abscess without hypopyon. However, the most common immunological graft reactions are endothelial, either acutely diffuse (here the graft becomes completely cloudy) or chronically focal (in these cases a so-called Khodadoust line spreads from one edge of the graft – typically with the occurrence of neovascularization – like wildfire over the whole graft to the opposite edge of the graft). In the case of an immune reaction, the patient must be treated immediately with local high doses of prednisolone acetate every half hour. An intracameral Fortecortin injection has proven successful. Typically, we also administer systemic steroids (e.g., 250 mg of Solu-Decortin H initially).

> The ophthalmologist in private practice should arrange an immediate follow-up appointment for a keratoplasty patient who calls in with problems.

Conclusions for Clinical Practice

- In addition to the situation-specific diagnosis and preoperative planning, the critical selection of the donor tissue, and the minimally invasive microsurgical technique, it is especially the indication-dependent close-meshed follow-up which plays an important role in the long-term success of penetrating keratoplasty.
- In the follow-up process, the repeated emphatic sensitization of the patient to alarming subjective symptoms and the informed

involvement of the ophthalmologist in private practice providing the follow-up treatment must be considered of crucial importance.
– "Treat them and street them" is certainly not the motto to follow!

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Immunology of Keratoplasty

7

Daniel Böhringer and Thomas Reinhard

Abstract

This is an overview of the current understanding of the pathomechanisms in graft rejection after keratoplasty. We discuss the experimental data on allorecognition and ACAID. We cover tissue typing of the human leukocyte antigen system and selected minor antigens. We give an overview of the clinical evidence in this field. The chapter ends with a recommendation on the best clinical practices with respect to tissue typing in keratoplasty.

Keywords

ACAID • Immune reaction • Allorecognition • HLA • MHC • Matching • HLAMatchmaker • Antibody • Minor antigens • Histocompatibility

Immunology of Keratoplasty

Basic Understanding

The pathophysiologic processes in corneal graft rejection have been thoroughly investigated in various animal models [13]. Interestingly, experimental grafts are well tolerated inside the anterior chamber in the first place. This is in sharp contrast to transplantation of, e.g., skin to other places. These grafts are readily rejected in all vertebrates. Moreover, antigens placed inside the anterior chamber may induce a specific systemic longlasting anergy, as has been demonstrated, e.g., in mice. This phenomenon has been termed anterior chamber-associated immune deviation (ACAID) [29]. Nevertheless, mouse models of keratoplasty with robust graft rejection have been developed [41]. A common model is the transplantation of BALB/c grafts to C57BL/6 recipients.

Current Model of a Graft Rejection

After transplantation, graft material is eventually internalized by antigen-presenting cells (APCs). Graft antigens are lysosomally fragmented inside the APCs. Some of the resulting small peptides are embedded into the binding groove of class II MHC molecules. These small peptides turn into transplantation antigens as soon as the peptide-MCH complex is integrated

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J. Hjortdal (ed.), *Corneal Transplantation*, DOI 10.1007/978-3-319-24052-7_7

into the outer cell membrane of an APC. APCs loaded in such a way migrate to the regional lymph nodes or the spleen. Here, they can activate lurking donor-specific T lymphocytes which clonally expand and fan out into the periphery [42]. Alternatively, the APCs stay within the anterior chamber. There, the specific milieu of the aqueous humor the APCs favors generation of allospecific regulatory T cells (Tregs). These Tregs are thought to be the major structural correlate of ACAID as they promote a longlasting anergy against their specific antigens [14]. Allospecific Tregs and effector lymphocytes are thought to constantly counteract each other until activated effector lymphocytes eventually reach the graft. Here, they conduct an inflammatory graft rejection by activating and recruiting other players of the innate and adaptive immune system [30].

Direct vs. Indirect Allorecognition in the Mouse Model

Current evidence suggests that donor antigens presented indirectly in the context of "own" MHC are the major trigger for graft rejections in a mouse keratoplasty model [21]. This mechanism (as detailed in the previous section) is known as indirect allorecognition. In indirect allorecognition, antigens are randomly picked out of the donor tissue by means of lysosomal fragmentation inside the APCs. However, the corneal graft comprises only a small percentage of MHC molecules. This renders indirectly presented donor MHC fragments subordinate for stochastic reasons. Consequently, matching MHC alleles (namely, HLA matching, HLA is the name for MHC in humans) would be of limited value in preventing indirect allorecognition. Actually, MHC matching has been demonstrated ineffective in the mouse model [37]. MHC matching would be of larger impact if functioning donor-derived APCs could interact with recipient leukocytes directly. This is known as direct allorecognition. Here, intact donor MHC class II molecules "talk" to T-cell receptors on recipient leukocytes. This crosstalk is postered by similarities between donor and recipient MHC overlap. This phenomenon is known as MHC restriction and has its roots in thymic T-cell development [2].

Direct vs. Indirect Allorecognition in the Clinical Setting

Both anatomical properties and morphologic appearance of graft rejections differ vastly between the rodent models and the human situation, e.g., the anterior chamber is much shallower in mice than in humans. This brings the graft into closer proximity to the iris vasculature. Furthermore, in humans the opacity from graft rejection is mostly due to stromal edema, whereas in mice the opacity is due to cellular infiltrates. Therefore, the data from the mouse model are most likely not directly applicable to the clinical situation. The direct pathway may thus still play a rather significant role in humans despite the negative evidence from animal models. In humans, experimental insights into graft rejection are ruled out by ethical concerns. However, organ culture and the recent rise of lamellar grafting incidentally shed some light on allorecognition in humans.

Clinical Clues Toward Direct Allorecognition

Direct allorecognition has two premises: firstly APCs have to be located in the graft in the first place. Secondly, APCs need to be able to migrate out of the graft and reach the lymphatic organs intact. Both premises are actually supported by histopathology and clinical observations [27]. APCs have been demonstrated in the graft epithelium as well as in the graft stroma [4]. Tissue density of APCs reduces during graft storage. Interestingly, long storage intervals have been reported to be protective toward immune reactions [36]. This points toward graft APCs actively migrating into the lymphatics and promoting alloreactions there. Furthermore, it came to a surprise that immune reactions are only rarely observed after Descemet membrane endothelial keratoplasty (DMEK) [1]. These grafts comprise only of endothelium and Descemet membrane. Graft APCs are therefore not present. On the other hand, the target antigens on the endothelium do not differ much from penetrating kerato-

plasty. Both observations are supporting the hypothesis that graft APCs play an important role in eliciting graft reactions in humans.

Tissue Typing

Major Histocompatibility Antigens (HLA)

The HLA system plays an outstanding role in eliciting graft rejections, i.e., when direct allorecognition is involved. This genetic complex is located on chromosome six. The proteins from this complex are subdivided into three classes [25]. Only class I and class II are directly relevant to transplantation immunology, though. Class I molecules are located on the membrane of all nucleated cells. They comprise of a heavy and a light chain (b2 microglobuline). Class I molecules can embed an antigen peptide of nine amino acid residues. The major class I loci are A and B. Further loci are C, E, F, G, K, and L. These are either strongly genetically linked to the A/B haplotypes or not much polymorphic. For this reason, only the A and B loci are commonly considered in HLA matching in solid organ transplantation. Class II molecules are located mostly on the membrane of APCs. These present peptides of 12–24 amino acid residues cleaved from external antigens. The locus DR is of particular importance to transplantation immunology. Further important loci are DP, DR, DM, and DO.

HLA Typing and Nomenclature

HLA typing was originally based on complement-dependent cytolysis. Here, the cells to be typed are incubated against a standardized selection of test antibodies from the International Histocompatibility Workshop (IHW). This library of IHW test sera was constantly extended as new alleles had been discovered. However, the first-generation test sera were not able to differentiate between some related alleles. Monoclonal antibodies later enabled to subdivide many of the original HLA alleles. These more specific entities were termed "splits" of the (original) "broad" HLA antigens. Nowadays, HLA typing is performed with highly specific multiplex assays. These can detect up to 100 unique alleles at once. The HLA allele naming is somehow confusing because "broads" and "splits" share the same number range in the nomenclature. Higher numbers had been sequentially assigned to the newly discovered alleles without removing the broad equivalents. The polymerase chain reaction (PCR) enabled the typing of truly distinct alleles. The accuracy and precision of the molecular typing increased the number of known HLA alleles vastly. In the current "star notation," each allele is a distinct colon segmented number [26]. However, the lower resolution splits or the original broads are still in clinical use and can be inferred from the star notation. For example, the HLA class II broad antigen HLA DR3 can be split into DR17 and DR18. These in turn can be subdivided into the alleles DRB1×03:02 and DRB1×03:03. The molecular methods are essential for typing corneal donors. These pose a special challenge to serologic HLA typing because the blood sample may be collected up to 72 h after onset of clinical death. This limits the detection of cell-bound antigens by antibodies because the cell membranes suffer from incremental autolytic damage. Therefore, some alleles are prone to be falsely not detected or to cross-react with other alleles. This has actually been an issue in the past [20].

HLA Matching

The HLA pool is highly variable in any given population. More than 100 alleles have been documented at each HLA locus. This results in millions of possible HLA phenotypes. However, HLA alleles are inherited in haplotypes. This means fixed combinations of alleles on the loci A, B, and DR often occur together. Nevertheless, it is nearly impossible to pick an HLA identical individual just by chance. This can only be achieved by means of a concerted sequential search in a donor population. HLA matching is the search for a donor who exclusively possesses HLA alleles that are also present in the recipient. All "foreign" HLA alleles of the donor are coined HLA mismatches. In solid organ transplantation, the loci HLA A, B, and DR are exclusively considered for matching. This limits the maximum count of mismatches from a single donor to

six. In bone marrow transplantation, more loci are matched. This is only possible because of worldwide donor pools. Aside from bone marrow transplantation, matching is not routinely performed at allele resolution but at the resolution of "splits" or even still the original "broad" HLA alleles. Another current matching approach is HLAMatchmaker [16]. This method considers only the HLA antibody epitopes. Most HLA alleles share a substantial percentage of these epitopes. The HLAMatchmaker assumption is that any given recipient will not generate antibodies against own HLA antibody epitopes uncoupled from the HLA alleles. This allows to increase the donor pool by distinguishing "harmless" and "dangerous" mismatches by counting only the "foreign" antibody epitopes from each mismatch.

Hindrances to HLA Matching in Keratoplasty

The usefulness of HLA matching is undisputed in kidney and especially in bone marrow transplantation [34]. Here, HLA matching is part of the clinical routine. This is not the case in keratoplasty, though. Only very few centers currently offer HLA-typed donors routinely. Two major reasons may motivate this reluctance. Firstly, the current evidence does not clearly support HLA matching. Secondly, the additional and unpredictable waiting time for HLA matching hinders the patients' personal planning and complicates surgical scheduling.

Evidence on HLA Matching

The evidence on HLA matching on keratoplasty is contradictory at first sight: the one and only randomized clinical trial (CCTS) failed to demonstrate efficacy of HLA matching [the collaborative corneal transplantation studies (CCTS) [38]]. By contrast, several nonrandomized investigations uniformly observed a beneficial effect of HLA class I matching (Table 7.1). The outlier position of the CCTS is underpinned by inaccurate HLA typing in that trial. The CCTS was based on HLA typing that differed in 55 % from retyping with molecular techniques [20]. The importance of accurate HLA typing for successful HLA matching was investigated by means of statistical simulation: even 5 % of faulty HLA DR typing obscured the beneficial matching effect [40]. Another methodical downside of the CCTS was the high postoperative dosages of topical steroids. This probably further obscured any HLA effect. A closer review of Table 7.1 renders a beneficial effect of matching at the HLA class I rather likely. For HLA class II, the situation is less clear. Both adverse affects of matching the HLA DR and benefits have been reported [40]. Interestingly, the largest and most recent retrospective investigation observed a statistical interaction between matching at HLA class I and HLA class II when it comes to preventing immune reactions. A protection against graft rejections was observed when the epitope agreement between the HLA class I loci A and B was

Table 7.1 Evidence on HLA matching in keratoplasty

Authors	References	*n*	HLA loci	Evidence level	Remarks
Hoffmann et al.	[19]	20	A, B/DR	II	Beneficial
Boisjoly et al.	[11]	438	A, B	II	Beneficial
CCTS	[38]	419	A, B/DR/AB0	I	No effect, typing inaccurate
Hoffmann et al.	[18]	248	A, B/DR	II	Beneficial
Vail et al.	[39]	602	A, B/DR	II	A/B: beneficial; DR: adverse
Munkhbat et al.	[28]	81	DR, DQ, DP	II	Beneficial
Baggensen et al.	[5]	74	DR/DQ	II	Beneficial
Völker-Dieben et al.	[40]	1681	A, B/DR	II	Beneficial (i.e., DR)
Khaireddin et al.	[24]	459	A, B/DR	II	Beneficial
Bartels et al.	[6]	303	A, B	II	Beneficial
Reinhard et al.	[32]	48	A, B/DR	II	Beneficial
Reinhard et al.	[31]	418	A, B/DR	II	Beneficial
Böhringer et al.	[8]	1561	A, B/DR	II	A/B: beneficial; DR: depends on A/B matching

poorer than at the DR locus. This was a benefit independent of the additional HLA class I matching effect.

Prediction of the Waiting Time in HLA Matching

HLA matching inevitably prolongs the time on the waiting list. This is because all grafts but the first with very few HLA mismatches are rejected for the patient. Quality of life usually limits acceptable waiting periods to one year at maximum. The additional waiting period strongly depends on the HLA type. Patients with more common HLA phenotypes usually receive a match after few months. This is because their HLA alleles are also common among the donors. However, patients with a rare HLA alleles (i.e., when additionally homocygotic) may remain on the waiting list for years. It is nowadays possible to identify these patients in advance with a computer program and a database of the haplotype frequencies in the donor population [9]. This method is essential for discussing HLA matching with the patients as early as when discussing the indication of keratoplasty with them.

Evidence on Anti-HLA Antibodies in Keratoplasty

Anti-HLA antibodies had been originally detected in macro-agglutination assays. In this method, the patient serum is incubated with HLA-coated test erythrocytes. After adding patient serum, the test erythrocytes agglutinated in the presence of specific antibodies against that HLA allele. Nowadays, flow-based bead assays are used to detect anti-HLA antibodies. Donor-specific anti-HLA antibodies are presumed to deteriorate the prognosis of penetrating keratoplasty [15]. On the other hand, several other investigations failed to observe an antibody effect. A new method for reliable detection of these antibodies has recently strengthened the hypothesis that donor-specific anti-HLA antibodies play an important role in graft rejections after penetrating keratoplasty [35]. This is in line with the success of HLAMatchmaker in keratoplasty. This method (detailed in a previous section) is based on antibody epitopes. However, HLA crossmatching is still not performed as part of the clinical routine in keratoplasty. This is most likely due to lack of clear level I evidence at the time of writing.

Minor Histocompatibility Antigens (H Antigens)

Graft reactions may occur even when all HLA loci are perfectly matched. In some transplantation models, these graft reactions take a milder course in comparison to rejections caused by HLA mismatches. The underlying antigens have therefore been coined minor antigens [17]. Later, these have been identified as the aforementioned targets of indirect allorecognition embedded in MHC class II molecules on APCs. Another source of minor antigens are the intracellular fragments that are embedded into the HLA class I molecules of all nucleated cells. These convey a proteomic cellular fingerprint to the outer membrane. The antigens originate from somatic proteins that are constantly degraded by proteasomes. Proteasomes are organelles that recycle the amino acids of freshly synthesized and sorted out proteins by means of enzymatic fragmentation. Sometimes the proteasomes fuse with the endoplasmatic reticulum. Here, the peptides are placed in the binding groove of freshly synthesized HLA class I molecules with the help of tapasin. The endoplasmatic reticulum eventually fuses with the outer cell membrane and exposes the loaded HLA molecules to the aforementioned methods of allorecognition. It is important to note that each HLA allele has a specific repertoire of minor antigens that it can hold. This specificity is a consequence of the physical properties of its binding groove.

Discussion on Selected H Antigens

H-Y

The Y chromosome encodes several cytosolic proteins. These give rise to the H-Y group. Male grafts can thus be rejected by female recipients. H-Y antigens are supposedly expressed in the human cornea. H-Y antigens can be embedded into HLA A1 or HLA A2. A 20 % reduction of graft rejections was observed in 252 keratoplasties when avoiding the HLA A1/H-Y mismatch. In the same trial, the HLA A2/H-Y epitope

was not relevant [10]. The prevalence of HLA A1 male donors is only 13 % in, e.g., Germany. For this reason, generally avoiding transplantation of male donors to female recipients does not make sense [22]. However, allocating male HLA-A1 donors to female recipients may be a discrete risk factor for immune reactions after penetrating keratoplasty.

HA-3

The HA-3 epitope is also HLA-A1 restricted. This epitope is derived from the lymphoid blast crisis (Lbc) oncoprotein and H antigen that has been expressed in the cornea. The HA-3 epitope comes in two alleles: VTEPGTAQY (HA-3 T) and VMEPGTAQY (HA-3 M). However, only grafting into the direction of HA-3 T is considered immunogenic. This does not seem to be highly relevant in penetrating keratoplasty, though [10].

Blood Group (ABO)

Blood group antigens are sometimes also referred as minor antigens. The allelic nature of the synthesizing enzyme gives rise to the ABO system. These are immunogenic glycoproteins attached to erythrocyte membranes but also present on a wide variety of human tissues. The ABO antigens are not physiologically expressed in the corneal stroma and corneal endothelium. However, they have been detected in failed corneal grafts [3].

The evidence on blood group matching in keratoplasty is controversial. A beneficial effect has been observed in high-risk penetrating keratoplasty, but not in normal-risk keratoplasties [12,23,38]. Other blood group antigens may play a role in normal-risk keratoplasties [33]. More research is warranted to work out the exact mechanisms in blood group histocompatibility. These retrospective nonrandomized results may well be confounded by peptidic H antigens originating, e.g., from the ABO-specific glycosyltransferases or other factors.

Conclusions and Recommended Clinical Practice

The recent rise of lamellar grafting certainly reduces the need for tissue typing in the clinical routine. It is nowadays possible, e.g., to replace a failed graft endothelium with Descemet membrane transplantation. This almost completely avoids subsequent rejection episodes. However, tissue typing still makes sense for specialty centers that deal particularly with high-risk transplantations in vascularized grafts or with limbal allografts. Here, all corneal donors should be HLA typed at least at the loci A, B, and DR. DNA typing is the method of choice. An alternative source of typed grafts is, e.g., Bio Implant Services, Leiden, the Netherlands. Blood groups may be additionally typed. The potential benefit from this is at a lower level of evidence, though. Lobbying is still required because costs from HLA typing of the donor are poorly reimbursed in most health systems. However, from the payer's perspective, the additional cumulative costs from HLA typing have been recently calculated as low as 4.62 EUR per additional day of graft survival after penetrating keratoplasty [7].

Patients awaiting penetrating keratoplasty should be provided a histocompatible graft (HLA and AB0) whenever possible. This is especially true for high-risk keratoplasties. The expected time on the waiting list should be calculated and discussed with the patient in advance. The HLAMatchmaker algorithm can help in discriminating between "harmless" and "dangerous" mismatches to increase the donor pool and shorten the waiting time.

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Post-operative Management

8

Sing-Pey Chow and D. Frank P. Larkin

Abstract

Corneal transplantation has been successfully performed for over 100 years. Despite HLA typing and systemic immunosuppression not being routinely undertaken, 5-year survival rates exceed 90 % in corneal grafts with no current or past history of inflammation. However, graft survival decreases dramatically in the presence of risk factors that place it at high rejection risk, and immunological graft rejection remains the leading cause for graft failure. Post-operative management of corneal grafts requires stratification according to the risk for rejection and addressing this with appropriate prophylaxis. It is critically important to recognise corneal graft rejection early and initiate appropriate treatment, as a delay in diagnosis and treatment will result in failure to reverse rejection, or at least shorter graft survival if rejection is reversed.

Keywords

Graft rejection • Rejection prophylaxis • Post-operative care • Systemic immunosuppression

Corneal Transplant Rejection

The Cornea and Anterior Chamber as Sites of Immune Privilege

The cornea and the underlying anterior chamber have long been recognised as sites of relative immune privilege [1, 2]. Various anatomical, physiological and immunological factors contribute towards this via modulation of the afferent and efferent arms of the immune response. Firstly, absence of blood vessels and lymphatics in the normal cornea and the presence of tight junctions

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J. Hjortdal (ed.), *Corneal Transplantation*, DOI 10.1007/978-3-319-24052-7_8

forming the blood-eye barrier result in the relative sequestration of the cornea from potential systemic immune responses [3]. Secondly, the aqueous humour contains a rich milieu of immunomodulatory molecules which downregulate immune responses which are potentially harmful to transplanted donor cornea. Some of these contribute towards the downregulation of systemic immune responses, in particular alloantigen-specific suppression of delayed-typed hypersensitivity (DTH) responses, which was first described over 30 years ago as anterior chamber-associated immune deviation (ACAID) and shown in rodents to reduce the impact of corneal allograft rejection [4, 5]. Thirdly, the cornea itself possesses several mechanisms that neutralise elements of the immune effector response. These include the expression of cell membrane-bound molecules on the epithelial and endothelial surfaces such as (*i*) Fas ligand (CD95L), which induces apoptosis of neutrophils and lymphocytes that ligate these molecules on the cornea during inflammation [6]; (*ii*) programmed death-ligand 1 (PD-L1) that inhibits T lymphocyte proliferation, induces T lymphocyte apoptosis and prevents T lymphocyte production of interferon-ɣ (IFN-ɣ) [7, 8]; and (*iii*) both membrane-bound complement regulatory proteins (CRP) on corneal epithelial cells and soluble CRP in the aqueous humour that buffer the capacity of complement-fixing antibodies to produce corneal injury in rejection [9, 10]. The corneal endothelium is also unique in its paucity of major histocompatibility complex (MHC) class I molecules [11]. This would usually trigger natural killer (NK) cells as members of the innate immune system to kill any cells that fail to express MHC class I molecules – termed "missing self hypothesis" – as many neoplasms downregulate their expression of MHC class I molecules to escape cytotoxic T lymphocyte (CTL)-mediated immune surveillance. However, this has not been convincingly demonstrated in the cornea to date and has been attributed to the presence of at least two molecules that inhibit NK cell-mediated cytolysis in the aqueous humour bathing the corneal endothelium, namely, macrophage migration inhibitory factor (MIF) and transforming growth factor-β (TGF-β) [12–15].

Clinical Factors Predisposing to High Risk of Corneal Graft Rejection

A corneal allograft becomes predisposed to rejection when facets of immune privilege are overwhelmed by pre-transplant alterations in recipient tissue or breached by post-transplant clinical events.

The Australian Corneal Graft Registry with a database of over 23,000 grafts reports the overall survival of corneal grafts as 87 % at 1 year and 73 % at 5 years. For some indications such as a first graft for keratoconus, graft survival is 97 and 95 % at 1 and 5 years, respectively [16]. However, this dramatically decreases in the presence of risk factors that confer the graft at high risk of rejection. For example, 5-year graft survival for corneal grafts with 2 or more quadrants of stromal vascularisation ranges from 45 to 58 %, and grafts with ocular inflammation at time of surgery range from 50 to 56 % [16].

Factors that have been consistently demonstrated to affect prognosis of corneal grafts include (i) two or more quadrants of stromal vascularisation in the graft recipient bed, (ii) inflammation at the time of surgery and (iii) history of a previously rejected corneal graft in that eye [17–23].

Corneal vascularisation is an almost invariable consequence of acute or chronic inflammation. The extent of vascularisation of the recipient cornea, as categorised as quadrants of blood vessel growth, at the time of transplantation correlates strongly with graft survival (Fig. 8.1) [18, 22]. However, it is worth noting that recent research has shown that it may be the presence of lymph vessels rather than blood vessels that robs the corneal allograft of its immune privilege. Dietrich et al. reported that administration of a molecule antagonist of α5β1 integrin or anti-VEGFR3 antibody that preferentially inhibits lymphangiogenesis, but not haemangiogenesis, produced a significant enhancement of graft acceptance in murine hosts who were pretreated with sutures to stimulate highly vascularised graft beds [24]. However, the long-held hypothesis that the presence of blood vessels in the corneal graft bed increases the risk of rejection is still valid as

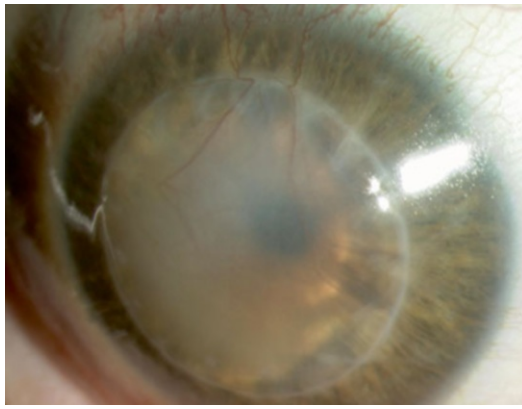

Fig. 8.1 Corneal vascularisation with graft failure secondary to graft rejection. Vascularisation of the recipient corneal bed is the most significant single risk factor for graft failure on multivariate analysis in all published reports (Reproduced from Niederkorn and Larkin [1], with permission of Informa Healthcare)

murine models of penetrating keratoplasty have demonstrated that the stimuli that induce blood vessel ingrowth also stimulate lymph vessel ingrowth and the infiltration of resident antigen-presenting cells, both of which conspire to promote immune rejection [1].

Inflammation is also an independent variable associated with corneal graft failure due to rejection. Using immunohistochemical staining analysis in 107 recipient corneas, Williams et al. found an inverse relationship between leucocyte counts in the graft bed and 3-year actuarial graft survival [20]. Hence, corneal transplantation is best avoided in an actively inflamed eye where possible, although it is important to note that even a history of inflammation alone without activity at time of transplantation results in a less favourable 5-year graft survival of 64 % compared to 91 % in an eye without any history of inflammation and 56 % in an eye without a history of inflammation but active inflammation at time of transplantation [16].

A previously rejected corneal graft implies allosensitisation with relative loss of immune privilege and has been demonstrated to increase risk of rejection in a subsequent allograft, even if the recipient cornea is avascular [22]. The number of preceding transplants in the recipient eye is also a prognostic factor for graft survival, with decreasing graft survival rates for subsequent allografts.

Other factors that have been shown to increase the risk of rejection include grafts in children, large-diameter grafts [25, 26] and the presence of atopy [27, 28]. The presence of non-ocular atopic disorders, even in the absence of clinically evident conjunctival allergy, appears to confer a higher risk of graft rejection. The mechanisms underlying this are not fully understood. However, patients with atopic dermatitis have been shown to have a poorer graft prognosis [27, 29, 30], and murine asthma models have also demonstrated that airway allergen exposure alone increases corneal allograft rejection risk [31]. Studies of corneal transplantation in the setting of allergic conjunctivitis have demonstrated an increased incidence and swifter tempo of graft rejection and the presence of an eosinophilic component in the alloreactive effector population of rejected grafts that is only found in atopic graft recipients [28, 32].

Post-transplant events can also lead to subversion of immune privilege and hence increase the risk of rejection. Loosened sutures, suture-related infections and herpetic infection recurrence are local episodes of alloantigen-independent inflammation that lead to recruitment of alloreactive cells, angiogenesis, lymphangiogenesis and upregulation of MHC molecules on graft cells [1, 33]. This combination of events can lead to an acute-onset rejection response, which must be recognised early and promptly treated.

Clinical Features of Graft Rejection

In 1948, Paufique and colleagues used the term *maladie du greffon* (disease of the graft) to describe clouding of the graft after an initial period of clarity [34]. In 1969, Khodadoust and Silverstein demonstrated that each layer of the cornea – epithelium, stroma and endothelium – could manifest a rejection reaction [35]. The incidence of corneal allografts experiencing a rejection episode at some stage following transplantation has been reported as ranging from 18 to 21 % in large cohorts of graft recipients [36, 37].

Epithelial rejection is characterised by the presence of an elevated linear opacity that stains with fluorescein and often progresses from the periphery to the centre of the graft over the course of several days to a few weeks. The average onset of an epithelial rejection line was 3 months' post-transplantation with a frequency of 10 % in one series [38] .Stromal rejection is characterised by nummular subepithelial infiltrates, similar to those found in adenoviral keratitis (Fig. 8.1a). They can be seen concurrently with an epithelial or endothelial rejection line. Its average onset was reported to be 10 months' post-transplantation with a frequency of 15 % [38, 39]. Patients with epithelial and stromal rejection may be asymptomatic or have only mild ocular discomfort.

In contrast, patients with endothelial rejection tend to be more symptomatic and may present with visual disturbance and/or symptoms consistent with anterior chamber inflammation. If examined early, there may only be cells in the anterior chamber without any flare or graft abnormality. This will then be followed by aggregated alloreactive cells adherent to graft endothelium as keratic precipitates, the presence of an endothelial rejection line and an area of localised graft oedema (Fig. 8.1b) [35, 40, 41]. The average onset of endothelial rejection has been reported to be 8 months' post-transplantation with a range of 2 weeks to 29 months, although unequivocal endothelial rejection has been observed as late as 9 years' post-transplantation [38, 41]. A rejection episode results in loss of donor endothelial cells, which are critical for maintenance of corneal transparency. As human endothelial cells do not repair by mitosis, endothelial decompensation may ensue if the cell density is reduced at rejection below the threshold necessary to prevent stromal swelling. This may happen at the time of an irreversible acute graft rejection or manifest at an interval following one or more rejection episodes that were reversed with treatment. Risk factors for significant endothelial cell loss include a delay in presentation of more than 1 day between onset of symptoms and initiation of treatment and recipient age of greater than 60 years [42].

Pachymetry is useful in detecting an increase in oedema and deturgescence following the initiation of steroid treatment. Naacke et al. reported that apart from preoperative diagnosis, the only other factor found to be significantly associated with reversibility of graft rejection was graft thickness at time of rejection diagnosis [43]. The Collaborative Corneal Transplantation Study Group also reported that 49 % of eyes had an increase in corneal thickness of at least 10 % in association with the development of a rejection episode, and the likelihood of graft failure was predicted by a larger increase in thickness at 1, 3 and 6 months [44].

Management of Corneal Transplant Rejection

Treatment of Rejection

The leading cause of graft failure is immunological graft rejection. It is important to promptly recognise the clinical features and initiate treatment, as a delay in diagnosis and treatment adversely affects graft prognosis.

Treatment with intensive topical corticosteroid, such as dexamethasone 0.1 %, is successful at reversing most endothelial rejection episodes. In cases where topical steroids fail to reverse rejection, this has been attributed to the failure of the topical steroid to reverse effector components of the allogeneic response or a delay in recognition and initiation of treatment with resultant significant endothelial cells loss, ultimately leading to graft failure [42].

Regarding additional systemic steroid, Hill et al. found that a single intravenous pulse of methylprednisolone was more effective than oral prednisolone in reversing rejection in patients who presented with endothelial graft rejection within 8 days of symptom onset. Patients were also significantly less likely to undergo a further rejection episode if the graft survived, with 67 % of the oral cohort and 26 % of the intravenous cohort experiencing further episodes of rejection [45]. A second pulse of intravenous methylprednisolone given 24 h or 48 h later did not

demonstrate any advantage in addition to that of a single dose at diagnosis [46].

However, a subsequent prospective randomised trial by Hudde et al. did not demonstrate a statistically significant benefit in receiving a single intravenous methylprednisolone pulse in addition to intensive local corticosteroid in terms of reversal of the rejection episodes, later recurrence of graft rejection or graft failure with a follow-up duration of 2 years. The intensive local corticosteroid regime used in that study consisted of one dose of subconjunctival betamethasone (2 mg) and hourly dexamethasone 0.1 % for 24 h [47]. Another study reported a higher rate of rejection reversal in patients receiving subconjunctival triamcinolone (20 mg) versus a single dose of intravenous methylprednisolone in addition to topical prednisolone acetate 1 % [48]. Successful reversal of an endothelial rejection episode ranges from 51 to 92 % [43, 47].

Other studies have examined the use of topical [49, 50] and systemic [51] cyclosporine in the treatment of endothelial rejection: Poon et al. in a prospective randomised trial did not find a significant benefit in using a commercially available preparation of topical cyclosporine (0.05 %) in addition to intensive topical steroids [49].

Prevention of Corneal Transplant Rejection

Patients with Low Rejection Risk

In patients without risk factors for graft rejection identified prior to transplantation, typical post-operative immunosuppression consists of dexamethasone 0.1 % or prednisolone acetate 1 % four times daily for the first 2–3 months, reducing gradually to zero by 6–12 months following transplantation. There are no definitive randomised controlled trials into the optimal immunosuppression regime for low-risk grafts, although there is remarkable consensus worldwide regarding the need for prophylaxis post-operatively with topical corticosteroid as demonstrated by surveys of practice patterns [52–54].

Koay and colleagues in their survey of corneal surgeons in the Bowman Club in the United Kingdom reported that all surgeons used topical steroids post-operatively, with 50 % favouring prednisolone acetate 1 % and 36 % favouring dexamethasone 0.1 %. Average duration of topical treatment was 8.7 months, although 5.5 % of respondents continued treatment indefinitely in low-risk grafts [52]. This is in stark contrast to that reported by Price and colleagues, who surveyed 250 corneal surgeons attending an endothelial keratoplasty course at a tertiary referral centre between 2006 and 2008; the majority (87 %) of whom were from the United States. They reported that 46 and 22 % of respondents continued topical steroids indefinitely for pseudophakic/aphakic and phakic patients, respectively, in low-risk grafts [55].

Price and colleagues also found that the majority (76 %) of respondents used intraoperative corticosteroids, of which 72 % were delivered as sub-tenon or subconjunctival injections, 8 % were intravenous, 7 % were oral and 2 % were intraocular. Again, all surgeons used topical steroids post-operatively, with 95 % using prednisolone acetate 1 %. Most surgeons (57 %) used the same regimen regardless of lens status. However, 14 % of respondents who initially prescribed prednisolone acetate 1 % for phakic patients had switched to a lower-strength corticosteroid such as fluorometholone or loteprednol at 6 months, and 20 % had withdrawn their patients' topical steroid. In contrast, 10 % of respondents had switched their pseudophakic/aphakic patients to a lower-strength steroid, and 10 % had withdrawn topical steroids [55].

Nguyen and colleagues in a recent prospective randomised trial of 406 eyes following normal-risk keratoplasty reported significantly higher rejection rates in grafts where topical steroids were stopped at 6 months (9.1 %) compared to 12 months (4.9 %) [56]. The use of topical cyclosporine 0.05 % four times daily for 1 year has also been undertaken and found to be significantly less effective than historical controls using topical corticosteroid for a median of 7 months as rejection prophylaxis in low-risk grafts [57].

Patients with High Rejection Risk

There is much less consensus on the postoperative management of grafts with high rejection risk (Fig. 8.2). Due to the shortage of large comparative prospective studies into immunosuppression regimes, different centres use varying protocols based on individual clinical experience and informed by experimental evidence, small uncontrolled or retrospective clinical studies and extrapolation from what has proven effective in solid organ transplantation. This is compounded by the lack of a consensus definition of what constitutes a "high-risk" graft, which also makes direct comparison between studies difficult. Some reports include risk factors for graft failure independent of rejection as part of their "high-risk" definition, and others include a subset of patients who received HLA-matched donor corneas, a factor that may independently affect transplant outcomes with respect to rejection.

Furthermore, as corneal transplantation is not a life-saving procedure, ophthalmologists are hesitant to commit patients to long-term systemic immunosuppression due to the potential side effects and risk of developing malignancies. However, in cases where there is a high rejection risk and patients are reliant on graft survival in order to undertake activities of daily living, the risks of systemic immunosuppression may be more justifiable.

Both topical and systemic immunosuppressive agents have been evaluated for prophylaxis against graft rejection in high-risk grafts. However, systemic rather than local administration is justified by evidence in experimental models that alloantigen immunisation does not occur in the eye, but that transported corneal alloantigens lead to clonal expansion of alloreactive T lymphocytes in regional lymph nodes and possibly spleen [58–60].

The majority of reports on systemic immunosuppression as prophylaxis against corneal allograft rejection utilise one of the calcineurin inhibitors, cyclosporine and tacrolimus, as monotherapy. This is in contrast to renal transplant recipients, who commence dual- or triple-agent prophylaxis that typically includes prednisolone, mycophenolate mofetil and calcineurin inhibitors or sirolimus. Hence, the poorer prophylaxis outcomes in corneal patients compared to renal transplant recipients may be due to (*i*) low drug doses, (*ii*) short duration rather lifelong prophylaxis and (*iii*) the narrow spectrum of activity within the alloreactive cell phenotypes of monotherapy [61]. Monotherapy with calcineurin inhibitors, which block T lymphocyte clonal expansion by interfering with interleukin-2 gene transcription, may also be less effective as most graft-reactive cells in the anterior chamber after rejection onset in humans are CD14+ cells of monocyte-derived macrophage lineage rather than lymphocytes [62].

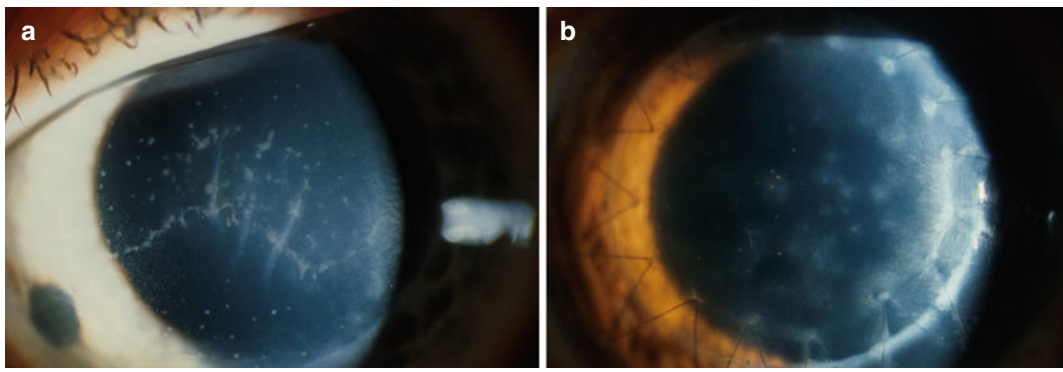

Fig. 8.2 (**a**) Endothelial rejection. A horizontal endothelial line, scattered keratic precipitates and Descemet membrane folds are shown. (**b**) Stromal rejection. Scattered anterior stromal infiltrates shown are restricted to donor cornea (Reproduced from Larkin [41] with permission of BMJ Publishing group)

Cyclosporin A

Cyclosporin A (CsA) is a calcineurin inhibitor that disrupts the signalling pathways necessary for the proliferation of activated T lymphocytes via interleukin-2 gene transcription. Various studies have evaluated the use of systemic CsA in addition to topical steroids in high-risk grafts. Direct comparison is limited by varying methodology such as their prospective versus retrospective nature, the inconsistent inclusion of HLA-matched grafts, varying intended CsA serum trough levels and the use of additional systemic corticosteroids in some studies. Survival of these high-risk grafts at 2 years has been reported to range from 67 to 74 % [63–67].

Hill and colleagues in their prospective series reported a significant reduction in rejection episodes in their CsA (49 %) group compared to controls (73 %) and noted that there was a significant higher rate of rejection reversal in patients on CsA 63]. Duration of CsA prophylaxis was also important; the group receiving CsA for 12 months had better rejection-free survival compared to those receiving CsA for 4 months or controls [68]. However, this contrasts with other studies that did not find a significant difference in graft rejection incidence or graft survival between CsA and control groups [65, 66].

Topical CsA has also been evaluated as rejection prophylaxis in high-risk grafts without convincing evidence of its efficacy. A prospective randomised trial did not demonstrate a significant difference in graft rejection incidence using CsA 2 % in addition to topical steroids, but did find a significantly higher proportion of reversibility in rejection episodes in the CsA group [69]. Other retrospective case series have reported significantly higher rejection-free graft survival rate in CsA 2 % versus control groups, but no difference in overall graft survival [70, 71]. Interestingly, one case series demonstrated blood levels of CsA after topical treatment [72].

Tacrolimus

Tacrolimus is a macrolide antibiotic isolated from the soil fungus *Streptomyces tsukubaensis*. Like cyclosporine, it is also a calcineurin inhibitor and has been successfully used in liver and renal transplantation [73]. Both topical [74, 75] and systemic [76–78] tacrolimus has been evaluated in high-risk grafts as monotherapy.

Joseph and colleagues reported the use of oral tacrolimus (aiming for a trough level of 1–12 μg/l) for 18–24 months in 43 patients. Five patients experienced rejection-related graft failure (12 %), whilst a further three patients experienced rejection episodes that were reversed [77]. Yamazoe and colleagues used a lower dosage of tacrolimus in their recent prospective study of 10 patients with a history of graft failure whilst on systemic CsA prophylaxis, aiming for a target trough level that was half of that used in renal transplantation (8–10 μg/l for 2 months then weaned to a maintenance level of 5–6 μg/l) for 18 months. Graft rejection occurred in 2 patients (20 %), both of which led to graft failure [78]. However, they reported significantly fewer graft rejection episodes on tacrolimus compared to cyclosporine in the same cohort of patients. Side effects were reported in 20–60 % of patients.

Mycophenolate Mofetil and Sirolimus

Mycophenolate mofetil (MMF) is a purine synthesis inhibitor that selectively inhibits proliferation of T and B lymphocyte proliferation. MMF has been shown to be effective and safe as prophylaxis against rejection following kidney, heart and liver transplantation [79–83].

Birnbaum and colleagues evaluated the efficacy of MMF for 6 months versus controls in a prospective, multicentre randomised trial involving 98 patients. Kaplan-Meier analysis demonstrated significantly higher rejection-free graft survival in the MMF group (83 %) compared to controls (65 %) with an average follow-up duration of 35 months. Rejection-related graft failure was 29 and 78 % in the MMF and control groups, respectively. Sixty-three percent of patients in the MMF group experienced side effects; 3.5 % of patients needed to be withdrawn from the MMF group due to severe side effects [84].

Studies comparing MMF with CsA have been conflicting. A prospective study of 56 patients did not demonstrate a significant difference in rejection rates [64], whilst a later retrospective study of 417 patients with 3-year follow-up

reported a statistically significant, stronger effect of MMF in terms of rejection-free survival [85].

MMF has also been compared with oral sirolimus (rapamycin) administered for 6 months as monotherapy without a significant difference in the incidence of rejection [86]. Sirolimus is a microbial macrolide that prevents G1 to S phase progression in the T lymphocyte cell division cycle and is active against T lymphocytes, B lymphocytes, dendritic cells, monocytes and macrophages.

Chatel and Larkin evaluated the efficacy of combination therapy with MMF and sirolimus in a prospective case series where patients were at high rejection risk but did not have any other risk factor for graft failure. Six patients received both sirolimus and MMF for 12 months, followed by sirolimus for another 2 years at trough serum levels used in prophylaxis following cadaveric kidney transplantation (sirolimus aiming for a blood trough level of 12–20 μg/l; MMF 2 g daily). Rejection episodes occurred in 3 patients (50 %), one of which led to transplant failure. Graft survival was 83 % with a minimum follow-up of 13 months, and only one patient required cessation of MMF due to significant adverse effects [61].

Endothelial Keratoplasty Following Failed Penetrating Keratoplasty

In cases where endothelial failure ensues subsequent to graft rejection, endothelial keratoplasty has become the preferred option for many surgeons to restore graft clarity where possible, particularly in cases where the failed penetrating keratoplasty has healed with a satisfactory refractive shape profile. Its advantages over a repeat penetrating keratoplasty include preservation of tectonic integrity and faster visual rehabilitation (Fig. 8.3).

Mitry and colleagues in a recent review of 246 eyes that underwent Descemet stripping automated endothelial keratoplasty (DSAEK) following failed penetrating keratoplasty (PK) across six sites in Europe, United States and Asia reported an estimated DSAEK survival rate of 89, 74 and 47 at 1, 3 and 5 years, respectively [87]. Other single-centre case series have also reported similar rates of graft survival

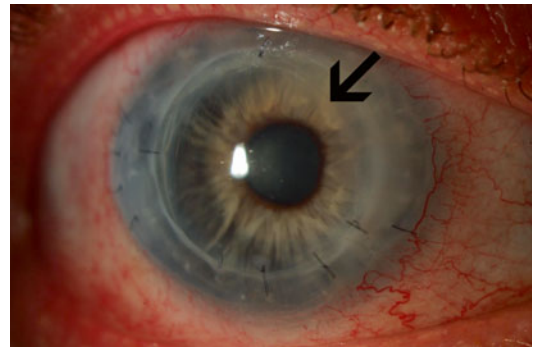

Fig. 8.3 Descemet stripping automated endothelial keratoplasty (DSAEK) following failed penetrating keratoplasty (PK) due to allograft rejection. The edge of the DSAEK graft can be seen beneath the PK (*black arrow*)

[88–90]. This is comparable to the 1- and 5-year graft survival rates for repeat penetrating keratoplasty of 80 and 58 %, respectively, for a second graft, and 71 and 47 %, respectively, for a third graft [16]. The Collaborative Corneal Transplantation Studies Research Group has also reported the increasing risk of graft failure with repeat grafts, from 17 % without a previous graft to 53 % with 2 or more previous grafts [18]. A promising finding was that a number of factors that increase the risk of graft failure following repeat PK, such as corneal vascularisation and number of previous PK, were not significant risk factors following endothelial keratoplasty [91].

Based on multivariate analysis, Mitry and colleagues reported significant pre-operative risk factors for DSAEK failure following failed PK as young recipient age, previous tube filtration surgery and rejection episodes before PK failure. It is important to note that any rejection episode prior to PK failure was found to be a significant predictor of post-DSAEK rejection, which in turn was a significant predictor of DSAEK failure [87]. This is in contrast to Anshu and colleagues, who did not find a DSAEK rejection episode to be a significant risk factor for subsequent graft failure, but concurred that previous tube filtration surgery is an independent risk factor [91]. Visual rehabilitation following DSAEK has also been reported to be comparable to that of a repeat PK [87, 91–93].

Future Prospects

Many questions still remain regarding the optimal immunosuppression regime for prophylaxis in grafts at high rejection risk. Prospective randomised controlled trials are required to identify the most effective agent(s) with the least side effects and the optimal duration of immunosuppression to balance graft survival with the potential risks of immunosuppression.

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Outcomes: Recurrence of Disease

9

Per Fagerholm

Abstract

The inherited diseases or true corneal dystrophies tend to recur in the grafts. The frequency and intensity of recurrence vary extensively. With time more sophisticated genetic analyses have made the old clinical classification less reliable and the IC3D classification system welcome. Heredity is part of the definition of a corneal dystrophy something that is rarely found in basement layer corneal dystrophy and in what is generally named Fuchs' dystrophy. Future studies need to accommodate to strict modern classification. Large variation in recurrence frequency can be found in clinical similar forms of granular dystrophy but having different genetic lesions. Keratoconus is overrepresented within many families, but the mode of inheritance is unclear. The disease can progress within the host cornea, the graft received may harbor keratoconus, and keratoconus may develop within an otherwise healthy graft. The exchange of cells between the host and the graft has been studied, but there are no strict rules of how stromal and endothelial cells carrying the genetic defect from the host will be distributed within the graft and cause a recurrence. A degeneration such as Salzmann's nodular degeneration can recur in a graft. Well known is also the recurrence of herpes simplex keratitis. Here prophylactic treatment postoperatively using oral acyclovir over long periods has proven beneficial. Corneal dystrophies with a distinct heredity, keratoconus with a presumed heredity, degenerations with no heredity, and infections like herpes simplex are examples of diseases that may recur in the graft.

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J. Hjortdal (ed.), *Corneal Transplantation*, DOI 10.1007/978-3-319-24052-7_9

Interpretation of the literature, especially of the corneal dystrophies, is complicated as scientific progress discloses so much new data. What started as one diagnosis later came to be many different mutations with varying phenotypes. We are presently in the midst of the molecular genetic revolution, and things may change further. It appears more important, in future studies, to carefully characterize both genotype and phenotype in the inherited diseases.

Keywords
Cornea • Corneal transplantation • Dystrophy • Degeneration • Herpes simplex keratitis • Recurrence • Reoperation

Corneal Dystrophies

Corneal dystrophy constitutes a fraction of the eyes undergoing corneal transplantation. Fuchs' dystrophy is most of the time presented as a separate entity and other dystrophies, mainly the stromal as another.

Fuchs' dystrophy constitutes between 0.5 and 27.9 % of grafted corneas. Generally the numbers varies between 7.8 and 21.2 % [1–20].

In several materials, but not all, the fraction of Fuchs' dystrophy is increasing [9, 10, 17].

Stromal dystrophy is a smaller group, sometimes so small it is not given a separate headline. The proportion of eyes grafted, for what is often referred to as stromal dystrophies, constitute about 2–5 % (range from 0.5 to 5.9 %) [4, 5, 9, 13–22].

Much is changing in the context of corneal dystrophies: A new classification of corneal system, the lC3D classification [23] based on genetic clinical and pathologic information, has been introduced. New genetic discoveries question old knowledge. The advent of excimer laser surgery has prevented or postponed corneal grafting for the dystrophies with mainly superficial opacification and erosive events.

The technique of corneal grafting offers new possibilities to exchange only the anterior part or the posterior part of the cornea. The latter technique, exchange of a posterior lamella, may be responsible for the increasing number of Fuchs' dystrophies grafted due to better results and a keen interest in the new technique.

It can be assumed that host cells repopulate the corneal graft and bring with them the genetic defect. It has been known for long that the epithelium is completely exchanged on the graft within the first year. Before the tissue bank era, eyes obtained at the morgue were brought to the operating theater, where the surgeon, to prevent infection, scraped the epithelium away before trephining the donor cornea. The host epithelium rapidly covered the defect.

Using the FISH technique, to differentially stain X and Y chromosomes, the donor and the recipient's cells could be traced provided there was a gender mismatch between the donor and the recipient [24]. Complete replacement of donor epithelium and endothelium was found in the 14 grafts examined. Donor keratocytes were found in only 3 out of the 14 graft after a mean follow-up of 4.5 years.

In two later studies of a larger material, gender mismatched grafts were analyzed with the FISH staining for X and Y chromosomes (52 and 36 excised corneal buttons, respectively). The follow-up time varied from 3 to 360 months [25, 26].

No donor-derived epithelial cells were found. Donor-derived stromal keratocytes were found in all corneal buttons in a proportion of 4–95 %. The numbers did not correlate to age.

The endothelium was analyzed in 35 corneal buttons. In 9 of those the donor endothelium was completely replaced by recipient cells. In 24 cases there was a mix of cells found, and in two corneal buttons, only donor cells could be

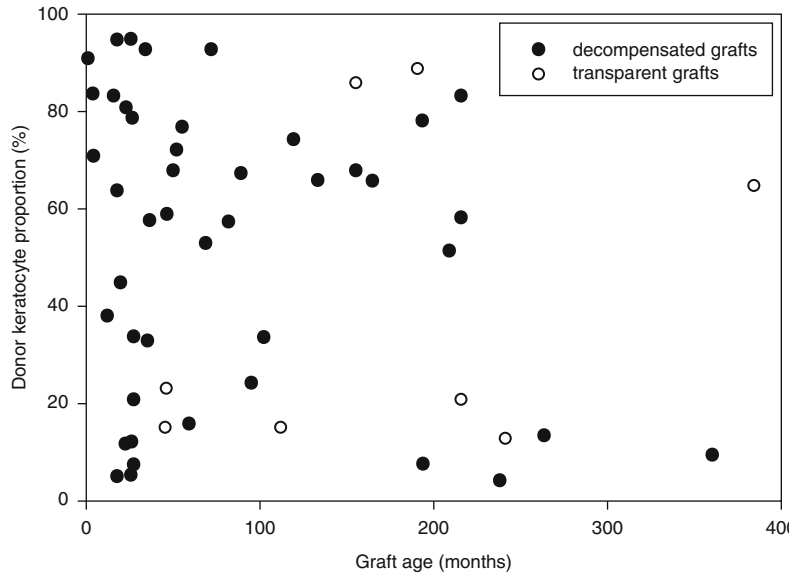

Fig. 9.1 Donor keratocytes as a proportion of total keratocytes counted in each of 52 corneal grafts, plotted against graft age. Subset of eight transparent grafts removed due to reasons other than endothelial decompensation. Donor keratocyte survival did not correlate with graft age (Reproduced from Lagali et al. [25]. doi:10.1167/iovs.08-2923. Epub 2009 Jan 17)

identified. In a two-dimensional study of the endothelium in the 36 corneal buttons, the distribution showed a surprising variability in the pattern and extent of the donor and recipient cell population, indicating a dynamic nature of the endothelium to a not expected degree (Figs. 9.1 and 9.2).

Interestingly, the mix of donor and recipient cells may be responsible for an altered phenotype of the recurrence of corneal dystrophies [27].

Recurrence Following Corneal Grafting for Corneal Dystrophies

In a classic review (1978), Waring 3rd et al. [28, 29] describe the corneal dystrophies and their tendency to recur after PKP. Due to advances in molecular genetics, the focus here has been placed on the more recent literature.

The Bowman layer dystrophies, the Reis-Bücklers and the Thiel-Behnke and the stromal dystrophies, the granular types, and the lattice types of dystrophies are subjected to PKP (penetrating keratoplasty) or DALK (deep anterior lamellar keratoplasty). The more superficial dystrophies, the epithelial, are generally treated with phototherapeutic keratectomy or by other means.

Modern molecular genetics have enabled the subdivision of the dystrophies based on gene location and mutation analysis. Several dystrophies emanate from different mutations in the same gene. Therefore, a more precise prognosis can be decided only on the basis of type of mutation in the individual [30].

In the literature, a recurrence of the inherited disease is either classified as signs of the original disease in the graft or as an actual reoperation caused by the recurrence.

With few exceptions, Bowman layer corneal dystrophies are the most anterior changes that are subjected to corneal grafting. Both Reis-Bücklers' (CDB1) and Thiel-Behnke (CDB ll) dystrophies are mutations in the BIGH 3 gene, and there are indications that the epithelium may be responsible for the pathologic changes. Both (there may be several subtypes) can recur in the graft.

In a study of 73 patients (110 eyes) grafted for corneal dystrophies originating in the BIGH 3 gene, 17 patients (27 eyes) were grafted for Reis-Bücklers' (R 124 L) and 8 patients (13 eyes) from Thiel-Behnke dystrophy (R555Q). Of the Reis-Bücklers' cases, significant recurrence was found in 24 of the 27 operated eyes within a mean time of 5.9 years. In the Thiel-Behnke eyes, dystrophic changes recurred in the graft in

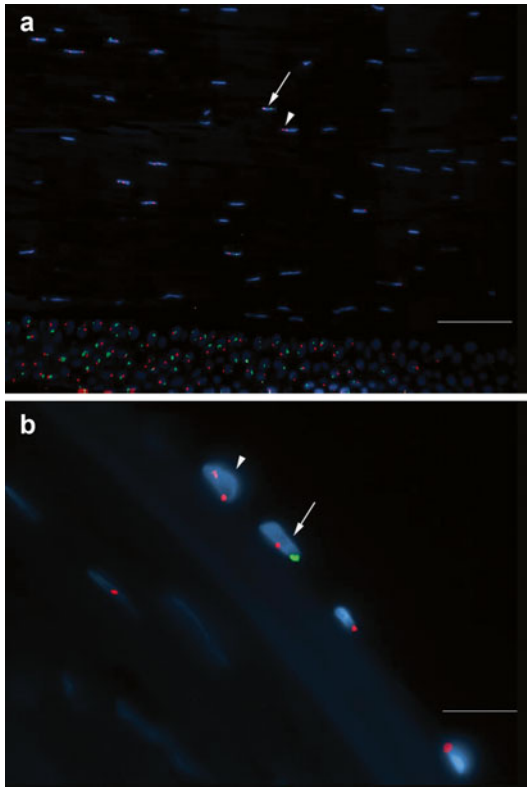

Fig. 9.2 Fluorescence microscope images used for FISH analysis of corneal sections. (**a**) Epithelial (*bottom*) and stromal (*top*) cells in a female donor corneal button removed from a male recipient. All epithelial cells with two distinct signals had one red and one green signal per cell. Keratocytes had either one red and one green signal (*arrow*) or two red signals (*arrowhead*) per cell. (**b**) Endothelial cells at the posterior surface of a male donor corneal button removed from a female recipient. Endothelial cells with one red and one green signal (*arrow*) or two red signals (*arrowhead*) per cell were observed bar: (**a**) 50 m, 20 objective; (**b**) 10 m; 100 objective. frequently observed adjacent to one another in the central cornea. Bar, 50 m (Reproduced from Lagali et al. [25]. doi:10.1167/iovs.08-2923. Epub 2009 Jan 17)

5 of the 13 operated eyes within a mean time of 15 years.

Three other BIGH 3-derived corneal dystrophies showed significant recurrences:

In classic granular dystrophy (C6CD1RSSSW), 13 out of 28 eyes showed significant recurrence after 10 years. Twenty eyes were grafted for lattice type 1 dystrophy (LCDI/R124C). Ten of these showed significant recurrence within 11.3 years. Diagnosis using mutation analysis creates small subgroups that can show more frequent and rapid recurrences compared to the main dystrophy type [30].

Of 61 PKPs in 39 patients with lattice type 1 dystrophy, 48 % of the grafts showed clinical signs of recurrence within 3–26 years. Subepithelial opacities and anterior stromal haze were the most common findings. Only 1 eye presented with lattice figures. Fifteen percent of the eyes needed regrafting [31] (Fig. 9.3).

In 54 PKP-operated eyes (37 patients) due to stromal dystrophies with a long follow-up (lattice dystrophy 21 eyes, granular dystrophy 19 eyes, and macular dystrophy 14 eyes), recurrences appeared earlier in younger patients. Macular dystrophy has a better prognosis than granular and lattice type I dystrophy in terms of recurrences. Seven out of the 21 eyes with lattice had recurrences, and fourteen out 19 eyes with granular had recurrences [32].

In a large material from England, the frequency of diagnosis (dystrophies) out of all together 3555 grafts, 1452 had to be reoperated. One hundred and one of the repeat surgeries or

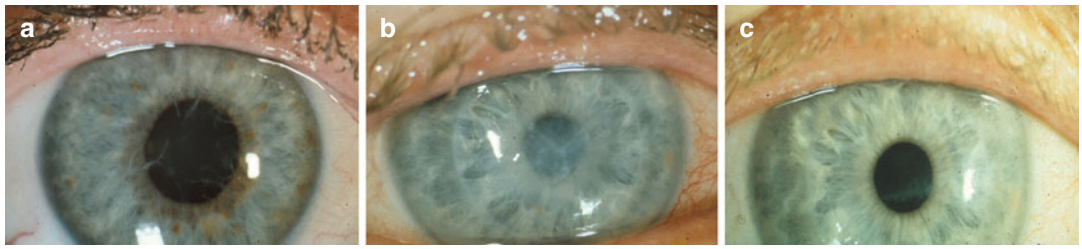

Fig. 9.3 Classic lattice dystrophy (**a**). Recurrence of lattice changes in the form of superficial opacifications without lattice lines (**b**). The superficial changes can successfully be removed with phototherapeutic keratectomy (**c**)

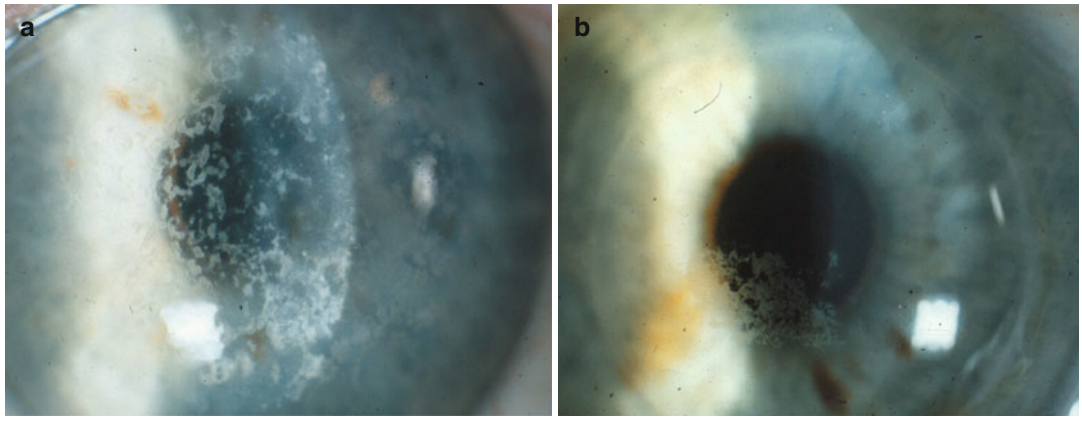

Fig. 9.4 A 56-year-old male with macular dystrophy in the right eye (**a**) and recurrence of the changes 6 years following a penetrating corneal graft (**b**)

7.2 % were due to recurrence of corneal dystrophies [33].

DALK has been evaluated as an alternative to PKP in lattice dystrophy l, and the outcome was compared to DALK in macular dystrophy [34]. Sixty eyes with lattice dystrophy and 24 eyes with macular dystrophy were operated on with a DALK technique. It was concluded that DALK was a favorable technique for lattice dystrophy, whereas for macular dystrophy, the DALK was not as good [34]. Endothelial density deteriorated faster in macular dystrophy. DALK has also been evaluated for granular dystrophy. Recurrences were common. Simple recurrence occurred in 5 out of 7 eyes (mean time to recurrence was 15.6 months). Clinical significant recurrence occurred 34 month after surgery in 3 out of 7 eyes. Two eyes showed no recurrence [35].

In another more recent material where DALK was performed in 9 eyes with granular dystrophy, 2 eyes showed a recurrence (22 %), one of which as early as after 14 months. The mean follow-up time was 43.5 months.

In the same material 1 out of 43 grafts with macular dystrophy (2.3 %) and 6 eyes with lattice dystrophy (35.3 %) recurred [36].

Macular Dystrophy

Opacities from macular corneal dystrophy recur less commonly than in lattice and granular dystrophies [28]. There is however a considerable variation in the literature.

In a cohort of patients operated on in Saudi Arabia with PKP for macular dystrophy (229 eyes in 141 patients) followed for a mean of 5.9 years, clinical significant recurrence was observed in 5.2 % of the grafts [37] (Fig. 9.4).

In two recent reports, a comparison of PKP and DALK for macular corneal dystrophy was analyzed.

The highest recurrence figures were reported in a retrospective study where PKP was performed in 57 eyes and DALK in 21 eyes. Mean follow-up time was 5.1 years. Seventeen percent of PKP-treated eyes showed recurrences and so did 42.9 % of the DALK eyes. The younger the onset of the disease and the younger at surgery, the higher the risk of recurrences [38].

In a randomized trial [31] (54 patients an 82 eyes), recurrence of disease (follow-up 30.5 months) was 4.8 % in the PKP group and 5.7 % in the DALK group. There was less endothelial damage in the DALK group [39].

In a British material, 16 patients who underwent 41 transplants in 31 eyes were followed between 25 and 408 months from the initial diagnosis. Six eyes of four patients were regrafted after recurrence of the disease. Clinical recurrence was observed in two more eyes. It was furthermore assessed that a larger graft showed less recurrence [40].

In a report from Iran 2009, 62 eyes of 39 patients underwent PKP for macular dystrophy and were followed for a mean of 52 months. Only one eye showed a minor recurrence [41].

The same low recurrence figures have been reported from Iceland where macular dystrophy is the most frequent indication for PKP. Both MCD types I and II exist. In none of the grafted patients has signs of recurrences been documented [42, 43].

Schnyder Dystrophy

In a retrospective case series of 115 individuals from 34 families with Schnyder dystrophy, it was found that the crystal component of the corneal changes was observed only in 54 % of the affected individuals.

PKP had been performed in 54 % of patients when older than 50 years and in 77% when older than 70 years. In 8 of 39 eyes that underwent PKP for Schnyder dystrophy, the disease recurred in the graft. There was no repeat surgery for dystrophy recurrence [44, 45].

In 77 eyes (48 patients) with stromal dystrophies, recurrence was most common in Bowman layer corneal dystrophy, Reis-Bücklers, and followed by granular and lattice dystrophies. Both macular dystrophy and Schnyder dystrophy were infrequent, and within the comparatively short follow-up (2–3 years), none had recurrences [46].

Avellino Dystrophy

Granular corneal dystrophy type 2 or Avellino dystrophy is caused by a mutation in the T6FB T I. Several reports exist on a reactive recurrence following PTK, LASIK, and LASEK. Early recurrence was noted after PTK and LASEK in a homozygous individual [47–49].

Holland et al. examined 27 family members with the disease. Of these, 3 had been grafted previously and two of those showed granular deposits in the grafts. The earliest changes were 9 year post-op. The granular deposits also precede lattice lines in the natural development of the disease [50].

Repeat Grafts

In one material, between 1990 and 1999, 1096 procedures were performed; 784 patient records were available for evaluation. Regrafting was the most common indication, accounting for 40.9 % of all cases [1].

Between 1989 and 1995, 16 % (271 of 1689) of transplants performed in Wills Eye Hospital were regrafts compared with 9 % (165 of 1860) in the period from 1983 to 1988 ($P < 0.01$) [51].

Of 243 repeat PKP performed in 210 eyes of 208 patients were included in the study. 5.7 % of the repeats were stromal dystrophies (constituting 4.9 % of the cases to begin with). Follow-up was 43 months. The best graft survival was in eyes with an original diagnosis of keratoconus (93.8 %), and the worst was in eyes with Fuchs' dystrophy (23.1 %). Overall, 29.6 % of eyes achieved a final visual acuity greater than 20/200, while only 4.8 % were 20/40 or better. The best visual prognosis was in eyes with an original diagnosis of stromal dystrophy and keratoconus [52].

150 repeat grafts at the Wills Eye Hospital in 1985–1995 were reviewed. Fuchs' dystrophy constituted 21 out of the 150 (14 %). Corneal dystrophies constituted 1.7 % of the original indications [53].

Fuchs' Dystrophy

Damage to the corneal endothelium or disease in the endothelium results in stromal edema and subsequently epithelial edema and then bullous keratopathy.

By definition, a corneal dystrophy is inherited. Spontaneous cornea guttata, followed by stromal edema and bullous keratopathy, is common, whereas true hereditary bullous keratopathy, or Fuchs' dystrophy, is fairly uncommon. In one US study 13.6 % had a documented family history [54]. If the many spontaneous cases are caused by gene defects remains to be shown. The present knowledge on the basics of "Fuchs'" dystrophy has recently been reviewed [55].

In most materials corneal edema is subdivided into two groups, postsurgical pseudophakic or aphakic bullous keratopathy and Fuchs' dystrophy. The latter group contains both hereditary and nonhereditary corneal edema. "Fuchs' dystrophy" constitutes a substantial part of the indications for corneal transplantation. The figures range between 0.5 and 23.8 %.

In two German materials and one US material, the indication for Fuchs' dystrophy increases [9, 10, 17] and diminishes in one over time [5].

Fuchs' dystrophy including what is known about the complicated heredity when present has been recently reviewed. There are at least two subforms, one with early onset starting around 30 years of age and one form with a late onset manifesting at about 60 years. Further genetic analysis is needed to clarify the varying aspects of disease [56]. It is an open question whether "Fuchs' dystrophy" recurs in the graft in the form of cornea guttata. New knowledge of the dynamics of the endothelium in the graft and host makes genuine recurrence possible [26]. The indication for regraft is generally corneal decompensation.

In a large material of PKP in 3993 eyes, Fuchs' dystrophy constituted 25 % of the primary surgeries. Graft survival after 5 years was 97 and 90 % after 10 years compared to keratoconus, 97 to 92 % [57].

Repeat penetrating grafts in Fuchs' patients constituted about 15 % of all repeat grafts. Fuchs' constituted 15 % of the indication for the first graft. The average time to the repeat grafts was 6.3 years. 7.7 % of the repeat grafts failed. The two main reasons were endothelial failure in eight out of the 21 failed regrafts and rejection in another eight 8 [53].

Fuchs' dystrophy constituted 3.9 % of indications in a large English material of 3555 cases. The group Fuchs' dystrophy constitutes 140 of the 329 corneal dystrophies undergoing PKP in 1971–1990 [33]. Repeat PKP for Fuchs' dystrophy showed a survival rate of 23.1 % after 43 months which is very low compared with eyes with the original diagnosis of keratoconus, 93.8 % [52].

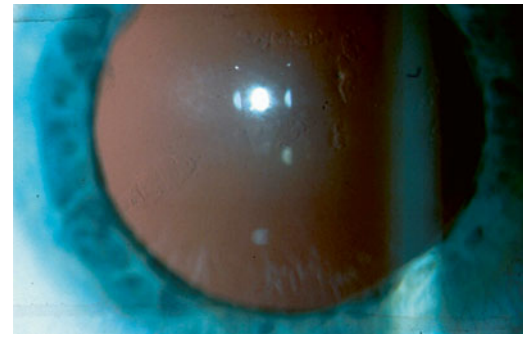

Fig. 9.5 Posterior polymorphous dystrophy in a 27-year-old female. Best spectacle corrected visual acuity was 0.3, and topography showed an concomitant keratoconus

In the Swedish National Cornea Register, the failure rate of the first operation was 15 % within 2 years, compared to 34 % for the regrafts. There was a higher rate of postoperative complications (suture related, glaucoma, etc.) following the regraft, 58 % compared to 34 % following the first graft [58].

The prevalence of Fuchs varies in different countries. Fuchs is rare in countries like Japan, Saudi Arabia, China, and Singapore. In the United States the prevalence is about 4 % [55].

Posterior Polymorphous Cornea Dystrophy

There are very few reports of corneal grafts performed for posterior polymorphous dystrophy. Two cases underwent PKP because of posterior polymorphous dystrophy at the age of 25 and 33 years, respectively. Six months and 18 months post-op, signs of recurrence, faint haze, or a dull appearance at the level of the endothelium were noted. Both eyes had to be regrafted 7 and 9 years after the first surgery [51]. Waring III in his review from 1978 states that no recurrences had been documented in grafts from posterior polymorphous dystrophy [29] (Fig. 9.5). The largest material described consisted of 120 patients. 13 of those were grafted in altogether 22 eyes. 9 of the grafted eyes opacified during the follow up period [59].

DSEK and SMEK

DSEK and DMEK are becoming the major grafting techniques for treating endothelial disease or "Fuchs' dystrophy." The shift from PKP has been fairly rapid. Prospective randomized studies comparing the old and the new technique have not been published.

Result from the Australian Corneal Graft Registry published in 2014 with a large number of corneal grafts (13,920) makes it possible to compare 858 DALK, 2287 endokeratoplasties with PKPs. The main outcome measure was graft survival. It was concluded that graft survival was worse in both DALKs and endokeratoplasties compared to PKPs over the same time frame. They also state that an evidence for a learning curve is unconvincing [60].

In 2011, in a Cochrane Review [61], the authors concluded that there was no high-quality evidence that endokeratoplasty was superior to PKP. Further randomized controlled trials of visual and refractive outcome need to be performed. A similar conclusion, although stressing the force of numerous published case series, was drawn 2013 in a German review. The authors conclude that Descemet's membrane endothelial keratoplasty (DMEK) is advantageous over Descemet stripping endothelial keratoplasty (DSEK) which in turn has better results than PKP [62]. The value of data in larger numbers in national registers was discussed in detail in a recently published editorial. These data also reflect the level of quality of care that is reached in the medical community in general [63]. Of 396 DSEK procedures, 20 failed, 40 % due to primary endothelial failure, 40 % due to progressive endothelial failure, and 20 % due to endothelial rejection. Repeat DSEK was performed on average 13 months after the first operation. The follow-up from repeat surgery was 27 months. The visual acuity outcome was satisfactory [64].

In 44 eyes with macular corneal dystrophy, 18 eyes with lattice dystrophy, and 12 eyes with granular dystrophy, DALK was completed in 69 cases (94.6 %). The mean follow-up period was 43.5 ± 23.9 months. Postoperative best spectacle-corrected visual acuity of 0.5 or better was present in 52 of 69 eyes (75.4 %). Lattice dystrophy recurred in 6 eyes (35.3 %) [36].

Keratoconus

Three indications for PKP are leading: pseudophakic bullous keratopathy, keratoconus, and regrafts. Regrafts have increased in the last decades, whereas keratoconus has declined in importance somewhat.

The keratoconus proportion of indications for corneal grafting is 2.5 % in Taiwan [11], 13 % China [16], 5.7 % China [19], 15.5 % Canada [14], 25.5 % Germany [9], 45.6 % N Zealand (1991–1999) [13], 26 % Greece [7], 12.1 % France [3], 16 % United States [8], and Great Britain 15 % [1].

In the Australian Corneal Graft Registry, survival rates of penetrating grafts after keratoconus were 89 % after 10 years, 49 % after 20 years, and 17 % after 23 years. After 15 years the survival rate was similar to that of all other penetrating grafts. Recurrent keratoconus caused failure in 4 % of the 4834 keratoconus grafts. In grafts surviving 15 years or more ($n = 235$), recurrent keratoconus constituted 12 % of the graft failures [65]. In a study of 112 eyes that underwent PKP for keratoconus, the rate of recurrence after 25 years was 11.7 % [66]. In another study, the probability of 20 years after surgery to suffer recurrence of keratoconus was 10 % [67]. The same study concluded that the greatest risk of rejection was during the first two postoperative years.

Recurrence of keratoconus is usually a late complication [68–71], in the majority of cases due to a progressive disease in the host cornea, being more accentuated if the original graft is small. Progressive, late development of astigmatism is a probable forerunner of recurrent keratoconus in the graft [71, 72].

The morphology of ectatic grafts is similar to that of keratoconic corneas [68–71, 73]. There is also a risk, although minimal, of grafting corneas from donors with keratoconus [74, 75].

Dalk has been used as an alternative to PKP in grafting for keratoconus. The experience of

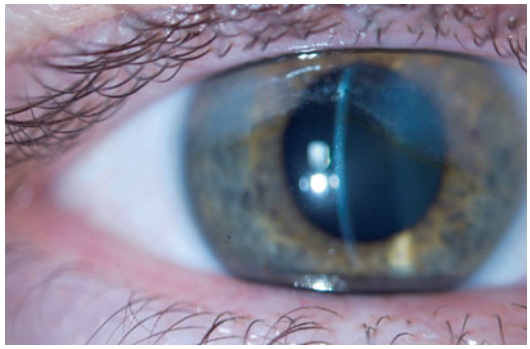

Fig. 9.6 Salzmann's nodular degeneration in a 47-year-old female. Iron lines are typical bordering the keloidlike changes

postoperative complications is not long, but the time to recurrent ectasia may be shorter [76, 77].

In corneal grafts (PKP) for keratoconus, the age of primary surgery was 33 years. Ectasia developed 22 years later on average. Two out 15 regrafts developed ectasia again [78].

Keratoconus can be associated with other corneal dystrophies as macula dystrophy [79–81], granular dystrophy [82], posterior polymorph dystrophy (Fagerholm P 2002), and lattice dystrophy (Fagerholm P 2005). If corrected visual acuity is worse than expected, concomitant keratoconus can be an explanation.

Salzmann's Nodular Degeneration

Salzmann's nodular degeneration is bilateral in 63 % of the affected and affects foremost women (89 %). The progression of the disease is accompanied by hyperopization and increasing astigmatism [83]. Decreased visual acuity is the most common symptom [84]. The disease is accompanied by meibomian gland dysfunction in 33 % of the cases, peripheral vascularization in 31 %, associated contact lens wear in 33 %, pterygium in 16 %, keratoconjunctivitis sicca in 10 %, and exposure keratitis in 4 % (Fig. 9.6).

These data spring from a cohort of 93 cases with the disease [83]. Recurrent erosions are not uncommon [85]. Impaired vision necessitated surgery in 85.5 %. In 79 % vision improved [83]. Most patients are operated on with manual keratectomy [86]. PTK has been evaluated [87–89]. Mitomycin has been added to both the manual and laser procedures with the motivation to minimize recurrences [86, 87, 89]. PKP is sometime employed, more so before the advent to mitomycin C and PTK. None the less, Salzmann's nodular degeneration can recur after PKP [90] but often after a longer time span and after lamellar keratoplasty [91].

Herpes Simplex Keratitis

Herpes simplex keratitis has been a constant but limited indication for corneal grafting in most materials. Opacities due to herpes simplex keratitis (HSK) have been more common as an indication in developing countries [22, 92, 93]. In statistics spanning over a longer period, the indication for PKP for herpes keratitis is diminishing [7, 21, 94]. In several materials the proportion of HSK cases that has been grafted constitute somewhere between 1 and 7.3 % [1, 3, 7, 18, 21, 84, 95].

It was shown that an increased virus load in the excised corneas made the prognosis worse for the graft. So did preoperative steroid treatment, severity at the time of surgery, and corneal neovascularization [96, 97]. The increased risk of allograft failure was also determined retrospectively from the histopathologic presence of corneal vessels [98]. The authors also found that vessel in the cornea increased the risk of recurrences. The latter conclusion contradicts the findings from a previous study [99] where no increased risk of recurrences from vessels present in the stroma was found.

In a Danish material from 1995 with 72 penetrating grafts for HSK without antiviral therapy, the recurrence rate after 2 years was 44 %. The 2-year survival rate of the grafts was 67 % [100].

In a retrospective study published the same year, the recurrence-free survival rate after 4 years was 51 %. Recurrence of HSV infection occurred in 18 of the 49 eyes. In 50 % of grafts with recurrences, the result was an opacified graft [101].

In a study from Germany (1993), the survival rate for 11 years after PKP for HSK was 68 %.

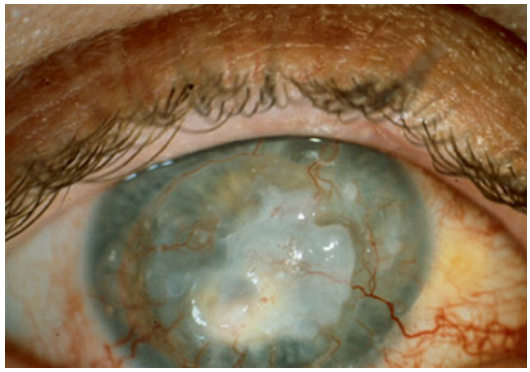

Fig. 9.7 Herpes simplex keratitis grafted á chaud in the era before antiviral therapy. Postoperative inflammation was intensive and the scaring produced a prominent keloid

Corneal inflammation at the time of surgery was found to be a negative prognostic factor [99] (Fig. 9.7).

In two other series, rejection was the principal cause of graft failure in 64 % and 46 %, respectively, whereas viral recurrence caused failure in 15 and 16 %, respectively [102, 103]. Graft survival after 2 years was 67 and 66 % [100, 102]. In the Australian Corneal Graft Registry, if recurrence-free, the grafts survive in 83 %. The report concludes that viral recurrence has a major impact on graft survival [104].

Graft survival following PKP has improved since prophylactic treatment with acyclovir was introduced.

In a study from 1994, the use of topical antivirals to reduce the risk of viral recurrence and graft rejection was compared to a group of patients given no prophylactic treatment [105]. Sixty-six (52 %) of the grafts received prophylactic postoperative topical antiviral treatment, and 59 (46 %) received no antiviral therapy. Postoperative prophylactic antiviral treatment was associated with decreased rates of herpes simplex keratitis recurrence and allograft rejection. In a prospective trial with oral acyclovir, the advantage of prophylaxis was evident [106]. In the small material, there were no recurrences of herpes simplex keratitis in any patient receiving acyclovir (mean follow-up of 16.5 months) compared with a 44 % (four of nine) recurrence rate in patients without acyclovir (mean follow-up of 20.6 months). Graft failure occurred in 14 % (2 of 14) of acyclovir treatment eyes compared with 56 % (five of nine) without.

Deep anterior lamellar keratoplasty has been compared with PKP when grafting for HSK opacities [107]. Fifty-eight eyes in 58 patients were operated on with DALK and 63 eyes in 63 patients with PKP. The follow-up time was about 46 months in both groups. There were no rejections in the DALK group, whereas 26 eyes (41.3 %) suffered rejection in the PKP group. There were 21 episodes of recurrence in the PKP group compared to 7 in the DALK group. Fourteen eyes failed in the PKP group compared to 1 in the DALK group [107]. The patient's received oral acyclovir for 12–18 months post-op.

A low graft rejection rate, 2.3 %, was reported after DALK in 44 patients given intravenous acyclovir and amniotic membrane prior to DALK. The follow-up time was 29.1 months (range 1–4 years). Fourteen percent developed recurrent HSK [108].

In another series of 52 eyes in 52 patients, DALK was performed and the patients were followed for a mean of 31 months. Both acyclovir and topical steroids were given for 1 year after surgery. No rejections or recurrences were noted [109].

On the other hand, a high percentage of postoperative complications were observed following DALK in another material of 18 patients. Six patients, or 33 %, experienced recurrence of HSK, 50 % experienced an episode of graft rejection, and 28 % (five cases) suffered graft failure. The patients were given oral acyclovir and topical dexamethasone for 1-year post-op [110]. A good effect of acyclovir prophylaxis was confirmed in a 5-year follow-up, placebo-controlled, randomized trial on acyclovir prophylaxis after keratoplasty [111].

Prophylaxis

In his commentary from 1998, Larkin concludes that oral antiviral prophylaxis for 1 year following

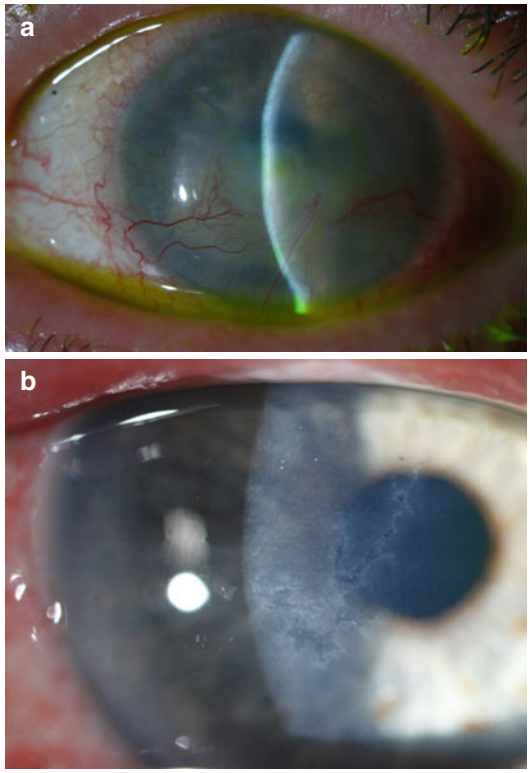

Fig. 9.8 Herpetic keratitis in the right eye of a woman with atopic dermatitis and keratoconjunctivitis (**a**). The sequel of a previous attack of herpes simplex keratitis is seen in the left eye (**b**)

PKP for HSK, accompanied by topical steroids, is a reasonable strategy [112].

This is confirmed in subsequent studies [113–117]. Oral antiviral therapy and postoperative steroids, furthermore, help in preventing relapse of neovascularization [118].

Herpes simplex virus keratitis is reported to be more common in patients with atopic disease [119, 120] (Fig. 9.8).

When comparing the outcome of PKP for HSK, it was shown that nonatopics had significantly more epithelial recurrences, although in general the incidences of recurrences were low and high graft survival numbers were reported [119]. Antiviral prophylaxis for HSV recurrences was proven more effective in reducing infections in atopics and less effective in reducing inflammatory episodes in atopics versus nonatopics. A recurrence of herpetic disease following PKP for HSK is likely to originate from the host, either via nerves from the trigeminal ganglion or from latency in the remaining host epithelium [121].

HSK can also originate from a de novo infection [122, 123]. The post-PKP eye is sensitive to infection due to microtrauma associated with sutures as well as immune superior with topical steroids [122, 124].

Although difficult to prove, herpes simplex virus can be transmitted from a donor cornea to a host [124–127]. Herpes simplex virus has also been blamed for primary graft failure after PKP [97, 128–131] and after DSAEK [132].

Adenovirus can contribute to epithelial defects following PKP for HSK [133]. CMV can cause endothelial disease and be mistaken for other reasons for corneal decompensation. Following DSEK, the endothelial disease can recur sometimes accompanied with retinitis. CMV can be treated if correctly diagnosed with valganciclovir, making a successful surgery possible [134].

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National Corneal Transplant Registries

10

W. John Armitage and Margareta Claesson

Abstract

National corneal transplant registries collect and analyze observational, longitudinal data and report outcomes on large numbers of patients across multiple transplant centres. Registry data are valuable for monitoring activity and outcomes, including rare events such as primary graft failure, and for showing the uptake of new surgical techniques. While randomized controlled trials (RCT) are considered to provide the highest level of evidence for comparative studies, the strict inclusion and exclusion criteria make generalization of the results and translation into routine practice at times uncertain. The greater heterogeneity of patient characteristics in registries provides a perhaps more realistic picture of expected outcomes. The same is true of carefully conducted single-centre case series, which can often provide benchmark data, but do not necessarily reflect the outcomes in routine practice in multiple centres. National registries provide an important source of information that contributes, along with RCTs, single-centre studies, expert opinion and meta-analyses, to a better understanding of corneal transplant outcomes.

Keywords

Corneal transplant registries • Corneal transplant outcomes • Corneal transplantation • Transplant outcomes • Registry data

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National corneal transplant registries, such as those in Australia [25], the United Kingdom [12] and Sweden [6], collect and analyze observational, longitudinal data and report outcomes on large numbers of patients across multiple transplant centres (Fig. 10.1). Although this chapter will focus primarily on these three registries, we do not wish to give the impression that these are the only, or indeed the only worthwhile, corneal

J. Hjortdal (ed.), *Corneal Transplantation*, DOI 10.1007/978-3-319-24052-7_10

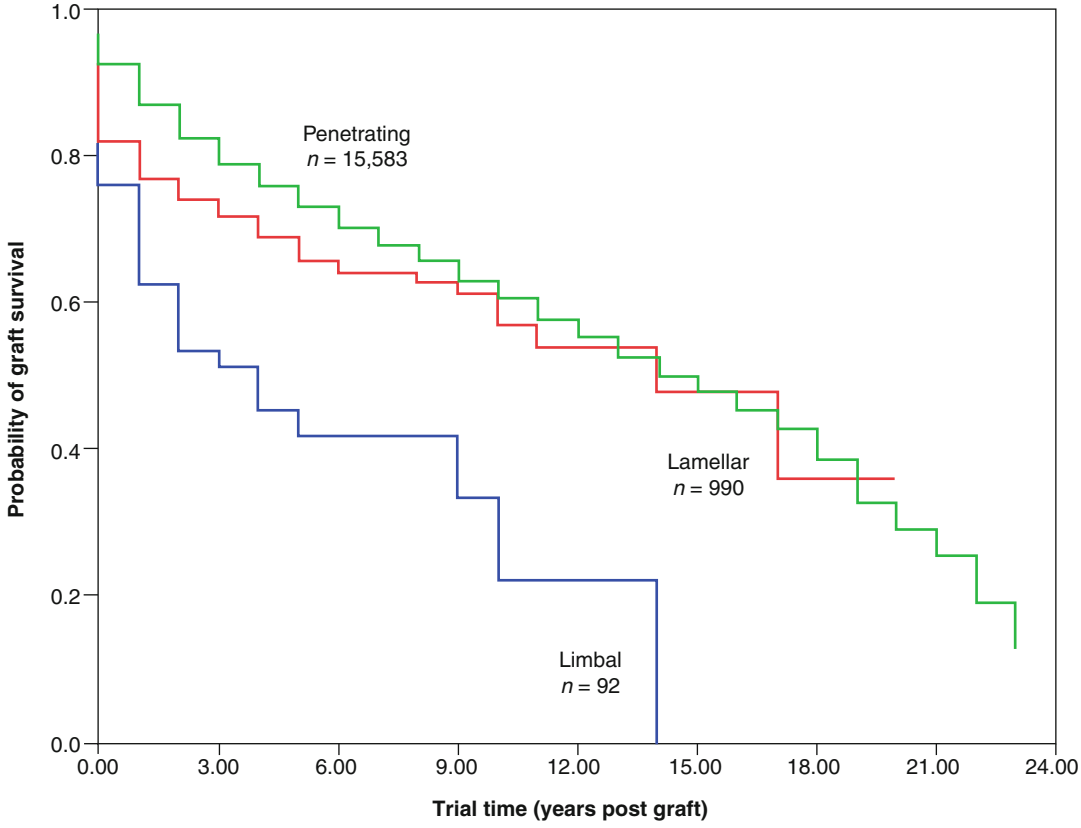

Fig. 10.1 An example of national registry data from the Australian Corneal Graft Registry (http://hdl.handle.net/2328/25860). Long-term survival of penetrating keratoplasty, lamellar keratoplasty and limbal allografts (Reproduced with permission of the Australian Corneal Graft Registry)

transplant registries: they are, however, either the most widely regarded (i.e. the Australian Corneal Graft Registry) or are the best known to the authors (i.e. the UK Transplant Registry and Swedish Cornea Registry). At their best, single-centre registries and case series provide valuable, often benchmark, data that have been rigorously collected by pioneering clinics at the forefront of new developments in surgical techniques and practice [11, 19, 22] (Fig. 10.2); however, given the large numbers of factors that influence corneal transplant survival and visual outcome, the value of some single-centre reports may be limited owing to small numbers of transplants and, as a consequence, unintended selection bias. The ultimate aim of data collection and analysis, whether through registries or single-centre case series, is to better inform surgeons about outcomes, which, in turn, will improve patient selection and postoperative management and extend the quality of information provided by surgeons to their patients.

Setting Up a Registry

Registry design, extent and mode of operation will be influenced by multiple factors ranging from individual surgeon preferences through to the availability of funding and appropriate infrastructure at local, regional and national levels. There is no 'best' way to organize a corneal transplant registry, which could be achieved through professional or academic organizations as well as with government support. Keys to success include: active surgeon involvement and

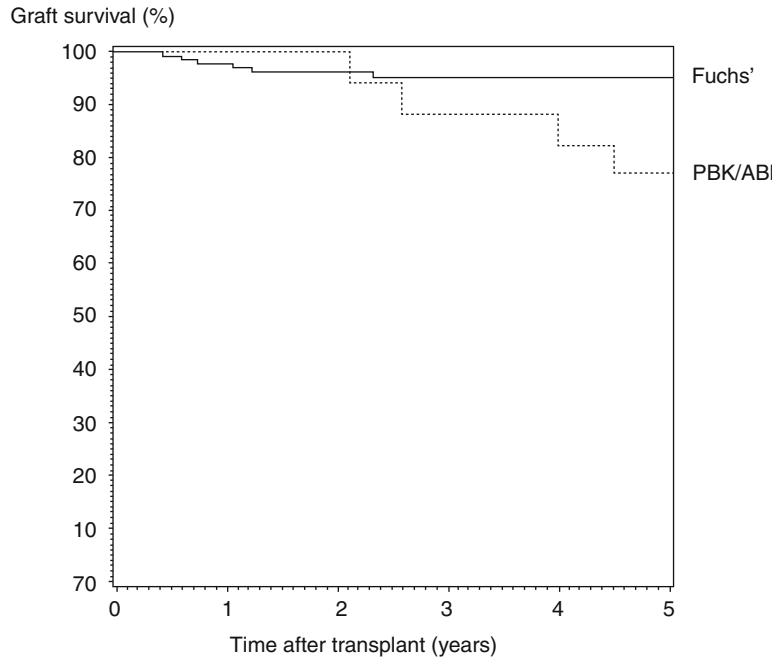

Fig. 10.2 An example of single-centre registry data from the Cornea Research Foundation of America (www.cornea.org) showing survival of endothelial keratoplasty for Fuchs endothelial dystrophy and pseudophakic/aphakic bullous keratopathy (Reproduced from Price et al. [19], with permission of Elsevier)

commitment to help define the clinical questions to be answered (which are likely to change with time) and to provide clinical data on their patients, expert statistical advice at all stages, staff to support and maintain the registry and an appropriate platform for the storage and analysis of clinical follow-up data. Other considerations will include the ethical and regulatory environments existing in different countries, for example, patient confidentiality, data protection and issues of consent for the storage of data for the greater good rather than being specifically linked to the treatment of an individual patient.

The three aforementioned national registries reflect these varying circumstances and, as a result, operate in different ways; for example, the Australian Corneal Graft Registry (ACGR) (http://hdl.handle.net/2328/25860), the oldest and largest of the three, which is based in an academic department, has been collecting data since May 1985 and can report very long-term survival data extending for more than 20 years [25]. The Swedish and UK registries both limit the follow-up of transplants to 2 and 5 years, respectively [2, 6]; however, longer-term follow-up studies are possible by collecting additional data on selected groups of patients rather than through continual collection of follow-up data on all patients without limit [4]. Not surprisingly, loss to follow-up, especially among the older patient population, becomes an increasingly important factor with extended follow-up times. Kaplan-Meier survival curves are used extensively for the analysis of graft survival [1]. Unfortunately, the right-hand side of Kaplan-Meier survival curves becomes increasingly less reliable at longer postoperative times as the numbers of transplants at risk declines through loss to follow-up and graft failures; moreover, the assumption that transplants lost to follow-up would have behaved in the same way as those available for examination becomes ever more uncertain.

In Sweden, there is substantial government support available for clinical registries across a range of clinical specialties. EyeNet Sweden (www.eyenetsweden.se), which was established in 2003, hosts the Swedish Cataract Registry [3] and the Swedish Cornea Registry (www.cornea.nu), which began collecting data in 1996. (NB The EyeNet website includes advice, in English, on setting up quality registers.) The Swedish registry collects data at two time points, viz., at the time of transplant and at 2 years postoperatively. In 2007, the Swedish Cornea

Registry abandoned paper-based data submission and implemented a web-based application through EyeNet with direct online data entry by each individual clinic. This greatly reduced the risk of transcription errors and improved the accuracy of the data held in the registry. The participating clinics, which include transplant units in Denmark and Norway, can access their own data on line and compare their outcomes and activity directly with national data.

The UK Transplant Registry is also supported by government funding. It is maintained by NHS Blood and Transplant (NHSBT) and holds national outcome data both for solid organ and corneal transplants. Data are collected at the time of surgery and then at 1, 2 and 5 years postoperatively using standardized follow-up forms; however, a move to online input of data is, at the time of writing, under discussion. This registry has the added benefit in that it includes donor and eye bank information for each cornea transplanted. As a result, it enables robust traceability between donors and recipients for the purposes of investigating serious adverse events and reactions in corneal transplant recipients, thus meeting the regulatory requirements set out in the EU Tissues and Cells Directive (2004/23/EC) and its accompanying Commission Directives (2006/17/EC and 2006/86/EC) (http://ec.europa.eu).

Uses of Data from Corneal Transplant Registries

Graft Survival

Because of their size, national registries provide a broader perspective than single-centre studies and can provide information not necessarily available through other means, for example, the routine monitoring and analysis of rare events and complications including primary graft failure and postoperative endophthalmitis. Registry data can be used to monitor transplant activity and outcomes, trends and patient demographics, which are not just of interest to surgeons but are important for informing the development of healthcare policies and resource planning. The data are an invaluable resource for clinical research, enabling large-scale studies that improve our understanding of the factors that influence corneal transplant outcomes. Indeed, registry data can help identify questions that would be best answered by controlled clinical trials and provide supporting information for their planning and design.

Table 10.1 Five-year graft (PK) survival by indication from the Australian Corneal Graft Registry (ACGR) and UK Transplant Registry (UKTR)

	5-year graft survival	
Indication	ACGR (%)	UKTR (%)
Keratoconus	95	93
Corneal dystrophy	82	80
Bullous keratopathy	56	59

Graft survivals reported in the registries are very similar (e.g. see Table 10.1 comparing Australian and UK data). All three registries have confirmed that the indication for transplantation, preoperative risk factors, such as vascularization and glaucoma, and postoperative complications, such as rejection, are the major factors influencing graft survival after penetrating keratoplasty (PK) [2, 6, 23, 25]. Registries also provide information relevant to the postoperative management of graft patients; for example, studies using UK Transplant Registry data have demonstrated that long-term topical steroid use reduces the risk of graft failure after PK for pseudophakic bullous keratopathy [20] and that oral antiviral treatment is more effective than topical treatment for reducing the risk of graft failure in patients undergoing PK for herpetic keratitis [10] (Fig. 10.3). The ACGR has also highlighted the negative impact of reversed rejection episodes on long-term graft survival: at 10 years, overall survival of transplants that have experienced no rejection is 68 % compared with just 35 % for those that have suffered one or more rejection episodes [8, 25].

Visual Outcome

The majority of corneal transplants are performed to improve a patient's vision. It is therefore of value to be able to use registry data to assess

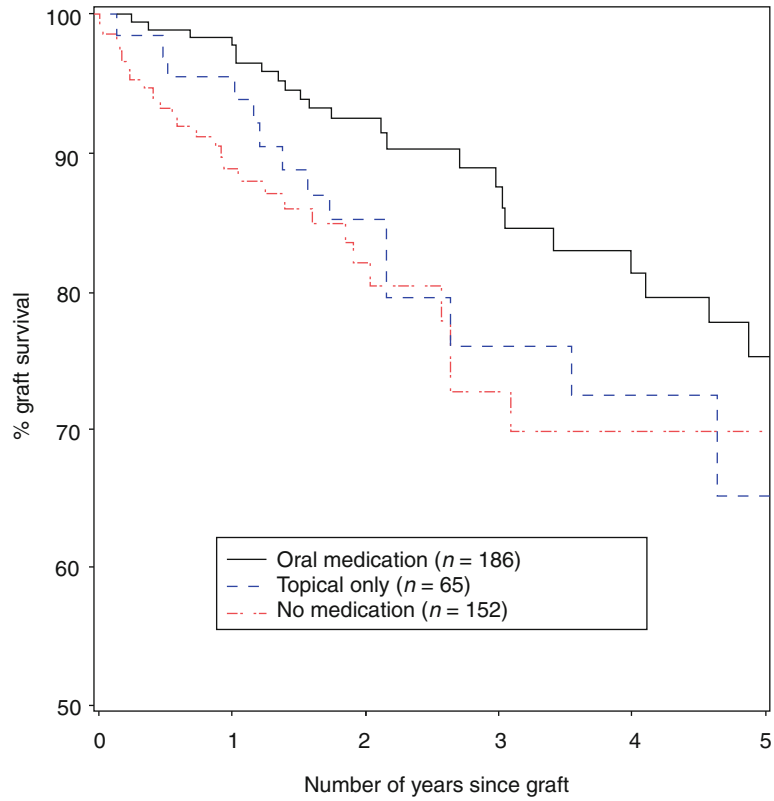

Fig. 10.3 UK Transplant Registry data showing the influence of oral vs. topical antiviral medication on 5-year graft survival (Reproduced from Goodfellow et al. [10], with permission of Nature Publishing Group)

outcomes in terms of vision rather than simply graft survival. The Swedish Cornea Transplant Registry was started in 1996 with the principal aim of reporting visual outcomes [6] (Fig. 10.4). These analyses have shown the dependence on indication of the expectations for postoperative visual rehabilitation, principally visual acuity (VA). While >80 % of patients had a preoperative VA ≤0.2 across all indications, almost 80 % of patients with grafts for keratoconus achieved a VA of ≥0.5 at 2 years after surgery compared with just over 50 % of grafts for Fuchs and only 20 % for bullous keratopathy. These results do not take into account the increasing occurrence of co-morbidity, such as retinal disease, in the older patients; however, this information is collected by the Swedish registry and can be included as a variable in analyses of visual outcome. Interestingly, for regrafts, the respective percentages of grafts achieving ≥0.5 VA for keratoconus and Fuchs were somewhat reduced at 55 and 19 %, respectively, compared with first grafts, while the outcome for bullous keratopathy regrafts was less affected but still reduced to about 10 % [5].

Patient-Reported Outcome Measures (PROM)

Registries and single-centre studies typically focus on clinical outcome measures (COM), such as graft survival, complications (e.g. rejection episodes) and visual outcome. There are, however, few studies of the impact of corneal transplantation on self-assessed, patient-reported outcome measures (PROM). These aim to determine improvement or otherwise in visual disability as perceived by transplant recipients. An early study from the ACGR looked at this important aspect of corneal transplant outcome [24], but little attention has since been paid to this area. The Swedish Cataract Register has conducted widespread studies on PROMs and compared

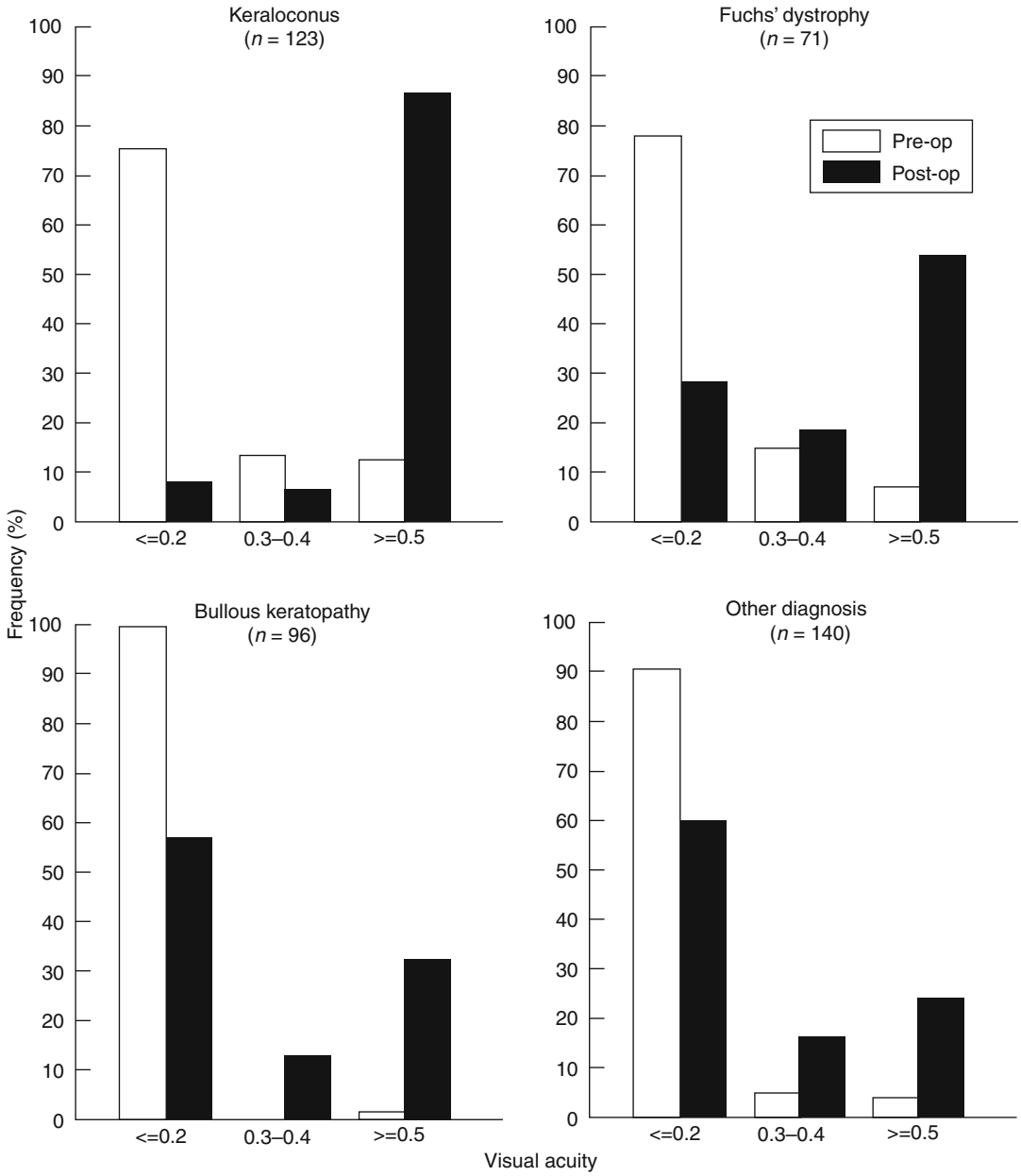

Fig. 10.4 Visual outcome data from the Swedish Corneal Transplant Registry showing percentages of grafts achieving VAs of ≤0.2, >0.2 and <0.5, and ≥0.5 preoperatively and at 2-year postoperative for keratoconus, Fuchs endothelial dystrophy, bullous keratopathy and 'other' indications (Reproduced from Claesson et al. [6], with permission of BMJ Publishing Group)

them to COMs [14]. These studies have used the Catquest 9-SF visual disability instrument, which consists of just 9 questions that patients complete before and after surgery [13] (Table 10.2). The answers are resolved by Rasch analysis into a single score of visual disability (high scores equate to greater disability), which are amenable to parametric statistical analysis. A study is currently underway in Sweden to complete the validation of Catquest 9-SF for corneal transplant

Table 10.2 Catquest 9-SF visual disability questionnaire

Difficulty items
Answers: No, no problems; Yes, some problems; Yes, great problems; Yes, very great problems; Cannot answer
1. Reading text in the newspaper
2. Recognizing faces of people you meet
3. Seeing prices of goods when shopping
4. Seeing to walk on uneven ground
5. Seeing to do needlework and handicraft
6. Reading text on TV
7. Seeing to carry out a preferred hobby
Global assessment items
Answers: Yes, very satisfied; Yes, fairly satisfied; No, rather dissatisfied; No, very dissatisfied; Cannot answer
8. Do you experience that your present vision gives you difficulties in any way in your daily life?
9. Are you satisfied or dissatisfied with your present vision?

Reproduced from Lundstrom and Pesudovs [13], with permission of Elsevier
Patients are asked a series of nine questions before and after surgery. The answers are resolved into a single Rasch score for statistical analysis

recipients and to apply this visual disability instrument to determine the factors that most influence PROMs as opposed to COMs. Patients will be asked to complete the questionnaire before surgery and at 2 years after surgery (Claesson M, personal communication, 2014).

New Surgical Techniques

Over the past few years, there has been a marked change in the surgical treatment of corneal disease with a move away from full-thickness grafts (penetrating keratoplasty, PK) to lamellar techniques that seek to replace only the dysfunctional part of the cornea, viz. deep anterior lamellar keratoplasty (DALK) for keratoconus and superficial corneal scars, and endothelial keratoplasty (EK) for endothelial dysfunction [15]. The evolution of these techniques has been pioneered by surgeons who have reported their outcomes, typically in single-centre case series, which show the benefit, especially of EK, in terms of graft survival, reduced risk of rejection and faster visual rehabilitation [11, 19]. Such studies show the potential that can be achieved with these newer techniques and provide a valuable benchmark for comparison, as well as a forum for sharing insightful advice. However, perhaps because national registry data report the outcomes across multiple centres and from patients with a broader case mix, the registry outcomes for DALK and EK do not reflect the excellent results reported from single-centre case series [7, 12]. This suggests that while many centres may well be achieving similar results to the single-centre reports, some will be falling short. This important information is crucial for the optimum translation of newer techniques into general, routine practice for the benefit of all patients and is an example where both single-centre reports and national registry data are needed for meaningful assessment.

The Value of Corneal Transplant Registries to Eye Banking

Eye bank standards for donor selection, including donor age, post-mortem times to corneal retrieval and preservation, preservation method, storage time and quality assessment based on endothelial cell density, are defined by eye banking organizations, such as the Eye Bank Association of America, the European Eye Bank Association, the Eye Bank Association of Australia and New Zealand and the Eye Bank Association of India, or left to the discretion of eye bank medical directors, usually a combination of both. When eye bank and donor information are included in corneal transplant registries, there is an opportunity to assess the influence of donor factors on both the suitability of corneas for transplantation and on graft survival [2, 25]. Large-scale analyses involving several thousand corneas and corneal transplants are not able necessarily to set standards, such as maximum acceptable death to preservation times or minimum endothelial cell density, but they can support and validate existing standards or suggest that standards should be raised. A study in the UK, for example, showed that the major factors affecting the suitability of corneas for PK (defined as a minimum endothelial

cell density of 2200 cells/mm^2) included donor age and storage time in organ culture while death to enucleation times up to 24 h had little influence on suitability. Provided corneas had an endothelial cell density of 2200 cells/mm^2, donor age (up to 90+ years) and storage time in organ culture (up to 4 weeks) had no influence on 5-year PK survival, which was dominated by recipient factors [2]. It is clear that there are differences in eye banking standards between different countries. Once a standard has been accepted, such as maximum donor age or post-mortem retrieval time, there is an understandable reluctance to introduce what may be considered to be a relaxation of a standard even though there may be evidence from registry data from other countries to support a change and where such a change may lead to an increase in availability of corneas for transplantation. The most rational way forward in this instance would be a prospective randomized trial, such as the Cornea Donor Study in the USA designed to determine whether corneas from older donors up to 75 years would be acceptable for PK [21]. However, as will be discussed later, randomized controlled trials require substantial organization and funding, and generalization of their results to reflect routine practice may not always be appropriate. Therefore, eye bank data recorded along with corneal transplant outcomes in registries are an additional important source of information for the validation of eye bank standards and practice.

Different Sources of Information Used for Decision Making

Evidence-based medicine is widely considered essential to the rational development of healthcare policies and clinical practice. In assessing the validity of the information/evidence provided by a study, the US Preventive Services Task Force methodology (www.uspreventiveservicestaskforce.org) uses a 'hierarchy of research design', which ranges from the strongest level of evidence, Level I, based on randomized controlled trials (RCT), down to Level III, which includes case reports, clinical practice and expert opinion. Registry data fall into Level II evidence, which includes well-designed trials without randomization and cohort or case-controlled studies.

The pre-eminence of RCTs is not universally accepted [18], and they do have a number of drawbacks and disadvantages. Randomized controlled trials can be expensive and difficult to design and implement, and techniques may undergo further development during the course of an RCT rendering its findings redundant [17]. Patient recruitment may be hindered through refusal of surgeons to randomize patients between the study groups because of ethical concerns or lack of resources. Another major problem concerns the wider application of findings from RCTs, which have strictly controlled inclusion and exclusion criteria, to routine practice where the patient population is far more heterogeneous, for example, greater diversity of disease severity at time or presentation/treatment and the presence of risk factors and co-morbidities, especially in older patients, which may be excluded from an RCT. There are, however, excellent examples of RCTs, such as the Cornea Donor Study in the USA, which has provided a wealth of information about the influence of donor age on PK outcome at 5 and 10 years after transplantation [9, 21, 27, 28]. This study was limited to PK and endothelial disease in moderate risk grafts without known risk factors for graft failure. It therefore leaves open the question of applicability to a wider range of indications including both low- and high-risk grafts. However, its conclusions are broadly supported by retrospective analyses of observational registry data, which show little influence of donor age on PK survival, provided corneas meet minimum criteria for endothelial cell density [2, 25]. The support is mutual, with the RCT affording credibility to the registry findings concerning donor age. However, direct comparisons and benchmarking studies between registries and RCTs and between studies in different countries need to be treated with caution as the definitions used for indications, risk factors and postoperative complications can and do vary.

Registries, as with other means of collecting data, do have limitations and are potentially vulnerable to errors stemming from inadequate data

accuracy and poor data return rates, causing selection bias. Data in registries are collected prospectively, and the usefulness of the information gathered depends on the willingness of centres to submit complete and accurate data with a high return rate; but this has to be done without necessarily having the motivation of a specific question to answer. Collection of high-quality data from large numbers of patients across many centres helps to correct bias from centres with poor return rates and/or inadequate data accuracy. A data collection initiative launched by the American Academy of Ophthalmology, which gathers data directly from electronic health records (www.aao.iris-registry), should also help address these concerns. Whereas RCTs and other well-controlled trials are designed with a specific question in mind, which in turn defines the clinical dataset required for the analysis, registries often lack such specific drivers for defining their datasets. They may, therefore, fail to seek specific relevant information that may have an important influence on the outcome measure of interest in a future analysis. However, the amount of data requested for each patient must take into account the time, effort and ability of surgeons to submit data: the amount of follow-up data requested needs to be balanced against the wider needs of clinics to treat new patients and minimize waiting times. It is partly for such reasons that the Swedish and UK registries have limited the follow-up periods to, respectively, 2 and 5 years. However, supplementary studies to gather additional information to answer specific questions can also be added to registries, for example, to investigate longer-term follow-up where the normal follow-up period is restricted [4]. Registries can also be used as a vehicle for collecting data for prospective controlled trials, such as the Corneal Transplant Follow-up Study II in the UK, which is investigating the influence of HLA class II matching in high-risk corneal transplants (Armitage WJ, personal communication, 2015).

While RCTs are the accepted gold standard for evaluating new techniques or interventions, they can be difficult to set up with adequate numbers of patients, and their results may not be generalizable outside the strictly defined inclusion/exclusion criteria. Registries provide a critical insight into how well outcomes from controlled trials and single-centre studies are reflected in routine practice [7, 18]. Moreover, data and analyses from registries can help to identify questions and guide the setting up of RCTs. When trying to answer questions such as, 'How well does this treatment work?' or, perhaps of greater importance, 'Has the patient benefitted from this treatment?', questions that are fundamental to the evaluation of new procedures and interventions intended to improve transplant outcomes, it is important for evidence to be gathered from as many sources as possible, including RCTs, registry data, well-controlled single-centre case series, case reports, expert opinion and meta-analyses such as the those sponsored by the Cochrane Eyes and Vision Group [16, 26]. It is clear, however, that national corneal transplant registries have an important role to play in the monitoring of outcomes in everyday practice across multiple transplant centres.

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Economic Evaluation of Keratoplasty

11

Isabelle Brunette, Catherine Beauchemin, and Jean Lachaine

Abstract

The economic evaluation of healthcare interventions is now a prerequisite in many jurisdictions. Adoption of new healthcare interventions cannot only be based on their efficacy and safety. In the context of limited healthcare resources we are facing, their economic impact should also be considered. To estimate the economic impact of health interventions, methods for economic evaluation have been developed and adopted. The main objective of these economic evaluations is to help the healthcare decision makers to select interventions that will support a better allocation of resources.

Alongside the development of different surgical techniques for corneal transplantation, economic evaluations have been performed. The new surgical procedures have improved the clinical performance of corneal transplantation, and in most cases these new interventions were shown to be cost-effective.

Only a few economic evaluations of corneal transplantation techniques have been performed in only a few different countries. Additional economic evaluations are needed to assess the economic impact of these interventions over many more contexts of use.

Keywords

Corneal transplantation • Economic evaluation • Cost-effectiveness analysis and cost-utility analysis

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J. Hjortdal (ed.), *Corneal Transplantation*, DOI 10.1007/978-3-319-24052-7_11

Nowadays, adoption of a health intervention not only depends on its efficacy and safety but also on its cost-effectiveness. In fact, given the relatively scarce healthcare resources, healthcare payers also include economic criteria in the decision about the adoption of new healthcare technologies. This is true for medications as it is for health technologies and medical and surgical procedures. The main objective in applying economic criteria in the decision process is to allow for a better allocation of healthcare resources. This explains the development of economic evaluations in all areas of health care, notably in ophthalmology and more specifically with respect to corneal transplantation.

Methods for Economic Evaluation (See Table 11.1)

An economic evaluation typically takes into account both the resources consumed by an intervention and the consequences of that intervention. The intervention of interest is also always compared to at least one alternative intervention. The following five methods can be considered when performing an economic evaluation: cost-consequence analysis, cost-minimization analysis, cost-effectiveness analysis, cost-utility analysis, and cost-benefit analysis [1, 2].

Cost-Consequence Analysis

The cost-consequence analysis is not the preferred method for economic evaluation, although it could be useful in specific cases. When an intervention produces many different outcomes that are difficult to aggregate into a combined measure of benefit, the cost-consequence analysis could be appropriate. In a cost-consequence analysis, costs and outcomes are listed in a disaggregated format. This forces a greater involvement of the decision maker, since he needs to weigh the relative importance of each individual outcome and consider the overall difference between interventions in terms of costs and outcomes.

Cost-Minimization Analysis

When interventions are considered similar in all relevant aspects, a cost-minimization analysis can be considered. In a cost-minimization analysis, the preferred intervention would be the alternative with the lowest cost. For this type of economic evaluation, the critical issue is to confirm that there are no meaningful differences between the alternatives for all important patient outcomes, including efficacy, adverse events, impact on quality of life, treatment adherence or convenience, etc. Once the equivalence of the compared alternatives is established, then the less

Table 11.1 Methods for economic evaluations

	Cost consequences analysis	Cost-minimization analysis	Cost-effectiveness analysis	Cost-utility analysis	Cost-benefits analysis
Cost	$	$	$	$	$
Outcomes	Many outcomes can be considered	Many outcomes can be considered All outcomes attained at same level	Only one outcome Main outcome of compared intervention Measured in neutral units	Quality Adjusted Life Year (QALY)	Many outcomes can be considered Measure in monetary value
Results of the analysis	Results for each outcome are presented separately	$	Cost per unit of results	Cost per QALY	$o–$c

expensive alternative should be selected. The necessity to demonstrate that alternatives are equivalent limits the use of cost-minimization analyses.

Cost-Effectiveness Analysis

The cost-effectiveness analysis is a very popular method for economic evaluation. In this type of economic evaluation, the interventions' outcomes or effectiveness are measured in terms of natural units. These comprise life-years gained, life saved, deaths avoided, clinical benefits obtained, or clinical events avoided. For a cost-effectiveness analysis, the selected outcomes have to be shared by the evaluated alternatives and should represent a significant outcome for these interventions. The result of a cost-effectiveness analysis is expressed in terms of incremental cost-effectiveness ratio (*ICER*). The ICER is calculated by dividing the difference in effectiveness by the difference in costs:

$$\text{ICER} = \frac{\text{Costs}_{\text{Intervention B}} - \text{Costs}_{\text{Intervention A}}}{\text{Results}_{\text{Intervention B}} - \text{Results}_{\text{Intervention A}}}$$

For example, if a new intervention costs $10,000 and is associated with a 10 life-years gain and if the alternative intervention costs $5000 and is associated with an 8 life-years gain, then the ICER for the new intervention would be $2500 per incremental life-year gained ($10,000–$5000/10 LYG – 8 LYG). There are some constraints with the cost-effectiveness analysis. Only one outcome can be considered when estimating the cost-effectiveness of an intervention. As most interventions produce multiple outcomes, the full impact of an intervention thus cannot be taken into consideration. Also, the ICER associated with an intervention cannot be easily compared with the ICER of other interventions. Even if the selected outcome is the same, for example, life-years saved, the life-years saved produced by one intervention may not be the same as the life-years saved by another intervention. The quality of life of these life-years saved may be different.

Cost-Utility Analysis

To overcome some of the limitations of cost-effectiveness analysis, the cost-utility analysis has been proposed. The cost-utility analysis is very similar to the cost-effectiveness analysis, except that the outcomes of interventions are expressed in terms of quality-adjusted life years (*QALY*). The main advantage with the QALY is that it enables the integration of a multitude of outcomes (positive or negative), including quality of life. A QALY is basically equivalent to a year in perfect health. As for the cost-effectiveness analysis, the result of a cost-utility analysis is expressed in terms of incremental cost-utility ratio (*ICUR*). The ICUR is calculated by dividing the difference in QALY between interventions by the difference in costs:

$$\text{ICUR} = \frac{\text{Costs}_{\text{Intervention B}} - \text{Costs}_{\text{Intervention A}}}{\text{QALY}_{\text{Intervention B}} - \text{QALY}_{\text{Intervention A}}}$$

For example, if a new intervention costs $10,000 and is associated with 10 QALY while the alternative intervention costs $5000 and is associated with 8 QALY, then the ICUR for the new intervention would be $2500 per QALY ($10,000–$5000/10 QALY – 8 QALY). Since the ICUR estimated for any intervention is based on a same outcome, the QALY, comparison can therefore be made between ICUR associated with different interventions. Cost-utility analyses have become very popular over the recent years and now represent, for most healthcare decision makers, the preferred method for economic evaluation.

Cost-Benefit Analysis

Finally, although very promising, the cost-benefit analysis is now less frequently used, as it faces many methodological issues. In a cost-benefit analysis, both cost and outcomes are expressed in monetary value. The main difficulty with this method is to derive the monetary value of health outcomes. For this, the willingness-to-pay

approach has been developed, but this approach is associated with significant difficulties, especially because it depends on individual's ability to earn income.

There are some specifics to consider when performing an economic evaluation. These comprise: the comparator, the perspective, the time horizon, and the generalizability.

Comparator

By definition, an economic evaluation is always comparative, thus at least two interventions are compared [3]. The appropriate comparator should represent the intervention to be eventually replaced by the intervention of interest. For example, lamellar keratoplasty was compared to penetrating keratoplasty (*PK*). As well, PK was compared to the absence of surgical intervention, since at the time PK was introduced, no other type of surgery was available.

Perspective

The perspective of the analysis is also an important consideration for an economic evaluation. It basically defines the point of view of the analysis. The most common perspectives are the societal perspective, the healthcare system perspective, and the third-party payers perspective. The selected perspective defines which cost would be considered in the economic evaluation. For example, the cost of the surgery will be comprised in all of these three perspectives, but the cost associated with the productivity losses while the patient is hospitalized would be included in the societal perspective only.

Time Horizon

An economic evaluation should encompass all relevant costs and health consequences associated with the intervention under evaluation. For this, the time horizon should be long enough to capture all related events and costs. For example when performing an economic evaluation of corneal transplantation, the time horizon of the evaluation should be long enough to capture all the costs and health consequences associated with the intervention but also all those associated with short-term and long-term complications and recurrences.

Generalizability

Generally in medicine, outcomes of an intervention performed in one location are expected to be replicable in other places. For example, the success of a medication or the rate of complications of a surgery is expected to be similar from one country to another, as long as the medication is used and the surgery is performed in similar conditions. Therefore, results of health intervention are in general considered to be generalizable. This is not the case with the results of an economic evaluation. Given the significant differences in cost structure and dispensation of care from one country to another, an intervention deemed cost-effective in one country may not be cost-effective in another country.

Interpretation of the Results

Another key consideration with economic evaluation is the interpretation of the results. Results of the cost-minimization and the cost-benefit analyses are easy to interpret. In the first instance, the least costly alternative is selected, while in the latter, the alternative with the highest net benefits will be selected. For the cost-consequence analysis, the decision maker has to determine which of the alternative interventions would be preferable after considering the various vectors of efficacy and costs. The cost-effectiveness and cost-utility analyses are the most frequently used methods for economic evaluation, and these analyses result in an ICER or an ICUR, respectively. The ICER and the ICUR basically estimate the incremental cost required to obtain an additional unit of health benefit. For example, results can be expressed in terms of $20,000 per life-year

gained or $5000 per surgical success or $40,000 per QALY. To determine if an intervention is cost-effective, the decision maker has to decide if the ICER or the ICUR is below its willingness to pay for the health benefit. If the decision maker is willing to pay $50,000 for a QALY and the ICUR for the intervention is $40,000, then this intervention would be considered cost-effective. In contrast, an intervention with an ICUR of $60,000 would not be considered cost-effective.

Corneal Transplantation

The cornea is one of the most commonly transplanted tissues, with more than 120,000 corneal transplantations performed each year all over the world, approximately 52,000 of which in North America only [4–6]. Such a high degree of corneal transplant activity represents a relatively high economic burden. Over the past decades, improvements in surgical procedures, development of pharmacological and immunological strategies, as well as changes in corneal storage and eye banking regulations have made corneal transplantation one of the most successful transplantations in humans.

Although corneal transplantation is associated with high success rates, it has practical limitations. Firstly, there is a shortage of corneal donor tissue, which in several countries impacts on the waiting time from diagnosis to surgery. Secondly, not rarely, there is insufficient access to operating room time, which also contributes to extend the waiting period. In Canada, wait times for corneal transplantation remains a challenging problem in several provinces, with more than 2300 patients waiting for a corneal transplantation in 2009, excluding the province of Quebec [5]. A Canadian study suggested that the average wait time for corneal transplantation was between 7 and 36 months in 2009 [5]. The waiting period for surgery is associated with anxiety, poor levels of visual acuity, and the negative impact on patients' quality of life is substantial. As demonstrated in several studies, reduced visual acuity highly correlates with low quality of life values [7–9].

The surgical techniques for corneal transplantation have been relentlessly evolving during the past decades. The paradigm of systematic full-thickness corneal replacement has been fundamentally revised, to be replaced by that of lamellar transplantation designed to replace only the diseased tissue while leaving intact the healthy corneal layers.

Endothelial Keratoplasty

A technique for posterior lamellar keratoplasty was described by Charles W. Tillett in 1956 [10], where the diseased posterior half of the edematous cornea of a 68-year-old patient with Fuchs corneal endothelial dystrophy was replaced by the manually dissected posterior half of a donor cornea. The graft was fixed using transcorneal sutures and intracameral air. Despite major postoperative complications related to the air bubble, anterior synechiae, and severe secondary glaucoma, corneal edema resolved, and the cornea remained clear for 1 year after surgery, which at that time constituted a major step forward.

In 1998, the technique was reintroduced by Gerrit R. J. Melles and al. [11, 12] of the Netherlands, with significant improvements characterized in particular by the absence of corneal sutures and a smaller limbal incision of 9–5 mm [13–17].

A few years later, after additional refinement of the surgical technique and instrumentation, Mark A. Terry and Paula J. Ousley performed a modified version of this technique in the United States and presented the first US clinical series in patients with corneal endothelial diseases [18, 19]. Through several clinical studies, these authors demonstrated that their new surgical technique, named deep lamellar endothelial keratoplasty (*DLEK*), was associated with rapid visual recovery, high endothelial survival rates, minimal astigmatism, and few postoperative complications [20–23].

In 2004, Melles et al. [24] proposed a simplified version of the technique consisting in preparing the recipient bed by simply stripping off Descemet's membrane and the endothelium

without stromal dissection, allowing implantation of the donor posterior lamellar button onto a smooth recipient posterior surface. Francis W. Price introduced technical improvements to further simplify the procedure and reduce the incidence of graft detachment [25], and he renamed the procedure Descemet's stripping endothelial keratoplasty (*DSEK*).

Mark S. Gorovoy [26] subsequently promoted the use of a microkeratome, which nearly eliminated the risk of donor tissue loss during donor preparation and also renamed the procedure Descemet's stripping automated endothelial keratoplasty (*DSAEK*). Eye banks have since then incorporated the microkeratome into their processing of donor tissue for DSAEK: precut tissue has eliminated the stress and financial risk to the surgeon of tissue loss during preparation [27].

Surgeons around the world rapidly adopted DSAEK as their preferred method of corneal transplantation for endothelial disease [6], because it was easier and faster than DLEK and better than PK, with a better visual outcome and increased patient satisfaction.

Soon after his description of posterior lamellar keratoplasty, Melles promoted the idea of transplanting only Descemet's membrane and its endothelium into a recipient bed where only Descemet's membrane and its endothelium have been removed, a technique that was later named Descemet's membrane endothelial keratoplasty (*DMEK*) [28]. Although theoretically ideal on an anatomical point of view and despite excellent visual results [29–31], surgeons are still reticent about DMEK, because it is technically more difficult than DSAEK, it takes too long to perform, the manual preparation of the donor tissue is more challenging, and it is overshadowed by what many surgeons view as unacceptable risks, including a higher initial postoperative complication rate, donor tissue loss, cancelation of the surgery, and associated financial loss [32]. Complications such as graft detachment and primary graft failure are higher than after DSAEK, although high-volume DMEK surgeons are now reporting complication rates that approach those of DSAEK. Contrary to DSAEK, total dislocation after DMEK usually requires graft replacement.

In conclusion, according to the published results on DLEK, DSEK, DSAEK, and DMEK, the advantages of the selective replacement of the posterior cornea – which has been dubbed "endothelial keratoplasty" – over standard PK are significant for patients with endothelial diseases.

First, the absence of corneal sutures associated with these techniques leads to greatly reduced levels of astigmatism and fewer suture-related complications, such as neovascularization, inflammation, and infectious keratitis.

Second, clinical data show that endothelial keratoplasty provides a greater and more rapid visual recovery compared to PK [33, 34]. This is related to the dramatically lower levels of induced astigmatism.

Third, endothelial keratoplasty is associated with lower rejection rates than PK; however, additional studies are needed to nuance the conclusions according to surgical technique, diagnosis, and risk factors [35]. Price et al. [36] found that the 3-year predicted probability of a rejection episode was statistically significantly less with DSAEK (9 %) than with PK (20 %). Hjortdal et al. [37] found similar results for patients with Fuchs endothelial dystrophy, documenting rejection episodes in 5 % of DSAEK and 16 % of PK eyes during the first 2 years after surgery. Ezon et al. [38] only found significant differences among non-glaucomatous eyes, for which fewer rejections were observed after DSAEK than after PK. Anshu et al. [39] demonstrated that patients undergoing DMEK have a significantly reduced risk of experiencing a rejection episode at 2 years compared with DSEK and PK performed for similar indications and using the same corticosteroid regimen.

Lastly, lamellar keratoplasty provides better accessibility to corneal transplantation, since it enables the use of donor tissues that would not be suitable for PK [40] and also because, theoretically, it could allow the preparation of more than one transplant from the same donor cornea [41, 42].

Deep Anterior Lamellar Keratoplasty

There has also been an increased interest in newer techniques for the selective replacement of the anterior layers of the cornea for vision restoration

in eyes where the posterior layers, and more specifically the corneal endothelium, remain healthy, as this is usually the case in keratoconus, for instance. The deep anterior lamellar keratoplasty (DALK) is a surgical procedure consisting in the removal and replacement of the anterior layers, down to Descemet's membrane.

Both observed and long-term predicted graft survival and endothelial densities are higher after DALK than after PK, making it a preferred technique for younger patients with corneal diseases not involving the endothelium [43]. The median predicted graft survival is 49 years in patients who underwent DALK and 17 years in patients who underwent PK and had normal recipient endothelium ($P<0.0001$) [44]. DALK is superior to PK for preserving endothelial cell densities, with an average 5-year postoperative endothelial cell loss of −22 % after DALK and −50 % after PK ($P<0.0001$) [44]. The risk of endothelial rejection is also eliminated, and the incidence of rejection episodes after DALK was reported to be 50 % less than after PK [45].

On the other hand, there are no advantages to DALK for refractive error and best-corrected visual acuity outcomes [46]. Overall visual acuity after DALK and PK is the same. It must be said, however, that DALK with a manual dissection technique results in lower visual acuity than PK (average difference of 1.0–1.8 line) or DALK using a big-bubble dissection technique (average difference of 2.2–2.5 lines) [44].

As an extraocular procedure, DALK has important theoretic safety advantages. However, DALK has not yet reach levels of popularity comparable with current endothelial keratoplasty techniques [6]. The standardization of the big-bubble dissection technique warranted to reduce the incidence of Descemet's membrane perforation [47] would increase the corneal surgeons' confidence in the technique.

Economic Evaluations of Corneal Transplantation Techniques
(See Table 11.2)

Although clinical results of lamellar keratoplasty have been extensively reported in the past years, little is known about its economic impact. Only few economic evaluations of corneal transplantation have been published. Six of these economic evaluations are cost-utility analyses, two are cost-effectiveness analyses, and one is a cost-minimization analysis. The adopted perspective of these economic evaluations was either a healthcare system perspective or a third-party payer perspective, and the time horizons ranged from 1 year to a lifetime period. Three economic evaluations were performed in Singapore, two in the United States, two in the Netherlands, one in Germany, and one in Canada.

A German study first reported in 2006 the costs and utility associated to PK in patients with poor binocular visual acuity [48]. In this study by Hirneiss and al., costs and patients' utility related to PK in one eye were evaluated using a 10-year time frame. Clinical data, in terms of patients' visual acuity, were obtained from a retrospective analysis of 60 patients with a mean age of 46 years. Costs included in this analysis comprised those associated with surgery, ophthalmic medications, ophthalmic medical evaluations, contact lenses, and disinfection solutions. Utility values were obtained by converting patients' best-corrected binocular visual acuity into patients' utility values, using the Brown and Sharma conversion chart [7, 48, 57]. The ICUR related to PK was estimated at US$11,557 per QALY, which represents a cost-effective strategy according to the authors.

An economic analysis by Roe and al. was conducted in order to evaluate the cost-utility of PK for patients with severe keratoconus, over a lifetime period [55]. Visual acuity data were extracted from a retrospective multicenter study that included a total of 123 patients with keratoconus who underwent PK. Postoperative complication rates, including graft failure, graft rejection, increased intraocular pressure, astigmatism, suture abscess, and cataract, were obtained from published clinical studies. Mean pre- and postoperative best-corrected visual acuities were used to estimate patients' utilities. The ICUR of PK for treating one eye, when compared to no treatment, was estimated at US$1942 per QALY.

Endothelial keratoplasty techniques were compared to PK in a few economic evaluations. The

Table 11.2 Economic evaluations of corneal transplantation

Author (year) Country	Type Perspective Time horizon	Comparators	Costs included	Base case results
Hirneiss et al. (2006) [48] Germany	Cost-utility Healthcare system 10-year period	PK versus no surgical intervention	Donor tissue preparation, surgery, follow-up, medications	ICUR PK versus no surgical intervention: US$11,557/QALY
Bose et al. (2013) [49] Singapore	Cost-utility using a decision tree Healthcare system 3-year period	DSEK versus PK PK versus no surgical intervention DSEK versus no surgical intervention	Medical charges associated with the initial procedure and related complications	ICUR DSEK versus PK: US$5209/QALY
Prabhu et al. (2013) [50] USA	Cost-utility using decision tree Third-party payer 5-year period	DSAEK versus PK	Donor tissue preparation, surgery, follow-up, postoperative complications, procedures, medications	DSAEK dominates PK
van den Biggelaar et al. (2012) [51] The Netherlands	Cost-effectiveness Healthcare system 15-month period (3 months pre-operation + 12 months post-operation)	PK versus FS-DSEK versus DSAEK	Preoperative costs, surgical procedures, hospitalization, follow-up visits, postoperative drugs	FS-DSEK dominated by DSAEK FS-DSEK dominated by PK ICER DSAEK versus PK: US$5920 per clinically improved patient (defined as a patient with a combined effectiveness of both a clinically improved best spectacle-corrected visual acuity and a clinically acceptable refractive astigmatism)
Van den Biggelaar et al. (2011) [52] The Netherlands	Cost-effectiveness Healthcare system 13.5-month period (1.5 months pre-operation + 12 months post-operation)	DALK versus PK	Preoperative costs, surgical procedures, additional procedures, hospitalization, follow-up visits, postoperative drugs	ICER DALK versus PK: US$13,768 per clinically improved patient on the 25-item National Eye Institute Visual Functioning Questionnaire US$9522 per patient with an endothelial cell loss of maximally 20 % within the first year
Koo et al. (2011) [53] Singapore	Cost-utility using decision tree Healthcare system 20-year period	PK versus no surgical treatment DALK versus PK	Medical costs associated with the primary cornea surgeries and any subsequent treatments for complications	ICUR PK versus no surgical treatment: US$3003/QALY ICUR DALK versus PK: US$2423/QALY

Table 11.2 (continued)

Beauchemin et al. (2010) [54] Canada	Cost-utility using Markov model Healthcare system Lifetime period	PK versus PLK techniques (including DLEK, DSEK and DSAEK)	Surgery, follow-up, postsurgical complications	PLK dominates PK
Roe et al. (2008) [55] USA	Cost-utility using decision tree Third-party payer 5-year period	PK versus no surgical intervention	Surgery, postoperative medications, follow-up visits, postsurgical complications	ICUR PK versus no surgical intervention: In 1 eye: US$1942 In the second eye: US$2228 In both eyes: US$2003
Tan et al. (2014) [56] Singapore	Cost-minimization Ophthalmic institution 1-year period	Tissue-engineered constructs versus donor tissue procured from eye banks for endothelial keratoplasty (EK)	Investment cost, amortization of investment cost, annual recurring cost	Tissue-engineered constructs for EK could be produced at a cost of US$880 per transplant Utilizing donor tissue procured from eye banks for EK required US$3710 per transplant

PK penetrating keratoplasty, *DLEK* deep lamellar endothelial keratoplasty, *DSEK* Descemet's stripping endothelial keratoplasty, *DSAEK* Descemet's stripping automated endothelial keratoplasty, *DALK* deep anterior lamellar keratoplasty, *FS-DSEK* femtosecond laser-assisted Descemet's stripping endothelial keratoplasty, *EK* Endothelial keratoplasty

first of these evaluations, by Beauchemin et al. [54], was performed in Canada and compared DLEK, DSEK, DSAEK, and PK using a cost-utility analysis based on a Markov model over a lifetime period. The health states included in the model comprised: waiting time for transplant, graft survival with or without complications, irreversible graft failure, non-eligibility, and death. In this economic evaluation, endothelial keratoplasty was more effective, providing more QALY, and was less costly than PK. Therefore, endothelial keratoplasty was shown to be a dominant strategy compared to PK. The robustness of the results was confirmed by deterministic and probabilistic sensitivity analyses.

Based on a retrospective study, Bose et al. [49] performed a cost-utility analysis comparing DSEK and PK. Improvement in best spectacle-corrected visual acuity was used to calculate the change in QALY over a 3-year period following the procedure. Cumulated costs over the 3-year period were slightly higher with the DSEK procedure, but the number of QALY gained was also higher. The ICUR for DSEK compared to PK was estimated at US$5209 per QALY, which is much lower than the threshold usually used in Singapore, where the study was performed. Therefore, it can be concluded from the results of this study that DSEK is a cost-effective procedure.

In the US study by Prabhu et al. [50], DSAEK was compared to PK in a cost-utility analysis with a 5-year time horizon based on published literature. The utility values used to estimate the number of QALY were based on visual acuity outcomes. The economic model developed to perform this economic evaluation considered complications, graft dislocation, early graft failure, graft rejection, and high intraocular pressure. After the 5-year period, costs associated with DSAEK were lower than with PK, and the number of QALY gained was higher with DSAEK. Therefore, based on this analysis, DSAEK is a dominant strategy compared to PK, being more effective and less costly.

van den Biggelaar et al. [51] in the Netherlands performed a cost-effectiveness study comparing femtosecond laser-assisted Descemet's stripping endothelial keratoplasty (*FS-DSEK*), DSAEK, and PK. This analysis was based on data from a multicenter randomized clinical trial and a non-comparative prospective study. The health outcome considered was the number of clinically improved patients. The analysis was based on a 1-year time horizon. The percentages of clinically improved patients were 52, 44, and 43 % with DSAEK, PK, and FS-DSEK, respectively. Mean total costs per patients were US$8416 with DSAEK, US$7942 with PK, and US$14,807 with FS-DSEK. Therefore, FS-DSEK is dominated by both PK and DSAEK, as it is more costly and less effective than the two alternatives considered. DSAEK was shown to be more costly than PK, but also more effective, with an ICER of US$5920 per additional clinically improved patient.

In another study, van Den Biggelaar et al. [52] performed a cost-effectiveness analysis comparing DALK to PK. This analysis was performed in the Netherlands alongside a randomized multicenter clinical trial with a time horizon of 13.5 month (1.5 months before and 12 months after surgery). The health outcome measures were the proportion of clinically improved patients on the 25-item National Eye Institute Visual Functioning Questionnaire (*NEIVFQ*) and the proportion of patients with endothelial cell loss of 20 % or less within the first year. DALK was more costly than PK, but it was also more effective than PK for both outcomes measures (NEIVFQ and cell loss) (NEIVFQ and cell loss). The ICER was estimated at US$13,768 per clinically improved patients on the 25-item NEIVFQ and at US$9522 per patient with cell loss of 20 % or less.

DALK has also been compared to PK in a cost-utility analysis performed in Singapore by Koo et al. [53]. One-year costs and outcomes were collected from patients seen for corneal graft between January 1991 and January 2009. Costs associated with DALK were higher than for PK, but the number of QALY gained were also higher with DALK. The ICUR of DALK compared to PK was estimated at US$3025 per QALY, which is much lower than the threshold that would usually be used in Singapore. Therefore, DALK can be considered as a cost-effective procedure.

Finally, Tan et al. [56] recently performed a cost-minimization analysis comparing a tissue-engineering strategy to a procured tissue strategy. They compiled all the cost associated with these two strategies according to the perspective of an ophthalmic institution in Singapore that possesses the surgical expertise to perform endothelial keratoplasty. The cost per transplant was lower with the tissue-engineering strategy ($880) compared to a procured tissue strategy ($3710). Therefore, based on this cost-minimization analysis, the tissue-engineering strategy would be cost-effective.

Conclusions

In a context of healthcare economic constraint, economic evaluations allow for a better allocation of resources. Although the number of economic evaluation on the many procedures for corneal transplantation is limited and not necessarily representative of their performance in all the settings they are used, some broad conclusions can be drawn. The few economic evaluations performed on PK compared to no surgery indicate that it is not only clinically beneficial to perform a corneal transplantation on these patients, but it is also cost-effective. Also, as the surgery procedures have evolved over time with the development of the endothelial keratoplasty techniques, the new procedures deemed to be cost-effective compared to PK. This seems to be also the case for the DALK, although there are fewer evidences available to support its cost-effectiveness. There was only one economic evaluation on the femtosecond laser-assisted procedure, but the results of this evaluation indicate that this type of laser-assisted technique was not cost-effective.

Many of the published economic evaluations on corneal transplantation procedures were cost-utility analyses. This type of evaluation can take into account all the consequences of an intervention and facilitates decision making. Indeed, the results of these analyses expressed in terms of cost per QALY are judged by the amount that the decision maker is prepared to allocate for a QALY or a year in perfect health. Although there may not be a consensus on the value of a QALY, the cost per QALY found in all the cost-utility analyses on corneal transplantation procedures was much lower than the usual threshold. Even more, in some cases the procedure was considered as dominant, being more effective and less costly than the alternative interventions.

Much progress has been made in recent years to improve the success of corneal transplantation interventions. Although other economic evaluations deserve to be made to learn more about the economic impact of these interventions over many different contexts of use, the results to this day indicate that most of these new interventions are cost-effective.

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Post-keratoplasty Astigmatism

12

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Abstract

Astigmatism is the leading factor to limit visual rehabilitation after otherwise successful keratoplasty. Reasons for post-keratoplasty astigmatism are multifactorial, and they can be divided into donor-related factors, recipient-related factors, intraoperative factors, and postoperative factors. In most reports the post-keratoplasty astigmatism is between two and four diopters after penetrating keratoplasty (PKP) and deep anterior lamellar keratoplasty (DALK). However, the amount of astigmatism varies and can be large enough to require surgical intervention to reach adequate optical correction. Endothelial transplantation does not usually induce astigmatism and surgical intervention is not necessary. The safest method to correct post-keratoplasty astigmatism is by use of spectacles followed by different types of contact lenses. Surgical correction of post-keratoplasty astigmatism includes astigmatic keratotomy, wedge resection, intracorneal ring segments, or excimer laser. Even higher amounts of regular corneal astigmatism can be corrected using toric intraocular lenses implanted both to phakic and pseudophakic eyes. Surgical treatment of post-keratoplasty astigmatism is always planned on individual basis and after careful examination. None of the single surgical methods fully correct the astigmatism, and often different surgical methods need to be combined. Here we have reviewed the reasons and different treatment modalities for post-keratoplasty astigmatism.

Keywords

Post-keratoplasty astigmatism • Toric IOL • Femtosecond laser • Astigmatic keratotomy • Wedge resection • Corneal transplantation

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J. Hjortdal (ed.), *Corneal Transplantation*, DOI 10.1007/978-3-319-24052-7_12

Introduction

Astigmatism is the leading factor to limit visual rehabilitation after otherwise successful keratoplasty. There are no differences in the amount of post-keratoplasty astigmatism between penetrating keratoplasty (PKP) and deep anterior lamellar keratoplasty (DALK). In most reports the astigmatism is between 2 and 4 diopters (D), although the variation can be large [41]. In most cases the optical correction can be made using spectacles or contact lenses, but surgical correction is often required. After Descemet's stripping endothelial keratoplasty (DSEK), the mean astigmatism is 1.5 D, and the surgically induced astigmatism is only 0.11 D [26], which usually does not necessitate surgical correction.

Reasons for Astigmatism

Reasons for post-keratoplasty astigmatism are multifactorial. Reasons can be divided into donor-related factors, recipient-related factors, intraoperative factors, and postoperative factors. Donor-related factors include power, astigmatism, and irregularities, which are normally not measured from the donor corneas. Peripheral uneven thickness of the donor cornea influences the apposition and healing of the graft-host junction. Donor corneas from infants are reported to induce more astigmatism and myopia compared to adult donor corneas in adult eyes after keratoplasty [21, 36].

Recipient-related factors also include peripheral changes in the recipient tissue, like astigmatism and uneven thickness, which may result in uneven peripheral support from the peripheral tissue and an unequal healing response in different parts of the graft-host junction [40].

Intraoperatively, external pressure to the globe caused by lid speculum, bridle sutures, or scleral rings can lead to corneal distortion and trephination of an oval opening in the host tissue [35]. Trephinations of both the host bed and donor button play an important role in relation to post-keratoplasty astigmatism. Eccentric trephination of the host bed or donor button flattens the axis in the direction of displacement [43]. Tilted trephination causes an oval host bed or donor button resulting in uneven distribution of tissues at the graft-host junction. This evidently leads to irregular forces at the graft-host junction and subsequently to irregular astigmatism [34]. Dull trephine may create irregular edges and an oval host bed or donor button [49].

Trephinations of the host and donor tissues are currently mostly done using suction trephines, which stabilize the tissues during the trephinations. The host cornea is trephinated from the epithelial side, while the donor button is often trephinated from the endothelial side. It has been suggested that the donor button should be trephinated similarly from the epithelial side using an artificial anterior chamber to give identical side cut compared to the host bed. Using a femtosecond laser it is now possible to perform identical side-cut configurations such as top hat, mushroom, or zigzag patterns, both to the host cornea and using an artificial anterior chamber to the donor tissue. In comparative studies, it has been shown that the astigmatism is lesser only during the early postoperative period up to 6 months using femtosecond (FS) laser-assisted side-cut incisions compared to conventional trephinations after PKPs [9, 11, 13, 19]. In one study, the lesser astigmatism after the FS laser use was noticed up to 12 months [27], and in one study no difference in astigmatism was observed [6]. After DALK, no difference in astigmatism was observed up to 12 months between FS laser-assisted operations and conventional operations [45].

Suturing the corneal transplant can be performed using interrupted sutures, continuous running single suture, or double sutures or a combination of interrupted and continuous sutures. Each of the techniques has advantages in management of astigmatism like selective suture removal of the interrupted sutures [47] and adjustment of the running suture [32]. It seems, however, that none of the techniques are superior compared to the others in astigmatism management. The surgeons' preference and experience

in a selected technique may be a more important factor for the outcome.

During the postoperative period, topical and systemic medications, inflammation, corneal vascularization, possible rejections, and wound dehiscence all affect wound healing and may affect postoperative astigmatism unequally and in an uncontrolled way [42].

Management of Post-keratoplasty Astigmatism

Spectacle and Contact Lenses

By far the safest method to approach post-keratoplasty astigmatism is by use of spectacles. Usually if astigmatism is less than 4 D and is mostly regular, spectacles offer the treatment of choice to treat refractive errors. Unfortunately, this is in most cases not so. Approximately, 30 % of patients undergoing PKP or DALK suffer from astigmatism of more than 5 D. Furthermore, problems arising from anisometropia and aniseikonia may limit the use of spectacles. Somewhat unexpectedly a large number of post-keratoplasty patients tolerate relatively large amounts of anisometropia and aniseikonia probably because the refractive status before grafting was problematic. Accordingly, spectacle trial should always be performed before further means to treat refractive errors are taken.

Rigid contact lenses, such as rigid gas permeable, hybrid, scleral, and piggyback contact lenses, provide a better means to treat an irregular corneal surface in post-keratoplasty patients, and also larger amounts of refractive errors can be addressed. The quality of vision is usually significantly improved with the aid of contact lenses. Yet, dry eye syndrome, contact lens fitting-related problems, graft size, graft location, and graft toricity as well as lifestyle may limit the use of these lenses. It has been estimated that 30–40 % of keratoplasty patients cannot tolerate contact lenses. If contact lens fitting is successful, it usually provides the best optical quality of visual rehabilitation.

Corneal Surgery

Astigmatic Keratotomy

Astigmatic keratotomy (AK) is a well-established method to address high degrees of post-keratoplasty astigmatism. Although a large number of different AK techniques have been suggested, the most common of these is probably arcuate incisions. Usually the relaxing incision corrects 4–5 D of post-keratoplasty astigmatism, and the effect is proportional to the preoperative cylinder. It has, however, minimal effect on the spherical equivalent. There are several advantages of this technique; most importantly they are safe and the incision site is at a constant distance from the visual axis allowing a better corneal contour. In short, the AK incisions are positioned perpendicular to the steep axis of the corneal topography. The effect of AKs can be augmented by placing compression sutures perpendicular to the AKs (i.e. to the flat axis). AK incisions can be made either by free hand, by specific devices such as the Hanna arcitome, or more recently by FS lasers. Usually two 90° length paired incisions to the graft-host wound are made when using mechanical devices or inside the graft when using FS lasers. If mechanical devices are used, the surface of the cornea is opened, whereas using FS lasers, AKs can be made intrastromally or by penetrating the corneal epithelium. In all cases, the somewhat poor predictability, corneal perforation, and wound gaps remain major problems.

It seems that there is no significant difference between mechanical and FS-assisted epithelium-penetrating AKs in reducing astigmatism. After mechanical AK, the reduction in refractive cylinder varies between 30 and 54 % [15, 16, 38] and after FS laser-assisted AK between 36 and 66 % [16, 25, 33]. Hovding [17] described a reduction of 49 % with transverse keratotomies. McCartney et al. [31] combined compression sutures with relaxing incisions and found a larger, 68 %, reduction in refractive cylinder. It seems that complications are quite rare with both methods, yet these populations are not large enough to differentiate between these methods.

We have recently shown that FS laser-assisted intrastromal AKs are safe, and the refractive and topographic results are comparable to epithelium-penetrating techniques [60]. We found significant 30–38 % improvements in topographic and refractive cylinders. Because both anterior and posterior topographic cylinders had a significant reduction in astigmatism, this indicates that the effect of intrastromal incisions extends throughout the whole cornea. The theoretical advantages of intrastromal relaxing incisions for treating astigmatism are its relative simplicity, less risk of postoperative infections, and reduced discomfort to the patient. In our patient material no infections were recorded and patients did not report pain or unpleasant effects due to incisions. It is not, however, possible to differentiate superiority between intrastromal incisions and epithelium-penetrating FS laser incisions or manual AK based on this population, as the other methods also have a very low rate of complications. Intrastromal relaxing incisions seem to be a good alternative to more penetrating methods of treating post-keratoplasty astigmatism. Based on our experience the effect of FS laser-assisted intrastromal AKs is good, the rate of adverse effects or complications is low, and reoperations are simple to perform when needed [60].

Wedge Resection

Troutman [54] was the first to introduce corneal wedge resections to treat high (over 10 D) post-keratoplasty astigmatism. The technique follows the idea of AKs, but instead of relaxing the strain in the cornea, wedge resections remove corneal tissue, and the opposing wound edges are sutured with 10-0 or 11-0 nylon sutures to initially overcorrect the astigmatism. As a rule of thumb, resection of 1 mm of corneal tissue should decrease astigmatism by 10–20 D. Obviously, the technique suffers from low predictability, and when performed manually the technique is very demanding. De la Paz and collaborators showed in their analysis of 21 patients that wedge resections were safe and decreased refractive, topographic, and keratometric astigmatism by more than 50 % in their 1-year follow-up [37]. Recently, a technique which utilizes FS laser has been introduced [14], but has not gained wide popularity. Krachmer and Fenzl [23] compared relaxing incisions ($N=16$) and wedge resections ($N=10$), finding a 43 % reduction with incisions and 59 % with resections.

Intracorneal Ring Segments

Intracorneal ring segments (ICRSs) provide another means to correct ametropia following keratoplasty. The idea behind this technique is the flattening effect to the cornea caused by the implantation of one or two intracorneal segments. Altogether four studies have addressed the efficacy and safety of these implants. Very recently, Lisa et al. [29] showed in their cohort study of 32 eyes that implantation of ICRS increased both uncorrected (UDVA) and corrected distant visual acuity (CDVA) as well as decreased both spherical equivalent and astigmatism. Likewise, Coscarelli and collaborators [10] in their larger retrospective analysis of 59 eyes showed that the corrected visual acuity improved by 1.5 lines, and the mean spherical equivalent decreased from 6.3 ± 3.4 D to 2.7 ± 2.5 D. The topographic astigmatism decreased from 3.4 ± 2.1 D to 1.7 ± 1.0 D. Somewhat similar results were obtained by Prazeres et al. in [39] and Arriola-Villalobos and collaborators in [5].

Excimer Laser

Photorefractive keratectomy (PRK) and laser-assisted in-situ keratomileusis (LASIK) have been widely used to treat post-keratoplasty refractive errors. Graft rejection as well as scar and haze formation has been reported after PRK limiting the applicability of this technique. LASIK has certain advantages over PRK in that larger amounts of spherical and astigmatic refractive errors can be treated with this technique, and it seems to provide somewhat predictable refractive outcomes and seems to be effective.

Bilgihan and colleagues showed that PRK reduced astigmatism by approx. 40 % in low to moderate post-keratoplasty astigmatism patients.

Yet, one-third of the patients suffered from significant haze which resolved in most cases, and roughly 10 % of patients experienced graft rejection [7]. Forseto Ados et al. studied the safety and efficacy of mitomycin C (MMC)-PRK in post-keratoplasty patients and found that the procedure had an index of success of 55 % in correcting astigmatism. Haze developed in roughly 10 % of patients [12]. Similarly, Ward and collaborators in their retrospective analysis of 20 patients found that MMC-PRK decreased astigmatism from 4.9 D to 2.0 D [58].

Kovoor et al. compared in their small clinical trial the efficacy of PRK and LASIK in reducing refractive errors after keratoplasty. Essentially, they found that both procedures were effective in treating post-keratoplasty refractive errors without significant differences between the two techniques. They found that both methods reduced astigmatism by 40–50 % [22].

LASIK is generally considered to be more effective than PRK in treating myopia and astigmatism in patients that have had a corneal transplant [24]. Yet, LASIK in post-keratoplasty patients is less efficient in treating the astigmatic component than the spherical component. Some authors, however, have reported up to 6 D reduction in astigmatism with LASIK [4, 59]. Post-keratoplasty LASIK patients are prone to complications with increased risk of graft rejection, graft dehiscence, epithelial ingrowth, and graft decompensation [24].

Intriguingly, LASIK can be performed as a 2-step procedure [3, 8]. In this technique a LASIK flap is created in one session, lifted, and allowed to heal. In theory, this could alleviate the strain within the corneal graft, thus reducing astigmatism. In the second step, the flap is lifted and refractive correction is performed to the stromal bed. To our knowledge, however, no conclusive studies have been performed to prove that the 2-step LASIK is more effective than the 1-step LASIK. Furthermore, it is unclear if FS laser-assisted LASIK flaps would offer any advantage over flaps made by microkeratomes or if wave-front- or topography-guided excimer procedures would offer advantage over conventional treatments.

Intraocular Surgery

Regular corneal astigmatism can be corrected using toric intraocular lenses (tIOLs). For eyes having normal crystalline lens, possible alternatives are in the anterior chamber implantable iris-claw or in the sulcus implantable collamer lenses. For pseudophakic eyes, iris-claw or in the sulcus implantable add-on supplementary lenses can be used. Supplementary add-on IOLs are designed to be implanted in the ciliary sulcus of a pseudophakic eye in addition to an IOL in the capsular bag. Product specifications for the toric anterior chamber or posterior chamber sulcus IOLs which have been used for correction of post-keratoplasty astigmatism are listed in Table 12.1. For implantation in the capsular bag after removal of the cataractous nucleus, ten different monofocal

Table 12.1 Product specifications for anterior chamber or sulcus implantable toric intraocular lenses (IOLs) or sulcus implantable supplementary add-on IOLs for correction of post-keratoplasty astigmatism

	Manufacturer	Material	Power range (D)
IOL			
Artisan®	Ophtec, the Netherlands	PMMA	Sph. −23.0 to (+7.5) Cyl. ±1 to (±7.5)
ICL™	Staar Surgical, USA	Collamer	Sph. −3.0 to (−23.0) Cyl. +1.0 to (+6.0)
Add-on IOLs			
Sulcoflex	Rayner, UK	Hydrophilic acrylic	Sph. −7.0 to (+7.0) Cyl. +1.0 to (+6.0)
Torica	HumanOptics/Dr. Schmidt, Germany	Hydrophobic MicroSil®	Sph. −30.0 to (+6.0) Cyl. +1.0 to (+30.0)

tIOLs are available [56]. Use of tIOLs for correction of post-keratoplasty astigmatism offers simultaneous option to correct spherical ametropia at the same time.

Anterior Chamber Iris-Claw Lens

The single-piece iris-enclavated Artisan® IOL is made of hard polymethyl methacrylate (PMMA). It can be implanted through a 5.5 mm wound, which subsequently requires suturing. Suturing the wound makes controlling the astigmatism more challenging and also often delays the final outcome of the surgery. Using this anterior chamber IOL also necessitates peripheral iridotomy or iridectomy. Iris-claw IOLs can be used in phakic and pseudophakic eyes.

Tehrani and Dick presented a report of a keratoconus patient who had 7.6 diopters of corneal astigmatism after keratoplasty and CDVA of 20/32 [51]. Using a toric iris-claw Artisan® lens, the UDVA improved to 20/20 after 6 months. In a larger material of 36 eyes of 35 patients, the refractive cylinder of 7.1 ± 2.0 D decreased to 2.0 ± 1.9 D at 3 years [50]. At the last follow-up, 28 % of the eyes had a refractive cylinder less than 1 D, 58 % of the eyes had a refractive cylinder less than 2 D, and 92 % of the eyes had a refractive cylinder less than 4 D. In 50 % of eyes the achieved cylinder correction was within 1 D and in 72 % of eyes within 2 D of the intended correction. The UDVA improved significantly, while the CDVA remained the same. Three eyes (8.3 %) lost CDVA more than 2 lines, three eyes lost CDVA 1 to 2 lines, in 22 (61.1 %) the CDVA remained the same or improved 1–2 lines, and in three eyes (8.3 %) CDVA improved more than 2 lines. Endothelial cell loss at 6 months postoperatively was 13.8 ± 18.7 % compared to preoperative values, and a progressive endothelial cell loss was observed at least up to 3 years. At 3 years the endothelial cell loss was 30.4 ± 32.0 % compared to preoperative values.

Posterior Chamber Sulcus Lenses

The Implantable Collamer® Lens (ICL™) is made of hydrophilic porcine collagen and hydroxyethyl methacrylate copolymer including ultraviolet-absorbing chromophore in the polymer chains. This lens can be implanted into the eye through a 3.0 mm corneal incision, and its implantation is indicated in phakic eyes. The latest model of the ICL™ has a central hole allowing aqueous flow from posterior chamber through the hole into the anterior chamber, which eliminates the need of peripheral iridotomy or iridectomy. Peripheral iridotomy or iridectomy was required when using previous model of ICL™.

The efficacy and safety of toric ICL™ for correction of post-keratoplasty astigmatism have been addressed in three publications. Results of altogether 14 eyes of 14 patients in two different publications have been presented [2, 18], and in one publication, a single patient was reported [1]. In these studies, the refractive cylinder decreased by 60–90 % at 12–24 months. From all 15 eyes, in five (33 %) the CDVA improved, in nine (60 %) remained the same, and in one (7 %) decreased one line. The refractive astigmatisms were reported to be stable during the follow-up periods, and no lens rotations or lens-related adverse effects were reported. The endothelial cell loss was reported not to differ from the expected endothelial cell loss after corneal transplantation [1, 2].

Two different models of toric supplementary add-on IOLs are available on the market (Table 12.1). The Torica (HumanOptics/Dr. Schmidt) is a three-piece IOL, in which the optic is made of silicone elastomer and the haptics of polymethyl methacrylate. It can be implanted through a 3.5 mm incision. Its toricity is available up to 30.0 D.

Thomas et al. described a case series of 20 patients implanted with add-on Torica IOL. The series included 15 eyes of 14 patients who had post-keratoplasty astigmatism [53]. Refractive cylinder decreased significantly in this subgroup of keratoplasty eyes from 9.7 ± 3.8 D to 2.4 ± 1.7 D at 2–6 months postoperatively. The UDVA improved significantly, while the CDVA remained the same. Postoperative surgical IOL rotation was performed in five eyes (24 %). In two eyes, graft failures occurred leading to re-graft. In addition, two case reports of Torica add-on IOL use have been reported with successful outcome

both in reducing the refractive astigmatism and improving the UDVA [28, 46].

Sulcoflex is a single-piece hydrophilic acrylic IOL that can be implanted through a 2.75 mm incision. The haptics are 10° posteriorly angulated compared to the optic to avoid pigment dispersion and iris capture of this sulcus-fixated lens. Use of Sulcoflex in correction of post-keratoplasty astigmatism in two eyes of two patients has been reported recently [46]. In both eyes, a reduction of refractive cylinder and an improvement of UDVA were achieved, while the CDVA remained constant. Excessive endothelial cell loss was not observed.

Posterior Chamber In-The-Bag Lenses

Delaying the possible cataract operation at the time of keratoplasty until sutures have been removed and refraction stabilizes offers an option to use tIOLs in the capsular bag after cataract removal to correct possible ametropias. Several reports of using tIOLs consisting of a single or a few patients are available.

Wade and coworkers reported a larger case series using toric acrylic AcrySof® IOL [57]. Results of 21 eyes of 16 patients with a mean follow-up of 14.7 months were reported. The preoperative topographic astigmatism of 4.6 ± 2.1 D was reduced to refractive astigmatism of 1.6 ± 1.3 after cataract removal and use of in-the-bag tIOL. The UDVA and CDVA improved significantly. The refractive astigmatism and the visual acuities remained constant over the follow-up period and up to 3 years for some patients. Possible rotational stability was not recorded in the study.

Using a silicon optic tIOL (MicroSil 6116 TU, HumanOptics), the astigmatism has been reported to decrease after in-the-bag implantation in two different reports from the preoperative topographic astigmatism of 9.2 ± 4.1 D to postoperative refractive astigmatism of 1.6 ± 1.5 D and from 10.2 D to 2.75 D, respectively [20, 55]. The follow-up times were 3.5 months and 1 month and the numbers of eyes were 11 and seven, respectively. In all cases both the UDVA and CDVA improved. The accuracy of the axis of the IOLs during the follow-up period were 4 ± 3° (range 0–8°) and 5° (range 0–9°), respectively. Customized tIOLs are also available by the same manufacturer, and reports of successful use of tIOLs with cylinders up to 15.0 D and 30.0 D have been reported [30, 52].

Using a tIOL made of hydrophilic acrylic optic (Rayner, UK), the post-keratoplasty refractive astigmatism has been shown to decrease in 1 month from 6.2 ± 2.7 D to 2.9 ± 2.2 D (eight eyes). The rotation of the IOL during the 1 month was 8.1 ± 9.4°, and two patients required operative realigning of the IOL [48]. In another report of three cases using the same customized tIOL, the refractive astigmatism decreased from 8.3 ± 2.1 D to 0.7 ± 0.6 D with a mean rotation of 3.3 ± 1.2° during the 12-month follow-up period [44].

Treatment Planning

Treatment planning for post-keratoplasty astigmatism is made on an individual basis, and therefore it is impossible to give definitive recommendations. The patient age, corneal graft prognosis, amount and regularity of astigmatism, endothelial cell count, lens status, and other ocular pathologies should be taken into account when planning the astigmatic and refractive error treatment. Furthermore, the status of the patients' other eye should be taken into consideration, and if it is healthy the purpose and goal of treatment should be reflected with the expectations of the patient. In almost all cases the visual performance after corneal grafting is certainly impaired compared to a virgin eye. Anterior segment optical coherence tomography (OCT) is a useful tool for analyzing the transplant contour and profile, eccentricity, and possible bulging, which all may affect the surgical decision. Comparing different surgical methods, it is obvious that none of the methods fully correct the astigmatism (Table 12.2). The realistic goal of treatment of the post-keratoplasty astigmatism is such an amount that can be finally corrected by spectacles or contact lenses, and not a plano refraction.

If the corneal graft is clear and the endothelial cell count is low, we usually approach corneal astigmatism by corneal interventions. In the case of very high corneal astigmatism, AKs followed

Table 12.2 Effect of different treatment methods on post-keratoplasty ametropias

Method	Astigmatism reduction (%)	Spherical equivalent change (%)	References
Wedge resection	Over 50	NA	[37]
AK			
Manual	30–54	−18	[15, 16, 38].
FS laser-assisted intrastromal	46	+14	[60]
LASIK	40–50	−60 to 80	[3, 8, 22]
IOLs			
Anterior chamber			
Artisan®	71	−68	[50]
Sulcus			
ICL™	60–75	−86 to 96	[2, 18]
Torica	75	NA	[53]
Sulcoflex	58	NA	[46]
Capsular bag			
AcrySof	53	NA	[57]
MicroSil	73–77	NA	[20, 55]
T-flex	53–92	−78 to 91	[44, 48]

AK astigmatic keratotomy, *FS* femtosecond, *LASIK* laser-assisted in-situ keratomileusis, *IOL* intraocular lens, NA not available

by wedge resection seem to be the treatment of choice. If this is unsuccessful, re-grafting can be justified. If the treatment provides a reasonable effect, this may be followed by re-AKs and later possibly by intraocular approach with tIOL.

In moderate levels of astigmatism, we have currently a multitude of different techniques. These include both corneal and intraocular approaches. Excimer laser surgery, intracorneal ring segments, manual or FS laser-assisted AKs, and FS-assisted intrastromal AKs seem all to produce relatively good results. The choice of treatment is based on corneal regularity, amount of astigmatism and other refractive errors, the surgeons' experience, and available instrumentation. It remains unclear if wave-front- or topography-guided excimer refractive surgery provides better results than conventional surgery, but this may be the best means to treat the irregular component of astigmatism.

If the endothelial cell count is high and the post-keratoplasty astigmatism is below 5 D, we would recommend trial with FS laser-assisted intrastromal AKs. If the effect is inadequate, one may consider manual AKs or either toric ICL™ or anterior chamber lenses provided that the lens is clear and the anterior chamber is deep enough. Yet, possible damage to the endothelial cells limits the usefulness of this approach. If the nucleus is less transparent, tIOL in the capsular bag may be the treatment of choice.

If the endothelial cell count is high and the graft shows moderate (less than 5 D) astigmatism, one has again a larger choice of treatment modalities that basically include all of the above. Our own approach in these cases is to first provide optimal reduction in corneal astigmatism followed by lens-based approach.

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Optics of Transplanted Grafts: IOL Calculation in Grafted Patients

13

Ugo De Sanctis

Abstract

Modern methods of intraocular lens (IOL) calculation have significantly reduced the risk of employing an improper IOL power. The development of theoretical formulas has improved the accuracy of predicting the effective lens position, while the use of laser partial coherence interferometry has increased the accuracy of axial length measurement. IOL calculation in grafted patients, however, is more challenging due to different and peculiar clinical situations. These situations fall into two main scenarios. The first comprises patients who are candidates to combined cataract extraction, IOL implantation, and corneal graft (the triple procedure). The second includes patients who have previously undergone corneal graft. In this second scenario, IOL calculation may be required for cataract extraction, piggyback IOL, and phakic IOL implantation.

This chapter examines the challenges inherent in each clinical situation and discusses the strategies that can be adopted to tackle these situations and optimize IOL power calculation.

Keywords

Intraocular lens power calculation • Triple procedure • Toric intraocular lenses

IOL Power Calculation in the Triple Procedure

Four potential sources of error affect the accuracy of IOL power calculation: corneal power measurement, axial length (AXL) measurement, effective lens position estimate, and calculation formula. In eye candidates for the triple procedure, the most significant source of error is

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J. Hjortdal (ed.), *Corneal Transplantation*, DOI 10.1007/978-3-319-24052-7_13

corneal power measurement. Before the intervention, the examiner must compute the keratometric readings to be inserted into the IOL calculation formula without knowing what the corneal power will be after the graft.

The advent of endothelial keratoplasty techniques, such as Descemet's stripping automated endothelial keratoplasty (DSAEK) and Descemet's membrane endothelial keratoplasty (DMEK), has greatly increased the predictability of post-graft corneal power. These techniques induce smaller changes of corneal power and, as a consequence, lead to more predictable refractive outcomes than is the case with penetrating keratoplasty (PKP); this is a major advantage of these procedures, which have been widely adopted in recent years.

The New Triple Procedure

The intervention comprising phacoemulsification, IOL implantation, and DSAEK has become the gold standard for the simultaneous surgical treatment of corneal endothelial diseases and cataract. What has been called the "new triple procedure" offers numerous advantages over the standard technique, which comprises PKP and cataract extraction. The main advantages of the new triple procedure are that surgery is performed through a small incision, corneal innervation and biomechanical strength are preserved, the change in corneal refractive power is moderate, and visual recovery is faster.

The advantages of the new triple procedure have increased patients' expectations of visual recovery, and these expectations can be met if the IOL power calculation is accurate. In eyes with corneal endothelial diseases, however, the accuracy of this calculation may be reduced due to the difficulty of determining the corneal power. Anterior surface irregularities caused by epithelial edema impede precise measurement of corneal power when keratometry or Placido disk-based topography is employed. Corneal tomographers that use slit-scanning or rotating Scheimpflug cameras might give reliable measurements in these cases (Fig. 13.1). However, the reliability of these instruments also decreases when the sharpness of the optical cross section acquired during corneal scanning is reduced by stromal edema. If reliable measurement of corneal power is impossible, the keratometric values of the fellow eye should be employed.

To calculate IOL power in candidates to the new triple procedure, the hyperopic shift induced by the endothelial lamellar graft must be taken into account. A number of studies have shown that microkeratome-prepared posterior lamellae change the postoperative sphero-equivalent of manifest refraction by between +0.62 and +1.26 diopters (D) [1–6]. The postoperative hyperopic shift is caused by the endothelial lamellar graft's decreasing the cornea power, by flattening the anterior cornea and steepening the posterior cornea [3, 4, 6–10]. The more significant changes occur on the posterior cornea. Microkeratome-prepared lamellae have a minus lens shape, which alters the corneal thickness profile and increases posterior curvature. In 23 consecutive patients who had undergone DSAEK, the microkeratome-prepared lamella graft was found to have decreased the anterior corneal power by -0.24 ± 0.61 diopters (D), on average, and increased the negative posterior corneal power by -0.96 ± 0.42 D [10].

A number of different methods for IOL power calculation have been proposed to compensate for this reduction in corneal power induced by the posterior lamellar graft. These methods include:

- Selection of an IOL power with predicted refraction more myopic than desired
- Adjustment of the keratometric (K) readings used in the IOL calculation formula
- Optimization of the IOL A constant.

The first method was used by Covert and Koenig [11], who selected IOL power with predicted refraction ranging from −0.50 to −1.15 D, and by Terry et al. [12], who selected implants with predicted refraction ranging from −0.80 to −1.25 D. This method led to accurate IOL power

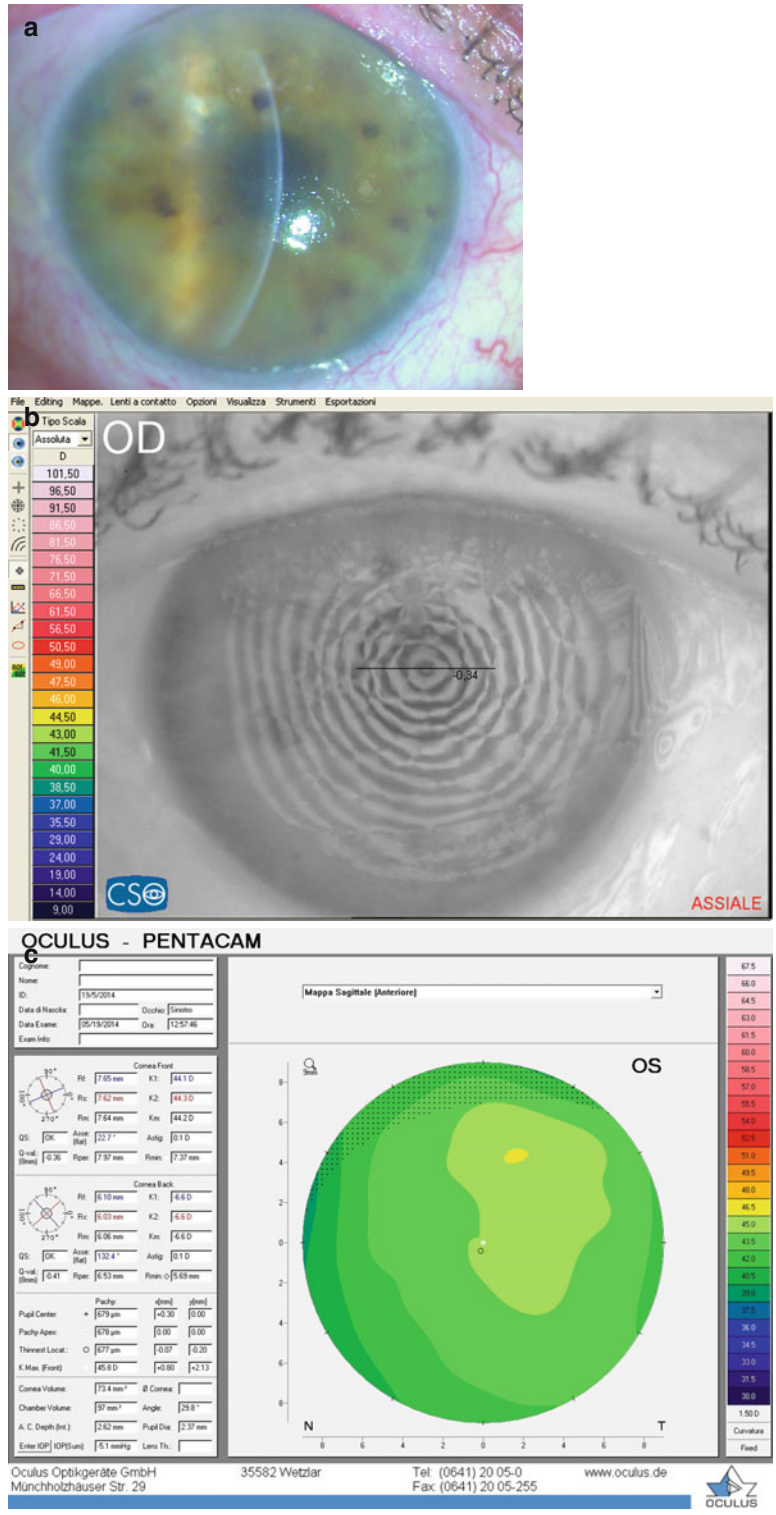

Fig. 13.1 In this eye with epithelial edema due to Fuchs endothelial dystrophy (**a**), irregularity of the corneal surface impeded analysis of the anterior curvature using Placido disk-based topography (**b**). Keratometric readings for intraocular lens calculation were taken using Pentacam HR rotating Scheimpflug camera (Oculus, Wetzlar, Germany) (**c**)

Table 13.1 Refractive error after cataract surgery combined with Descemet's stripping automated endothelial keratoplasty

Author	Number of eyes	Follow-up (months)	Absolute prediction error (D)	Proportion of eyes ±1.00/± 2.00 D of target refraction
Covert and Koenig, *Ophthalmology* [11]	21	6	NA	62 %/100 %[a]
Terry et al., *Ophthalmology* [12]	135	6	NA	74 %/97 %[a]
de Sanctis et al., *Am J Ophthalmol* [13]	39	6	0.59 ± 0.42	83 %/100 %
Bonfadini et al., *Ophthalmology* [14]	30	18.4 ± 9.8	0.61 ± 0.40	83 %/NA

NA not available
[a]Proportion of eyes with achieved refraction within ±1.00 and ±2.00 diopters (D) of target refraction or emmetropia

calculations. After surgery, 62–74 % of eyes were within 1.00 D of emmetropia (Table 13.1). However this method requires complex calculation. The change in corneal power induced by the lamellar graft modifies both the IOL power calculation and the predicted refraction, depending on the biometric characteristics of the eye. Thus, to optimize the use of this method, the degree of myopia in the predicted refraction must be calculated taking corneal curvature, anterior chamber depth, and axial length of each eye into consideration [15].

The adjustment of keratometric (K) readings used for IOL calculation should take into account the average expected reduction of corneal power induced by the endothelial lamellar graft [10]. This method was used in 39 consecutive patients operated for cataract and Fuchs endothelial dystrophy and gave predictable postoperative refractive results (Table 13.1). Six months after surgery, the absolute prediction error (absolute difference between predicted and achieved refraction) was 0.59 ± 0.42 D (range +0.05 to −1.52 D). The achieved refraction fell within ±0.50 D, ±1.00 D, and ±2.00 D of the predicted refraction in 55.5 %, 83.3 %, and 100 % of cases, respectively.

An optimized IOL A constant was used by Bonfadini et al. in 30 eyes undergoing the new triple procedure using pre-sectioned lamellar endothelial graft [14]. This approach significantly decreased the mean absolute error (from 1.09 ± 0.63 D to 0.61 ± 0.40 D; $p = 0.004$) and increased the proportion of eyes falling within ±0.50 (43 % versus 20 %) and within ±1.00 D (83 % versus 50 %) of the target refraction (Table 13.1).

The results of the above studies [11–14] highlight the fact that the refractive outcome of the new triple procedure is highly predictable, provided that the IOL power is calculated taking into account the postoperative refractive shift induced by the lamellar graft. The absolute prediction error is just slightly higher than that normally observed after phacoemulsification with posterior chamber IOL implantation. Seven highly experienced senior surgeons found a mean absolute prediction error of 0.25 D after simple phacoemulsification with posterior chamber IOL implantation [16], a result that is considered a benchmark of excellence for cataract surgery. In other studies, the mean absolute prediction error after phacoemulsification with IOL implantation was comparable to that obtained after the new triple procedure, varying between 0.32 and 0.71 D [17–21].

The accuracy of IOL power calculation, and thus the postoperative refractive outcome of the triple procedure, might further be improved by combining cataract surgery with DMEK. DMEK grafts, which contain only donor Descemet's membrane and endothelium, should induce very slight changes in corneal power. The minus lens effect cannot occur, because the grafts do not contain donor stroma. However, a postoperative hyperopic shift has also been reported using this technique [22, 23]. Ham et al. [22] analyzed

corneal power by Scheimpflug imaging and showed that the negative power of the posterior cornea increased on average by +1.00 D after DMEK. The study authors attributed this change to the postoperative de-swelling of the posterior stroma, which leads to a steepening of the posterior corneal curvature. Lasser et al. also reported a postoperative hyperopic shift in 61 eyes that underwent DMEK combined with phacoemulsification and IOL implantation [23]; they suggested selecting IOL power with a predicted refraction of −0.75, to optimize postoperative results. Using this approach, 54.5 % of eyes were within 1 D of emmetropia and 77.3 % were within 2 D of emmetropia, 6 months after surgery.

Cataract Surgery Combined with Penetrating Keratoplasty

Cataract surgery combined with PKP is routinely performed for the simultaneous surgical treatment of cataract and corneal stromal diseases, such as ectasia, postinfectious scars, traumatic leukomas, and dystrophies. In eyes scheduled for cataract surgery combined with PKP, IOL power calculation is truly challenging: the postoperative refractive power of the corneal graft is extremely variable, the eye's axial length may change after the procedure, and the reliability of theoretical formulas that calculate the effective lens position from the preoperative corneal curvature and axial length is reduced [24].

The great variability of postoperative corneal power is caused by the full-thickness trephination of the recipient cornea and the suturing of the donor tissue. After suture removal, the corneal power may be below 40 D or above 48 D. Since the postoperative corneal power is highly unpredictable, Katz and Foster have suggested using the keratometric readings of the fellow eye to calculate IOL power [25]. However, this approach is only suitable for patients with unilateral diseases and leads to unpredictable refractive results. Today, many surgeons use the average postoperative keratometric readings obtained from a previous series of corneal grafts; for this purpose, the series should comprise grafts performed using a surgical technique that is standardized in terms of trephination method, donor-recipient disparity, and suture technique. However, the resulting predictability of postoperative refractive outcome is only moderate. Davis et al. [26] report on a series that included 106 eyes; they found postoperative sphero-equivalent values in the range of – 6.00 D to +4.00 D and differences from the target refraction of ≥2.00 D in 48 % of cases. Javadi et al. reported similar results [27]; in a series of 76 interventions, the postoperative sphero-equivalent values ranged from −6.55 to +3.78 D and the difference from target refraction was ≥2.00 D in 54 % of cases.

The refractive results would be better if phacoemulsification with IOL implantation were performed as a secondary procedure, after the corneal graft [28]. However, the surgical trauma due to cataract extraction increases postoperative endothelial cell loss, and this two-step approach delays postoperative visual recovery, since cataract surgery is not usually performed until 12–24 months after PKP, when all sutures have been removed.

IOL Calculation in Patients with Prior Corneal Graft

Cataract extraction is the most frequent clinical situation that requires IOL power calculation in eyes with prior corneal graft; other special circumstances include piggyback IOL and phakic IOL implantation. In these clinical situations, IOL power calculation should be planned 2–3 months after suture removal, when serial topographical analysis demonstrates corneal curvature to be stable.

Cataract Surgery in Eyes with Previous Corneal Graft

Cataract occurs quite commonly in eyes with prior corneal graft, because of preoperative and postoperative intraocular inflammation, surgical trauma, and prolonged use of corticosteroids. Cataract is frequently associated with clinically

significant corneal astigmatism. The multicenter Corneal Transplant Follow-up Study showed that corneal astigmatism after keratoplasty was ≥4 D in 43 % of eyes and ≥6 D in 20 % of eyes [29]. In eyes with marked corneal astigmatism, postoperative visual recovery is generally only modest after phacoemulsification with monofocal IOL implantation. After surgery, anisometropia makes this refractive error difficult to correct fully by means of spectacles. Contact lenses are difficult to fit and frequently not tolerated and carry the risk of severe complications. Moreover, keratorefractive procedures to correct severe astigmatism on corneal grafts have moderate predictability and high complication rates.

Phacoemulsification with toric IOL implantation provides an opportunity to correct both corneal astigmatism and cataract with a single procedure. The first toric IOL for correcting post-PKP astigmatism was implanted during cataract surgery in 1999 [30]. It was made of PMMA and required a 6 mm incision. Since then, many toric IOL models, made of different materials and with different designs, have become available (Table 13.2). The surface adhesiveness of acrylic materials and the new designs that have been introduced have increased toric IOL stability in the capsular bag and decreased the risk of postoperative rotation [31].

A number of studies and case reports have found phacoemulsification with toric IOL implantation to be effective for the simultaneous correction of post-keratoplasty astigmatism and cataract [30, 32–37]. The largest series was

Table 13.2 Toric IOLs available in Europe

Toric IOL model	Material	Haptic	Diameter (mm)	Power (diopters)		Incision size (mm)
				Sphere	Cylinder (steps)	
AcrySof (Alcon)	Hydrophobic acrylic	Loop	13.0	+6.0/+30.0	1.5/6.0 (0.75)	2.2
AF-1 toric (Hoya)	Hydrophobic acrylic PMMA haptic tips	Loop	12.5	+6.0/+30.0	1.5/3.0 (0.75)	2.0
Acri.Comfort/ AT Torbi[a] (Zeiss Meditec)	Hydrophobic acrylic with hydrophobic surface	Plate	11.0	+10.0/+32.0	1.0/12.0 (0.50)	<2.0
Fil 611 T (Soleko)	Hydrophilic acrylic	Plate	11.8	+5.00/+30.0	1.0/6.0 (0.50)	2.0
Lentis Tplus (Oculentis)	Hydrophobic acrylic with hydrophobic surface	Loop/plate	12.0/11.0	0/+30.0	0.25/12.0 (0.75)	2.6
LAL (Calhoun Vision)	Silicone with PMMA haptics	Loop	13.0	+17.0/+24.0	0.75/2.0	3.0
MicroSil/Torica[a] (HumanOptics)	Silicone with PMMA haptics	Loop	11.6	−3.5/+31.0	2.0/12.0 (1.0)	3.4
Morcher 89A (Morcher GmbH)	Hydrophilic acrylic	Bag in lens	7.5	+10.0/+30.0	0.5/8.0 (0.25)	2.5
Staar (Staar Surgical)	Silicone	Plate	10.8/11.2	+9.5/+28.5	2.00 or 3.5	2.8
T-flex[a] (Rayner)	Hydrophilic acrylic	Loop	12.0/12.5	−10.0/+35.0	1.0/11.0 (0.25)	<2.0
Tecnis toric (AMO)	Hydrophobic acrylic	Loop	13.0	+5.0/+34.0	1.0/4.0 (0.5/1.0)	2.2

[a]Toric IOLs available with customized cylinder powers

reported by Wade et al. [38] and included 21 eyes with cataract and mean post-PKP corneal astigmatism of 4.57 ± 2.05 D. After phacoemulsification with implantation of a single-piece acrylic toric IOL (SN6AT, Alcon, Fort Worth, USA), the uncorrected distance visual acuity (UDVA) was ≥20/30 in 67 % of eyes, and the refractive astigmatism was within 1 D of the predicted value in 76 % of eyes.

Toric IOL with customized cylindrical power can correct very severe corneal astigmatism. A hydrophilic acrylic toric IOL (T-flex 623 T, Rayner, UK) with customized cylindrical power was used in 3 eyes with cataract and post-PKP astigmatism ranging from 6.75 to 8.75 D [35]. After phacoemulsification, rotation of the toric IOL was <5°. The UDVA was ≥20/40, and residual refractive astigmatism was less than 1.00 D in all three cases (Fig. 13.2).

Calculation of toric IOL power requires careful preoperative assessment of corneal astigmatism. Cases with irregular astigmatism are contraindicated. The magnitude and principal meridians of corneal astigmatism must be measured, using manual/automatic keratometers, Placido disk-based topographers, and corneal tomographers. Corneal tomographers analyze the contributions made by both the anterior and the posterior corneal surfaces. Working from multiple measurements, it is possible to compare data and to determine the principal meridians precisely. The angular position of the principal meridians is the point of reference for aligning the toric IOL during surgery; an error of 5° or 10° in positioning the IOL reduces the efficacy of cylindrical correction by 15 % or 30 %, respectively.

Surgically induced astigmatism must be taken into account. It varies with position (temporal/superior), site (corneal/scleral), and length of the incision. A surgeon should determine his/her personal value from a series of patients he/she has operated previously, using the same incision technique.

A number of companies have developed online software to calculate toric IOL power. The corneal power and astigmatism, surgically induced astigmatism, incision position, anterior chamber depth, axial length, and target postoperative refraction must be entered. Then the IOL spherical/cylindrical power and the residual refractive cylinder are automatically calculated.

Online software offers simple and fast access to toric IOL power calculation. However, some of the available packages suffer from a major limitation; they calculate IOL toric power using a fixed ratio between cylindrical power at the IOL plane

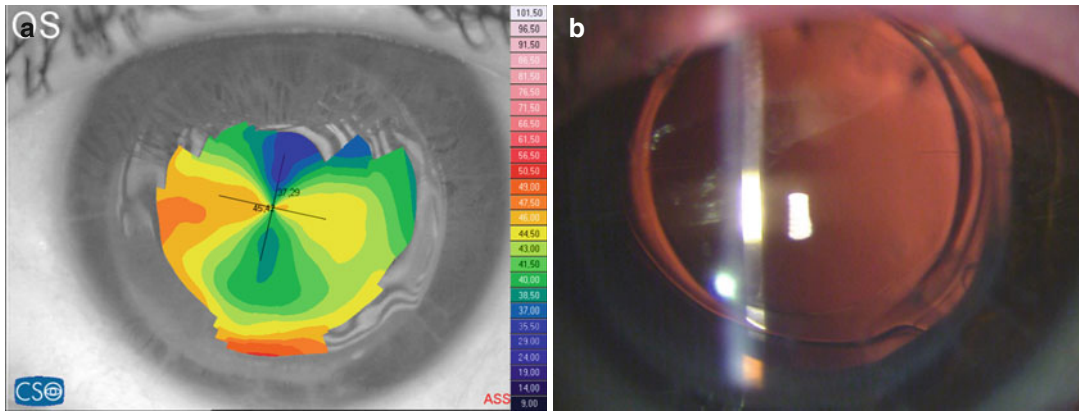

Fig. 13.2 This patient's left eye showed high corneal astigmatism after penetrating keratoplasty 8.13 D × 172°; (**a**) and cataract extraction. The uncorrected and corrected distance visual acuities were 20/200 and 20/40, respectively, and the manifest refraction was +1.00–9.00 × 80. Phacoemulsification with implantation of a customized +10.50 sphere/+11.00 cylinder T-flex 603 (Rayner, UK) was scheduled. After surgery, the customized toric IOL was well aligned (**b**). The uncorrected and corrected distance visual acuities were 20/30 and 20/25, respectively, and the manifest refraction was −1.00 × 70

and at the corneal plane. This method can cause significant errors in determining toric IOL power in eyes with short or long axial lengths [39]. The effective IOL cylindrical power at the corneal plane is a function of the effective lens position and the sphero-equivalent power of the IOL. More precise calculation entails converting the IOL cylindrical and spherical powers into the two principal lens powers [40]. Both lens powers are calculated to the corneal plane using a standard vertex formula. The difference between the two lens powers at the corneal plane is then used to select the IOL cylindrical power.

Piggyback IOL

The need for a primary piggyback IOL might arise in eyes with extreme hyperopia that undergo cataract surgery simultaneously with, or after, corneal graft. A secondary piggyback IOL, however, is more frequently required to treat post-keratoplasty refractive errors in pseudophakic eyes.

Secondary piggyback IOLs are placed in the sulcus between the anterior surface of the primary IOL and posterior surface of the iris. These IOLs should have a large optic diameter with rounded edge and haptic length sufficiently large for the size of the ciliary sulcus. For many surgeons, the Staar Surgical AQ5010 (powers from −4.00 D to +4.00 D) and AQ2010 (powers from +5.00 upward) have been the favorite models. They are 3-piece silicone IOLs with rounded edges, 6.3 mm optic diameter, and 13.5 mm haptic diameter. The Sulcoflex (Rayner, UK) is an IOL specifically designed to be placed in the ciliary sulcus as secondary piggyback. It is a single-piece hydrophilic acrylic IOL, with a round-edged 6.5 mm optic and 14 mm undulating round-edged haptics. The optic has a concave posterior surface to prevent contact with the IOL in the bag. The haptics have a 10° angle to prevent contact with the iris. The aspherical model is available in half-diopter steps from −10 D to +10 D. The toric model is available over the range of −6 D to +6 D, with up to 6 D of cylindrical correction in half-diopter steps.

The calculation of piggyback IOL power is independent of eye axial length; it is calculated from the sphero-equivalent of the patient's refractive error, using the Holladay refraction formula [41]. The sphero-equivalent is multiplied by 1.50, in the case of hyperopic error, and by 1.0 in the case of myopic error. Several variations of this formula have been proposed [42, 43].

Phakic IOLS

Iris-fixated and posterior chamber phakic IOLs are available for the correction of post-keratoplasty refractive errors in phakic eyes (Table 13.3). Calculation of the phakic IOL power is independent of the eye's axial length. It is provided by the manufacturer on receipt of the

Table 13.3 Phakic IOLs

Phakic IOL model	Material	Haptic	Diameter (mm)	Power (diopters)		Incision size (mm)
				Sphere	Cylinder (steps)	
Artisan (Ophtec) Verisyse (AMO)	PMMA	Iris claw	7.50/8.50 5.00/6.00	−23.5/+12.0	1.00/7.50 (0.50)	5.2/6.2
Artiflex (Ophtec) Veriflex (AMO)	Silicone with PMMA haptics	Iris claw	8.50/6.00	−2.0/−14.5	1.00/5.00 (0.50	3.2
Visian ICL[a] (Staar Surgical)	Collamer	Plate	–	−3.0/−23.0 +3.0/+21.0	1.00/6.00 (0.50)	3.0

[a]The toric model of Visian Implantable Collamer Lens is available only with negative spherical powers

following measurements: spherical and cylindrical error, keratometric readings of the principal meridian, anterior chamber depth, and postoperative refraction target. For the posterior chamber ICLs (implantable collamer lenses), white-to-white measurement is also required. This is a critical step for proper calculation of IOL sizing and vaulting in the ciliary sulcus. The horizontal white-to-white is measured using validated calipers and topographers. Ultrasound biomicroscopy and very-high-frequency ultrasound can be employed to measure the posterior chamber diameter.

Conclusions

The biggest challenge in calculating IOL power for grafted versus normal eyes is to determine the corneal power accurately. In eye candidates to the triple procedure, this problem has been significantly reduced by the advent of endothelial keratoplasty techniques. These techniques induce small changes to the corneal power; however, these must be taken into account when calculating IOL power. This approach leads to highly predictable postoperative refractive results, which are very close to those obtained after simple phacoemulsification.

In eyes with prior graft, IOL power calculation is required for cataract extraction and to correct postoperative errors. In these eyes corneal astigmatism is frequently high, and IOL selection comprises pseudophakic and phakic toric IOL models that provide the opportunity to correct both spherical and cylindrical errors simultaneously. Precise assessment of the magnitude and orientation of corneal astigmatism is crucial to optimize calculation of the power of these IOLs.

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Mechanical Microkeratomes

14

Elena Albé and Massimo Busin

Abstract

During the last decade several techniques of lamellar keratoplasty (LK) have been developed with the purpose of retaining the advantages of penetrating keratoplasty (PK) while avoiding the removal of healthy portions of the cornea, thus selectively replacing the dysfunctional parts, limiting the rate of rejection, and increasing long-term graft stability. This chapter will review the different instruments and techniques to prepare donor tissue for endothelial keratoplasty (EK) and deep anterior lamellar keratoplasty (DALK). Descemet's stripping automated endothelial keratoplasty (DSAEK) foresees the transplantation of a donor graft consisting of endothelium, Descemet's membrane, and a variable amount of posterior stroma in case of eyes with decompensated endothelium. In order to optimize visual rehabilitation, the present trend is toward minimizing the amount of stroma transplanted, and this can be done with both single- and double-cut procedures. DALK has been gaining popularity as the optimal approach for treating non-endothelial disorders affecting Bowman's layer and stroma. Hand dissection of the stroma is technically difficult, and the quality of the surfaces obtained is rarely compatible with optimal vision, while pneumatic dissection technique as the "big bubble" is difficult to learn and can be complicated by micro-macro perforations making a conversion to PK necessary. As an alternative, microkeratome-assisted LK has the advantage of being a standardized, technically easy procedure, yielding extremely smooth dissected surfaces, therefore compatible with 20/20 vision.

Keywords

Microkeratome • Artificial anterior chamber • Descemet's stripping automated endothelial keratoplasty • Deep anterior lamellar keratoplasty • Microkeratome-assisted lamellar keratoplasty

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J. Hjortdal (ed.), *Corneal Transplantation*, DOI 10.1007/978-3-319-24052-7_14

Introduction

During the last decade several techniques of lamellar keratoplasty (LK) have been developed with the purpose of retaining the advantages of penetrating keratoplasty (PK) while avoiding the removal of healthy portions of the cornea, thus selectively replacing the dysfunctional parts, limiting the rate of rejection, and increasing long-term graft stability.

At present the main use of microkeratomes for therapeutic keratoplasty is related to the preparation of donor tissue for endothelial keratoplasty. Descemet's stripping automated endothelial keratoplasty (DSAEK) foresees the transplantation of a donor graft consisting of endothelium, Descemet's membrane, and a variable amount of posterior stroma. In most countries, this technique has quickly replaced PK for the treatment of eyes with decompensated endothelium, and in the USA DSAEK has become the keratoplasty procedure performed most frequently. In order to optimize visual rehabilitation, the present trend is toward minimizing the amount of stroma transplanted, and this can be done with both single- and double-cut procedures. To date, other techniques utilizing either femtolaser-assisted or manual dissection have not proven as effective as microkeratome-assisted DSAEK in achieving comparably smooth surfaces and are not used routinely by any high-volume keratoplasty surgeon.

Instead, despite the obvious advantage of keeping in place the healthy recipient endothelium, deep anterior lamellar keratoplasty (DALK) has been slowly gaining popularity as the optimal approach for treating non-endothelial disorders affecting Bowman's layer and stroma. Hand dissection of the stroma is technically difficult, and the quality of the surfaces obtained is rarely compatible with optimal vision, while the pneumatic dissection utilized for the so-called "big bubble" technique is difficult to learn and can be complicated by micro-macro perforations making a conversion to PK necessary. As an alternative, microkeratome-assisted LK has the advantage of being a standardized, technically easy procedure, yielding extremely smooth dissected surfaces, therefore compatible with 20/20 vision. However, microkeratomes are relatively imprecise, and in most cases the surgeon cannot control adequately both the diameters and the thickness of the cuts by using interchangeable heads. In general, to minimize the risk of intraoperative perforations, the surgeon should plan on leaving a recipient residual bed at least 100 μm in thickness. In keratoconic eyes, to reestablish a physiologic corneal thickness, the donor graft should be about 100 μm thicker than the excised lamella. Also, matching the diameter of the lamella removed from the recipient cornea with that of the donor tissue prepared from a cornea mounted on the artificial chamber is difficult. Therefore, it is advisable to simply punch the donor tissue to the diameter required after measuring the diameter of the excised lamella.

Artificial Anterior Chamber

In 1976 Ward and Nesburn described a way to trephine the donor cornea from the anterior surface when the donor corneal-scleral tissue was mounted on an instrument that formed a seal around the scleral rim of the excised donor cornea, allowing the endothelium to be supported physically by the liquid storage medium [1, 2]. The artificial anterior chamber (AAC) protected the donor endothelial cells from damage as if they had been still in an intact globe and was subsequently modified and improved to allow trephination of the donor tissue up to any desired depth.

AACs are presently used mainly for both manual and automated lamellar dissection of the donor corneas. The goal is to obtain a corneal lamella of the required thickness and diameter, by means of an easy procedure with minimal risk of complications.

ACCs are available on the market both as reusable and disposable devices. The former can be used repeatedly and include the Moria (Antony, France) and the Bausch & Lomb (Rochester, NY) AACs (all for manual dissection) and the Moria ALTK system (for microkeratome-assisted dissection). The Moria AAC utilizes the Evolution 3 console (Moria, Inc.) that is fully compatible with

all Moria microkeratomes (LSK, M2, CB units). It has two pumps that provide a quick and stable vacuum for the procedure. The Moria ALTK system can be used for automated therapeutic lamellar keratoplasty or for DSAEK. In this system the high-speed, high-power turbine (30,000 cuts/min) creates a smooth keratectomy for a seamless-edge margin. The single-piece construction of the microkeratome heads are pre-calibrated for various depths of cuts (90–400 μm). Both Bausch & Lomb and Katena ACCs can be used for manual dissection of donor lamellar tissue. They are not designed to be used with a microkeratome. The pressure inside the chamber is optimized by injecting fluid from the attached syringe and closing the valve to maintain the system pressure. The Barron AAC (Katena, Inc., Denville, NJ, USA) is a sterile, disposable device consisting of three pieces.

Microkeratome

Initially, microkeratomes were developed to treat refractive errors in a safe and reproducible way. The first manual microkeratome was developed in 1958 by José Ignacio Barraquer, the initiator of modern corneal refractive surgery. The device had a cutting angle of 0° for keratophakia and keratomileusis [3]. Until 1984 the microkeratome was used to cut a free corneal flap, which was frozen, cryolathed on its posterior surface, thawed, and then sutured back in place [4]. In 1985 Krumeich and Swinger introduced the non-freeze keratomileusis technique. The lenticule was processed unfrozen on its stromal side with a BKS 1000™ refractive set [5]. In 1986, Ruiz developed the in situ keratomileusis technique, in which a microkeratome was used to make two consecutive cuts with a diameter and depth that varied according to the degree of ametropia, using a set of suction rings of different sizes, calibrated applanation lenses, and various plates [6]. In 1991 Dr. Ioannis Pallikaris introduced the concept of corneal hinge [7]. Motorized microkeratomes, of which the Castroviejo electrokeratome (unveiled in 1963) was the precursor, became available in the same year. Ruiz and Lenchig's Chiron automated corneal shaper (ACS), equipped with a system of gears to ensure a constant rate of movement on the suction ring, was developed to create a more reproducible corneal flap with a nasal hinge. The height of the suction ring could be adjusted to vary the diameter of the second cut, avoiding the need of changing rings. However, this automated lamellar keratoplasty (ALK) technique was imperfect and poorly reproducible; it was abandoned in 1995 in favor of LASIK, in which photoablation with a 193 nm excimer laser replaced the second refractive mechanical cut [8]. In 1996 the Carriazo-Barraquer pivoted rotating microkeratome (CB) was presented to the ophthalmic community with the theoretical advantage of allowing the hinge to be placed wherever required. The use of an upper hinge limited the risk of flap displacement during blinking. Moria, the company that manufactured the device, also markets disposable adaptable microkeratome heads. In 1997, Chiron released the Hansatome automated microkeratome, that also produces a corneal flap with an upper hinge. In 2001 Carriazo unveiled the first generation of pendular microkeratomes (Carriazo-Pendular, Schwind). The cutting head of this device moves vertically, rather than horizontally, and the pendular motion requires a slightly lower cutting pressure than do conventional microkeratomes, leading to less mechanical friction and smaller size.

Over the last decade, femtosecond laser-assisted dissection has replaced microkeratome-assisted dissection in the hands of most refractive surgeons. Instead, therapeutic keratoplasty, especially DSAEK, has become the main field of use for these instruments, with new devices being marketed recently (Gebauer, Horizon).

Description of Mechanical Microkeratome

The last several years have seen many microkeratome models. Manufacturers continue to offer new and improved versions of equipment in an attempt to improve results. However, a microkeratome still consists of few basic components: a peripheral

part, a central unit, and connections/accessories. The microkeratome itself is the actual peripheral component with three main parts: the suction ring, the microkeratome cutting head, and the drive unit. The *suction ring* fixates and stiffens the globe during the keratectomy. The choice of suction ring depends on the corneal curvature and on the flap diameter required. The upper part of the suction ring, called "plate," can have variable diameters and allocates the protruding cornea during the cut. The microkeratome head tracking system is part of the plate: it consists of two parallel dovetail grooves for rectilinear translation or a single arciform rail (smooth or geared). In the latter case the rotational axis is an eccentric plot located diametrically opposite the rail. A stopper is used to create hinges with variable diameters. The central part of the suction system is connected to the suction chamber of the ring by a supple silicone tube either fixed to a hollow hand grip or connected directly to the ring. The skirt is the vertical outer part of the suction ring. Its lower edge must ensure hermetic sealing to allow proper suction. Some manufacturers offer a choice of suction rings with different angulations of the lower edge of the skirt. The correct choice of ring minimizes the risk of complications during flap creation in eyes with extremely low or extremely high keratometric values. The ring also determines the diameter of the flap. In some models the diameter of the aperture of the ring may vary; in others the diameter of the flap to be obtained is determined by the ring thickness. Some manufacturers provide a nomogram to help choose the ring according to the required flap diameter and to adjust the hinge support.

The *microkeratome cutting head* consists of a non-vibrating block and an oscillating blade/blade holder unit. The latter unit is either assembled on the operating table or delivered premounted and sterile (for single use). The block is composed of a tracking system, an applanation plate, and cavities. The first one matches the suction ring and can be dual (linear microkeratomes) or single (pivoted rotating microkeratomes). The applanation plate is the part of the head that precedes the oscillating blade and flattens the cornea, allowing a constant angle of engagement for the blade, which in turn yields constant flap thickness. Each head is designated by a number in microns corresponding to the height of its guard (space between the blade and the applanation plate). This height does not always correspond exactly to the predicted flap thickness. Variations within few microns from the predicted values are considered tolerable. There are usually two cavities to accommodate the oscillating blade/blade holder unit and the flap itself. Some microkeratomes use disposable sterile heads with preassembled blades. The blade is usually made of stainless steel or sometimes of chrome-platinum. The angle of tissue engagement varies from 24° to 30°, and the standard oscillation speed of the blade is approximately 15,000 rpm. The head translation could be *linear* when the cutting head is guided by two parallel rails in the horizontal plane and can create only a nasal hinge, *arciform* when the cutting head is guided in the horizontal plane around an eccentric axis by a single smooth or geared arciform rail offering a wide choice of hinge positions, or *pendular* as with the Carriazo-Pendular microkeratome, which has a pendulum-like cutting action via a horizontal axis located above the corneal apex. Many other factors can influence the flap thickness. It varies proportionally to the intraocular pressure (IOP) reached while cutting and to the preoperative pachymetry and inversely to the oscillation blade and head translation rates. The excised lamella may be often 30–40 microns thicker than planned due to tissue compression while passing through the microkeratome slit.

The motorized *drive unit* is connected on one side to the unit; the other side is clipped or screwed to the microkeratome head in order to ensure that the drive axis fits the blade properly and oscillates correctly. The drive unit consists of a single or dual electric motor or a gas turbine for blade oscillation in models with manual head translation.

The central unit delivers the calibrated energy necessary to power the motors and creates the vacuum between the eyeball and the suction ring. One pedal is used to start/stop the vacuum pump. A second pedal controls blade oscillation and in some automated microkeratomes the forward translation of the head.

Head propulsion can be automated or manual. The first has the advantage of offering a regular speed of cut and therefore, in theory, a constant flap thickness. In contrast, if premature blade stop occurs, withdrawal can be difficult with a high risk of flap damage. Manual drive is strictly surgeon dependent: the surgeon determines the speed of head translation, which can be inconsistent and create an irregular flap thickness and bed surface.

To reduce the incidence of infections, single-use, sterile components have become increasingly popular and include the head alone (equipped with a preassembled blade), both head and the suction ring, and all peripheral components (suction ring, preassembled head and blade, and handpiece).

Technical Considerations

Several variables are important in determining the overall bed smoothness and depth consistency. These variables include blade quality, engagement angle of the blade into the cornea, blade translation rate and consistency across the cornea, suction pressure and overall globe fixation, blade oscillation rate, and cut mechanism.

- Overall blade quality and sharpness play a role in the quality of flap cut. Electron microscopy has been performed on blades from several different manufacturers, showing substantial difference [9]. Several reports also demonstrate that blades used on the second eye yield up to 10 % thinner flaps. Theoretically, this would be due to some dulling of the blade during the first pass.
- Variations in blade engagement angle exist from 0° to 30°. A steeper angle of blade engagement allows the predetermined flap depth to be reached more rapidly; therefore, a "gutter" is created at the initial incision site. As a result, the flap has a more uniform thickness and is easier to align at the conclusion of the procedure. A more shallow blade engagement angle results in thinner flap edges and may subsequently limit flap stability. Aside from the Innovatome (Innovative Optics) at 6° and the Hansatome (Bausch & Lomb) at 14°, all other instruments fall between a 25° and 30° angle of engagement.
- Theoretically, a quicker pass could result in a thinner flap. The exact difference in the pass speed resulting in a substantial difference in flap thickness has yet to be determined. Automated drive models would intuitively seem to provide a more consistent excursion rate, but no data have shown a clinically relevant advantage. Independent motor drives for blade movement rate and oscillation are ideal and are the standard in today's market. Manual drive models have rather smooth sliding mechanisms but are obviously dependent on the surgeon to provide smooth and consistent blade movement. Blade oscillation is essentially independent of travel, but a quicker pass by the surgeon could result in a thinner flap as the blade "skims" through the cornea. A more rapid pass could also lead to increased resistance, thereby relatively slowing the cut rate.
- Most systems require elevated intraocular pressure created by a vacuum ring or device to stabilize the cornea. Variations from eye to eye do exist in the vacuum obtained. It is generally considered that an intraocular pressure (IOP) higher than 65 mmHg is necessary to assure a high-quality dissection, but some reports have shown that IOP may rise much higher in some instances. In addition, relative barometric pressure does vary based on altitude and could have an impact on final flap characteristics. The correlation between the amount of vacuum generated by the suction ring and the true intraocular pressure at the time of dissection on one side and flap thickness on the other side is as yet unclear.
- The blade cutting rates range from 2500 to 20,000 oscillations per minute. While a minimum speed probably does exist below which flap quality is negatively affected, rates above 10,000 rotations per minute are used routinely and do seem to dissect the tissue adequately.
- All traditional microkeratomes in use today employ surgical stainless steel blades. Chrome-platinum alloy and crystalline blades

made of sapphire or diamond were used in earlier models but have fallen out of favor. Water under high pressure in the form of a "blade" is also available (HydroKeratome, VisiJet, Inc.). This mechanism should dissect the tissue, at least theoretically, along lamellar planes, thus minimizing damage. However, issues related to tissue hydration have not allowed perfection of this system into clinical use. More recently, femtosecond lasers (Pulsion FS, IntraLase) have been introduced. This technology allows mid-stromal vaporization of corneal tissue with a seemingly limitless list of potential applications, besides its application for flap creation.

Flap Thickness Considerations

Most surgeons agree that consistency of flap depth is paramount during refractive procedures. The 250 μm stromal bed thickness minimum is resoundingly regarded as the thinnest allowable unadulterated cornea that will maintain its long-term structural integrity. Unfortunately, there is simply no accurate way to determine flap or residual stromal bed thickness. Ideally, to avoid ablating too much stroma, the surgeon could verify the residual bed thickness intraoperatively. However, ultrasonic pachymetry requires some surface fluid and can be inconsistent. By subtracting the residual bed thickness measurement from the preoperative full thickness, the surgeon can occasionally find significant discrepancies. While confocal microscopy and Orbscan topography may be helpful, neither technology can be used intraoperatively, when the surgeon needs it most. Assembly has been adequately simplified, and nearly all systems offer some type of self-diagnostic program requiring adequate vacuum and gas pressure (if appropriate) prior to proceeding. Newer instruments offer a one-piece design in which the fixation ring and microkeratome head are assembled prior to placement on the eye. This serves the purpose of simplifying surgery and may shorten the learning curve.

Microkeratome Complications

Several studies have been carried out considering microkeratome complications on a large number of patients undergoing LASIK, which is the most used technique in which the flap is cut by means of a microkeratome [10]. Jacobs and Taravella showed that there was a cumulative complication rate of 0.30 % in over 28,000 cases, including failure to achieve the appropriate intraocular pressure (IOP), partial flaps, buttonholes, thin or irregular flaps, and free flaps [11]. A larger study by Nakano et al. on more than 47,000 eyes also showed that different microkeratome platforms have statistically different intraoperative complication rates, being more common with the automated corneal shaper (1.26 %) than with the Hansatome (0.63 %) and MK-2000 (Nidek, Inc., Fremont, California, USA) (0.63 %) [12]. The most recent review (NIDEK-MK-2000) of 26 600 eyes also showed a low rate (0.24 %) of similar complications [13].

Recently, several studies have attempted at identifying risk factors influencing the occurrence of complications. One main target of microkeratome critics has been the inconsistent flap thickness obtained with their use. Consistent predictable flaps are important because flap thickness is a relevant variable; flaps constructed with the 130 μm microkeratome head exhibited a significantly lower rate of epithelial defects than the 100 or 150 μm head. Some studies also found that epithelial defects were less likely to occur with disposable heads than with reusable heads [14–16].

Other reported risk factors for epithelial defect formation during LASIK include increasing patient age (especially over 40 years), preoperative hyperopia, years of contact lens wear, and intraoperative epithelial damage in the first eye during simultaneous bilateral LASIK [17, 18].

Preoperative keratometric values were found to affect the incidence of intraoperative complications as well. Eyes with flatter corneas tended to have more free caps and incomplete flaps, whereas eyes with steeper corneas were associated with more epithelial abrasions and thin or irregular flaps [19].

Technique: Microkeratome-Assisted Lamellar Keratoplasty for Keratoconus

A radial marker stained with gentian violet is used to obtain radial marks on the recipient cornea. Then a suction ring is applied to the eye and the intraocular pressure increased over 65 mmHg. BSS is instilled on the corneal surface, and a hand-driven microkeratome (Carriazo-Barraquer, ALTK, Moria, Paris, France) is advanced in the track until anterior corneal lamella is completely severed from the underlying recipient stroma (Fig. 14.1). During surgery maximal care is taken to sweep the microkeratome very slowly across the cornea, thus letting the instrument safely engage the recipient tissue and avoiding formation of buttonholes. The instrument is a manual, translational microkeratome. It offers a single-piece head with easy assembly. Different head slit widths and suction rings allow a customized keratectomy. The 200 μm microkeratome head is used in corneas with a minimum corneal thickness above 300 μm, whereas for thinner corneas the 130 μm microkeratome head is preferred. The diameter of the excised lamella is measured using a caliper. Then the microkeratome with a 300 μm head is employed to prepare the lamellar graft from the donor cornea (a corneoscleral button with at least 2 mm scleral margin on each side), which is placed and centered on the artificial anterior chamber of the ALTK system (Moria, Paris, France) and then locked. The pressure inside the artificial anterior chamber is raised up to approximately 60 mmHg. The lamella is cut as large as possible and then punched to the desired size. The quality of the donor tissue is checked under the operating microscope and if it is found to be unsatisfactory, a new graft can be prepared. Finally the lamellar graft is sutured in place under tension.

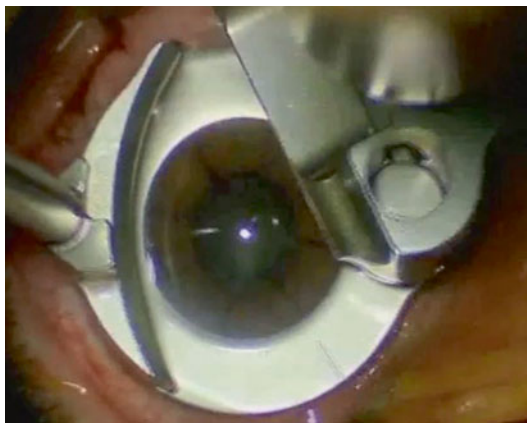

Fig. 14.1 Automated lamellar microkeratome 300 μm cut in a patient with granular dystrophy

A more recent modified technique of microkeratome-assisted LK consists of removing the anterior lamella from the host cornea and performing a partial trephination of the recipient bed with a 6.5 mm trephine. Then the Descemet's membrane is exposed with a big bubble technique and donor tissue is sutured in place. The donor graft is cut about 100 μm thicker than the excised corneal lamella.

Technique: Donor Preparation for DSAEK and Ultrathin DSAEK

The following surgical technique is broadly described in Busin et al. [20, 21]. Central corneal thickness (CCT) is initially measured using ultrasound pachymetry. During the whole procedure, the ideal pressure in the artificial anterior chamber (AAC) is maintained by raising the infusion bottle at a height of 120 cm and clamping the tubing at about 50 cm from its entrance into the AAC. The first *debulking* cut is performed using a Carriazo-Barraquer (Moria, Antony, France) microkeratome with a 300 μm head. Pachymetry is then performed again to determine the residual tissue thickness. The second *refinement* cut is made with a 90, 110, or 130 μm microkeratome head, depending on the residual tissue thickness, with the goal of ultimately creating a graft that is approximately 100 μm or less (Fig. 14.2). For the second cut, the dovetail of the AAC is rotated by 180° in order to perform the second cut from a direction opposite to that of the first cut. In fact the depth of dissection is maximum at the beginning of the cut, and insisting with both cuts on the same spot would increase the risk of perforation and produce grafts

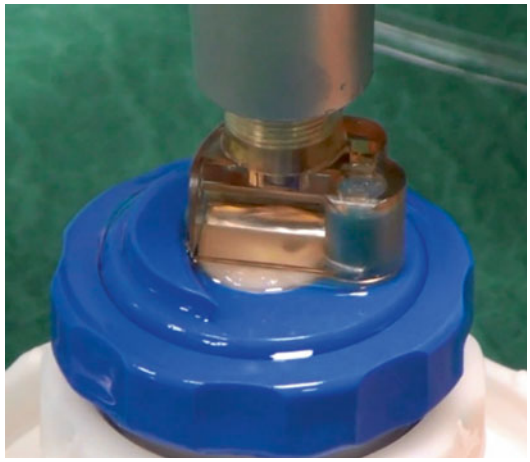

Fig. 14.2 Refinement cut during UT-DSAEK procedure

of uneven thickness. Instead, the planar grafts obtained with this procedure are very thin but unlike DMEK grafts do not tend to roll onto themselves, thus allowing a relatively easy manipulation. The tissue is placed on a Barron punch with the endothelial side up and cut to the desired diameter (8.5–9.0 mm), and the stromal side can be marked to facilitate correct intraoperative orientation of the graft. A dedicated mini-glide is used to deliver the UT graft. The tissue roll obtained with UT grafts can pass easily through a small opening without being squeezed or damaged, and the mouth of the glide can be therefore inserted into a 3 mm wound to allow optimal tissue delivery.

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Technology: Femtosecond Laser in Keratoplasty

15

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Abstract

Femtosecond lasers offer a controlled, precise means of disrupting clear ocular tissue, facilitating full thickness and lamellar corneal transplantation. This provides an opportunity to create reproducible and accurate incisional depths, lamellar stromal beds, and potentially the ability to follow the curvature of the cornea. The femtosecond laser has been employed in penetrating, anterior lamellar and endothelial keratoplasty. To date, the greatest promise has been demonstrated in the ability to create improved wound configurations with faster recovery and reduced astigmatism. Final visual outcomes are currently comparable to conventional surgery for femto-assisted penetrating keratoplasty (PK). For both Femto-PK and deep anterior lamellar keratoplasty (DALK), there is evidence of a faster rate of astigmatic correction and visual recovery, in part because of novel interfaces and the ability to remove sutures earlier. The technology appears to be safe with regard to corneal endothelial cell preservation in PK and DALK, but the exact limits of trephination remain to be determined. The promise with endothelial keratoplasty (EK) however is currently limited by concerns regarding the effects on the endothelium and stromal bed smoothness and there is little long-term data on corneal graft rejection. At present, a major barrier to its wider application is cost, the nature of the applanation device and optimization of the imaging systems that will facilitate real-time enhancement of lamellar trephination. Although this technology is relatively new, its full potential has yet to be realized.

GPW is supported by the Peel and Rothwell Jackson traveling fellowship and a Pfizer/Royal College of Ophthalmologists Ophthalmic Fellowship.

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J. Hjortdal (ed.), *Corneal Transplantation*, DOI 10.1007/978-3-319-24052-7_15

Keywords
Femtosecond • Femto- • Laser • Keratoplasty • Transplantation

Introduction

Lasers have historically facilitated corneal transplantation as ancillary tools, for example, the use of excimer lasers to correct postoperative astigmatism. Historically, the process of cutting tissue during keratoplasty procedures has been restricted to trephination, the use of semiautomated or fully automated microkeratomes and for lamellar procedures, dissection, or pneumatic separation of tissue planes [60]. Global trends in corneal transplantation have evolved rapidly over the last 15 years (see Chap. 20). With an increased uptake of lamellar corneal transplantation including anterior lamellar and endothelial procedures, the potential for femtosecond laser-assisted keratoplasties has come to the fore.

The femtosecond laser offers a controlled, precise means of disrupting clear ocular tissue such as the cornea and lens. This has been utilized in the refractive field as an accurate means of creating corneal flaps, for example, in LASIK surgery and more recently for the creation of extractable lenticules [2]. Femtosecond lasers have also been utilized for cataract surgery, potentially expediting the procedure through enhancement of controlled wound construction, capsulotomy, and lens fragmentation [61]. This technology also offers a new and more accurate means of facilitating lamellar and full-thickness corneal transplantation including reproducible and accurate incisional depth, creating a smooth stromal bed and potentially the ability to follow the curvature of the cornea. In this chapter, we explore the principles underpinning this technology, the platforms available, their respective applications, clinical outcome results, limitations, costs, and future technologies.

Femtosecond Laser Principles

Nd:YAG lasers such as those used for lens capsule disruption rely on energy being delivered in the nanosecond range (10^{-9} s, near-infrared range 1064 nm). Unlike excimer lasers (operating in the ultraviolet range), this results in photodisruption of tissue, inducing cavitation and the production of bubbles. By reducing the time taken to deliver an energy pulse, collateral tissue damage may be reduced, and the advent of lasers operating in the picosecond range (10^{-12} s) were first described as an alternative means of generating a LASIK flap [37]. By contrast, the femtosecond laser relies on the generation of a femtosecond of energy, 10^{-15} s, ≅1050 nm, and its mode of action is akin to a cutting blade. It is precise to a level of 1 μm, and the reduction in pulse time (usually measured in *KHz* or *MHz*) increases the energy delivered in a given time.

Despite recent interest in their potential, lasers operating in the pico- and nanosecond range to a large extent have been overtaken by femtosecond technology because of its lower energy and improved ablation [59]. The advantage over picosecond and nanosecond lasers in corneal applications was outlined over a quarter of a century ago when a reduction in tissue damage and improvement in corneal wound ultrastructure was demonstrated in a proof of concept study [59]. Subsequently, these platforms were evaluated in porcine and human cadaveric eyes as an alternative to the manual microkeratome, paving the way for its application in LASIK, commercially introduced in 2002 [38, 57]. With further applications in the refractive field, it became obvious that the femtosecond laser could augment corneal transplantation. This led to the development of a myriad of applications for both corneal transplantation and cataract surgery.

Femtosecond Laser Platforms

A number of femtosecond laser platforms have been developed and continue to evolve. The first commercially available Femto laser was the IntraLase system (Michigan, USA). This was

Table 15.1 Summary of femtosecond lasers employed for corneal applications including keratoplasty and LASIK

Platform	Company	Docking system	Repetition rate	Approximate energy pulse
IntraLase iFS	Abbott Medical Optics, USA	Flat applanation	60–150 kHz	1000 nj
LDV Z6	Ziemer, Switzerland	Flat applanation	5 MHz	< 50 nj
VisuMax	Zeiss, Germany	Curved applanation	200–500 kHz	170 nj
520 F (replacement for Femtec)	Technolas Perfect Vision (Bausch & Lomb), Germany	Curved applanation	40–80 kHz	4800 nj
Wavelight FS200	Alcon Laboratories, USA	Flat applanation	200 kHz	800 nj

followed by several others that have differences in their energy, interface, and mechanism of action (Table 15.1).

A common misconception in understanding the use and application of this technology is that there is uniformity in the underlying commercially available systems. Lamellar cutting may be achieved through raster patters, progressive side to side dissections such as the Ziemer Z6; centrifugal, circular "in to out" or centripetal, circular "out to in" such as the Technolas 520 F; or both, for example, Zeiss VisuMax during LASIK. The latter offers the advantage of allowing patients to track a target more efficiently and reduces the chance of suction loss or eccentric cuts by eye movement. A caveat to this is the ability to see a target under supraphysiological pressures created by the docking platforms.

The energy required to undertake anterior or side cuts is higher than for lamellar cuts. Experimental data suggests that the energy delivery between platforms influences the collagen disruption and this can be visualized at a nanoscale level by helium ion microscopy [53]. The IntraLase (high-energy, low-frequency pulse) system induces greater cavitation than the VisuMax (low energy, high frequency) system resulting in excessive tissue bridges and difficulty in flap elevation during LASIK [53]. Furthermore, wound healing and scar tissue formation may be reduced by platforms that deliver low-energy, high-frequency systems such as VisuMax and the Ziemer LDV Z6 for LASIK flap formation [52]. Further investigation in lamellar keratoplastic procedures may determine whether an improved interface recovery with reduced energy has a commensurate reduction in complications seen in LASIK such as light sensitivity and gas breakthrough.

Applications

Penetrating Keratoplasty

Penetrating keratoplasty (PK) forms the cornerstone of corneal transplantation and in some circumstances remains the only option for ocular preservation or restoration of sight. Potential problems with mechanical trephination techniques involved in PKs include divergent recipient cut angles and convergent donor cut angles resulting in tissue deficit at the posterior corneal plane resulting in potential misalignment. These problems may be compounded in eyes with narrow palpebral apertures. Femtosecond wound construction allows improved centration without undercutting. This may also help reduce damage to the endothelium, with evidence from animal and human studies demonstrating preservation of cell counts [3, 40].

Even in conventional PK, suction-based trephination systems allow better stability during trephination, are faster, and facilitate rounder trephination with less slippage. There are problems however with intraocular pressure elevation, centration, and in eyes with reduced scleral support such as in aphakia. The application of suction, common to most femtosecond platforms, may also be associated with elevated IOP. A demonstrable reduction in intraocular pressure variation in femtosecond laser-assisted PK has been shown in the VisuMax system compared with manual trephination [3]. There is

considerable variability in IOP elevation between platforms however, and this is discussed later in this chapter.

The data from clinical trials to date is relatively limited but increasing, summarized in Table 15.2. The variable results seen by Femto-assisted PK reflect the myriad outcome measures determined in individual trials or large case series including indication and graft size. Furthermore, many of the studies to date have evaluated the first commercially available platform, IntraLase, and data on many of the newer platforms is therefore restricted. Nonetheless, the principle considerations with regard to this technology relate to wound integrity and recovery, astigmatism and visual outcome, and endothelial cell preservation and rejection. These issues may well be addressed further by well-constructed randomized control trials.

The first major decision in choosing Femto-assisted penetrating keratoplasty over conventional grafting is better wound strength and alignment. The strength of the wounds constructed with femtosecond laser has been shown to be resistant to leakage even with less sutures [42]. The second outcome to contemplate (and related to the first) is astigmatism and visual recovery. A retrospective series directly comparing a straight-cut conventional PK and Femto-assisted PK (n = 20 in each group) suggested that there was less induced astigmatism (6.06 vs. 4.06 D; p 0.04) and faster visual recovery with Femto assistance, but the overall visual outcomes were similar (0.39 vs. 0.22; p = 0.8 LogMar) [33].

Femto-trephination can also facilitate novel and potentially more stable wounds with a theoretical reduction in astigmatism. Improved wound construction and alignment with a femtosecond laser assistance has been proposed with a number of methods including zigzag shapes, mushrooms, top hat (see above), dove and tail, decagonal and lock, and key designs among others [23, 26, 39, 46, 49, 58]. The advantages with shelved or stepped interfaces are a potential reduction in the number of sutures and faster postoperative recovery. Although a reduction in astigmatism by improved tissue apposition has been shown with zigzag configurations in the initial postoperative period (between 8.4 and 5.8 D at 4–6 months), this difference was not seen after 6 months [13, 25]. Other studies have failed to demonstrate an improvement compared to conventional surgery in astigmatism, albeit paradoxically with improvement in vision [27]. It is worth considering that the same early effect was seen with straight cuts as outlined above [33].

Third, there appears to be variable results in the reduction in endothelial cell loss compared to conventional PK. Kamiya and colleagues' series with the VisuMax platform did not demonstrate a difference between Femto-PK vs. conventional PK [33]. Other clinical trials have shown postoperative endothelial cell counts in the range of 1200–2000 cells/mm^2 at 6–12 months [19, 30, 46]. Graft rejection rates in most series have been variable, but it is worth noting that few studies have follow-up data for greater than 12 months, compounded by the variable timing of suture removal. Larger series have suggested that complete suture removal can be achieved earlier than in conventional PK [7].

Finally, all three parameters must also be preceded with a fundamental question regarding the choice as to whether one should undertake PK over a lamellar procedure. This has not been fully addressed and will be considered in the following sections of this chapter.

Anterior Lamellar Keratoplasty

Targeted replacement of the anterior stromal layers of the cornea by anterior lamellar keratoplasty may involve the superficial layers (by manual dissection or microkeratome) or deeper layers (through manual dissection or the use of a big bubble). Deep anterior lamellar keratoplasty (DALK) has the advantage of facilitating an extraocular procedure and theoretically reduces the risk of both rejection and endothelial cell damage where the endothelium is unaffected. To date, outcomes from large national datasets in the United Kingdom and Australia have indicated worse visual outcomes and survival than for penetrating keratoplasty [21, 31]. Advocates of DALK argue that the published data relates to (relatively)

Table 15.2 Summary table of clinical trials involving femtosecond-penetrating keratoplasty

Title	Authors	Journal	Platform	Study design	Outcome
Laser welding in penetrating keratoplasty and cataract surgery in pediatric patients: early results	Buzzonetti L et al. [10]	*J Cataract Refract Surg*. 2013 Dec;39(12):1829–34	IntraLase	Prospective cohort (n = 7) Sutureless diode welding in Femto-PK	No wound leaks at 3 months and minimal astigmatic change
Economic evaluation of endothelial keratoplasty techniques and penetrating keratoplasty in the Netherlands	van den Biggelaar FJ et al. [62]	*Am J Ophthalmol*. 2012 Aug;154(2):272–281.e2	IntraLase	Cost evaluation of 118 eyes	DSAEK most cost-effective, Femto DSAEK least cost-effective
Quality of vision after femtosecond laser-assisted Descemet's stripping endothelial keratoplasty (FLEK) and penetrating keratoplasty: a randomized, multicenter clinical trial	Cheng YY et al. [20]	*Am J Ophthalmol*. 2011 Oct;152(4):556–566.e1	IntraLase	RCT in 80 eyes. Femto DSAEK vs. conventional PK	Straylight and contrast sensitivity improved with FLEK VA improved with PK
Femtosecond laser-assisted decagonal penetrating keratoplasty (PK)	Proust H et al. [49]	*Am J Ophthalmol*. 2011 Jan;151(1):29–34	Femtec	Nonrandomized CT in 16 eyes	Decagonal PK demonstrated mean improvement in VA and low astigmatism
Efficacy and safety of femtosecond laser-assisted corneal endothelial keratoplasty: a randomized multicenter clinical trial	Cheng YY et al. [18]	*Transplantation*. 2009 Dec 15;88(11):1294–302	IntraLase	RCT in 80 eyes. Femto DSAEK vs. conventional PK	Improved astigmatism, decreased VA and ECC in Femto-group
Femtosecond laser versus manual dissection for top-hat penetrating keratoplasty	Bahar I et al. [6]	*Br J Ophthalmol*. 2009 Jan;93(1):73–8. 2008 Oct 16	IntraLase	Nonrandomized CT (n = 94) comparing Femto-PK vs. top-hat PK vs. PK Improved	VA and ECC improved with Femto-PK

CT control trial, *ECC* endothelial cell count, *VA* visual acuity

historical series when more surgeons were undergoing the learning curve, and this may be borne out by higher rates of primary graft failure in the early weeks posttransplantation for this group. This issue is one of contention however and an ophthalmic technology assessment undertaken by the American Academy of Ophthalmology published in 2011 (albeit predating the work by Coster and colleagues) found that there was no difference between DALK and PK with regard to visual acuity, but endothelial cell counts were better preserved with DALK [50].

An apparent advantage with DALK is cost-effectiveness. Two cost-utility analyses to date have demonstrated that despite the higher costs of undertaking DALK (in part due to increased operative time), there are financial longer-term benefits [35, 63]. The rates of lamellar (including anterior lamellar surgery) continue to rise internationally and we will therefore consider how the femtosecond laser may enhance the application of this procedure.

In addition to the problems seen in Femto-PK relating to wound configuration and astigmatism, the major consideration relating to femtosecond-DALK, like conventional DALK, is the technical challenge presented with creating a clear graft-host interface and leaving the minimal amount of residual tissue bed. This difficulty in theory should be circumvented by the automated lamellar dissection offered with femtosecond laser, which may offer a smoother interface. A caveat however is that with deeper dissection, there is enhanced light scatter and potentially a less smooth surface. Differences in collagen disruption have also been determined with the construction of differential laser firing during refractive lenticule construction [51]. Whether this will have an influence on lamellar construction in the context of keratoplasty is yet to be determined. Visual outcomes in DALK may in part be explained by the thickness of the host tissue bed [5]. Notwithstanding the effects of scatter, Femto-dissection could create potentially thinner dissections without the inherent risk posed by manual dissection alone.

Although microkeratomes facilitate anterior cuts, pneumatic dissections for deep lamellar procedures carry a risk of perforation. The femtosecond laser offers theoretical improvement in control in creating an interface during tissue separation. Laboratory data shows that the interface created by femtosecond lasers is smoother, and as previously discussed, low-energy high-frequency platforms may offer an enhanced role for Femto-assisted DALK [51–53]. Femto-assisted lamellar trephination also lends itself to treating superficial pathology and no difference was seen at 12 months follow-up when comparing the visual acuity of Femto-anterior lamellar keratoplasty cut at <250 μm and >250 μm [1].

There is a paucity of clinical trials evaluating Femto-DALK, but a number of series have been published regarding the technique. Both Farid and Price separately presented a zigzag adaptation of a "debulking" big-bubble technique where a zigzag side cut is combined with a 300 or 250 m lamellar dissection, respectively. This facilitates a big-bubble separation of the residual stroma [24, 47]. Buzzonetti and colleagues also describe an adaptation of Anwar's original big-bubble procedure [4] whereby the IntraLase femtosecond laser is employed to facilitate a side cut of 50 μm and a lamellar cut of 100 μm anterior to the thinnest point [11, 12]. This in turn is supplemented by the injection of air to separate Descemet's in a technique termed Intra-Bubble. Outcomes from a 1-year case series (n = 11) by the same group determined that the best-corrected distance visual acuity was 0.52 ± 1 with a refractive outcome of −1.50 ± 1.7 diopters (D) sphere and 2.00 ± 2.6 D cylinder (two attempts were converted to PK at outset) [12]. Longer follow-up also shows mean BCVA of 0.3 ± 0.1 at 24 months (n = 12) and a mean cylinder of 1.7 ± 1.4 D [56].

The bespoke interfaces used in femtosecond-penetrating keratoplasty such as a top-hat configuration may improve the speed of recovery in femtosecond-DALK [14]. The integrity of zigzags, top-hat, and mushroom configurations has been evaluated in an experimental model to determine burst pressure with these cuts [36]. Although the pressure required to induce wound burst was variable, direct comparisons were not undertaken, and it is therefore difficult to draw definitive conclusions regarding the optimal technique.

Sutureless techniques have also been described with variable mean uncorrected and best-corrected visual improvement [8, 67]. Evidence from retrospective series has indicated a faster visual recovery when comparing mushroom configurations with conventional straight cuts undertaken with the IntraLase system but with no difference in overall visual recovery or astigmatic outcome, similar to the findings in Femto-assisted PK [55]. Although conventional DALK is undertaken to prevent endothelial cell loss, it is worth considering the potential effects of the femtosecond laser due to the application of energy in the host bed.

The uptake for Femto-DALK has been limited in part due to the technical difficulty of achieving a safe dissection in the context of severe ectatic disease and reflected by the absence of controlled trials to date. This is further highlighted when the surgeon is faced with existing posterior stromal scarring, a situation that complicates previous hydrops. Limitations in visualizing the cornea in real time by OCT and Scheimpflug imaging raise legitimate concerns about proceeding with femtosecond laser-assisted surgery following docking, as small movement may have catastrophic consequences on the already-friable host bed. High-resolution intraoperative OCT has been shown to enhance the ability to undertake DALK safely by conventional methods [22]. It is hoped that recent improvements in imaging platforms attached to femtosecond platforms may offer an improvement in this regard and a safer option to undertake femtosecond-DALK.

Endothelial Keratoplasty

In contrast to anterior lamellar keratoplasty, the uptake of Femto-assisted dissection of graft material for endothelial keratoplasty has been more widely adopted. Endothelial keratoplasty, like its anterior counterpart, potentially facilitates smoother and more accurate cutting of the desired tissue bed. This is particularly important when minimizing the residual stromal bed transplanted.

Manual or microkeratome dissection has traditionally been employed for Descemet's stripping automated endothelial keratoplasty (DSAEK). The accuracy of depth of microkeratome cuts may be less consistent than with Femto-assisted dissection. Femtosecond laser-assisted ablations have been shown to have a mean deviation in attempted depth of 15 μm [44]. Further adaptations such as double-pass techniques may consistently achieve sub-150 μm grafts with improved visual acuity however [9].

Femtosecond beds have been shown to be smooth under histological evaluation [17, 43]. Another study evaluating the effects of the IntraLase 30 kHz femtosecond laser has demonstrated the mechanical microkeratome may improve the depth and smoothness of the cut [32]. The "rougher" interface created by the femtosecond laser was postulated as having a potentially improved interface for maintaining adherence – however, the rate of graft dislocation has previously been shown to be as high as 20 % when undertaking Femto DSAEK [16]. Whether this relates to surgical technique or the smooth tissue bed created remains to be elucidated. Furthermore, the type of laser may influence the interface created and another comparisons using the IntraLase platform found rougher surfaces were created with the femtosecond laser compared to microkeratomes [45].

Endothelial cell loss is an important consideration in judging the safety of Femto-assisted endothelial keratoplasty. Both the aforementioned studies comparing smoothness of the interface created by microkeratome found no difference in the reduction of endothelial cell count [32, 45]. Inverse cutting techniques have also been proposed as a means of safely maintaining endothelial cell counts when creating lamellar cuts [29]. A study comparing 50 vs. 150 μm dissection in rabbits using the Wavelight FS200 however showed significantly higher rates of endothelial cell damage and apoptosis with thinner cuts [41]. A large randomized control trial also found that rates of endothelial cell loss were higher with Femto-assisted endothelial keratoplasty compared to conventional penetrating keratoplasty (1200 vs. 2150 cells/mm^2 at 3 months) [18]. The difficulty in comparing two separate techniques and by laser and conven-

Table 15.3 Summary table of clinical trials involving femtosecond endothelial keratoplasty

Title	Authors	Journal	Platform	Study design and objective	Outcome
Economic evaluation of endothelial keratoplasty techniques and penetrating keratoplasty in the Netherlands	van den Biggelaar FJ et al. [62]	*Am J Ophthalmol*. 2012;154(2):272–281. e2	Intralase	Randomized CT: Cost evaluation in 118 eyes	DSAEK most cost-effective, Femto DSAEK least cost-effective
Quality of vision after femtosecond laser-assisted Descemet's stripping endothelial keratoplasty (FLEK) and penetrating keratoplasty (PK): a randomized, multicenter clinical trial	Cheng YY et al. [20]	*Am J Ophthalmol*. 2011;152(4):556–566. e1	IntraLase	Randomized CT in 80 eyes	Straylight and contrast sensitivity improved with FLEK VA improved with PK
Efficacy and safety of femtosecond laser-assisted corneal endothelial keratoplasty (FLEK): a randomized multicenter clinical trial	Cheng YY et al. [18]	*Transplantation*. 2009 15;88(11):1294–302	IntraLase	Randomized CT of 80 eyes	Astigmatism better with FLEK VA better with PK ECC better with PK

CT control trial, *ECC* endothelial cell count, *VA* visual acuity

tional means makes this more difficult to interpret. Furthermore, the authors concede that the method by which the graft was inserted (by forceps with a folded graft) will have contributed to the endothelial cell attrition. The limits by which safe dissection can be achieved need to be evaluated further. This of course represents a challenge for undertaking the very thin cuts needed to facilitate ultrathin Descemet's stripping endothelial keratoplasty (UT-DSEK).

Outcomes of randomized clinical trials involving femtosecond laser-assisted endothelial keratoplasty are also sparse but summarized in Table 15.3. It is interesting to note that the visual acuity was reduced in the large trial comparing conventional PK and may also reflect the quality of the interface [20].

These findings were supported in another large case series comparing microkeratome and femtosecond laser DSAEK where the visual outcome was worse in the femtosecond laser group although this was smaller ($n=6$) than the microkeratome group ($n=41$) and four of the six had significant preexisting visual comorbidities [28]. Other studies have shown more favorable results with femtosecond dissection in a technique involving femtosecond followed by microkeratome cutting [54]. This may add more weight to those advocating the use of microkeratomes over the femtosecond laser in the context of endothelial

keratoplasty. Again there is a need for directly comparable randomized trials between the two techniques as well as between conventional and femtosecond EK.

Limitations and Costs

The immediate and future application of femtosecond lasers offers exciting opportunities to enhance corneal transplantation. There are however several limitation both in the flexibility of the technology and their cost implications. An example is the effect of corneal edema and scarring have not been fully evaluated, and the limits by which femtosecond laser platforms can achieve reliable cuts warrant further investigation. The problem of judging the efficacy of different platforms is also compounded by the mixture of underlying pathologies compared in many of the current studies, and clear disease-orientated criteria cannot be established as yet.

The reliance on creating suction and the associated elevation in intraocular pressure mean there is a limited role in tectonic or emergency transplantation, in particular in the context of infections with associated severe thinning or impending perforation. Intraocular pressure (IOP) rises are higher during microkeratome suction compared to femtosecond docking with the VisuMax system in a rabbit model (mean 141 ± 20 vs. 62 ± 3 mmHg, $p < 0.001$) [15]. By contrast, a study undertaken with porcine eyes with the IntraLase platform showed no difference in IOP elevation compared to the microkeratome (135 ± 16 mmHg vs. 152 ± 24 mmHg) [66]. Other studies have shown higher pressures with the IntraLase platform (when directly compared with VisuMax) have also been demonstrated with the Femtec and Ziemer LDV models [64, 65]. In part, this may reflect the effect of a flat applanation system with the IntraLase and LDV Z6 systems. The effects of liquid interface systems for keratoplastic procedures remain to be determined.

The closest approximation to human keratoplasty has been demonstrated in a rabbit model of IOP in PK. Direct comparisons between suction base trephine and the VisuMax revealed similar IOP but greater variation during the procedure with manual suction trephination [3]. Clearly patients with glaucoma or those at higher risk from IOP fluctuation will represent a relative or absolute contraindication when considering patients for Femto-keratoplasty. The considerable variation between platforms and the length of the docking procedure in addition to maximal IOP must be considered.

The effects of femtosecond laser systems on the corneal endothelium have been discussed earlier in this chapter. Safety concerns regarding the application of femtosecond laser in relation to undertaking future transplantation were considered by Klingler and colleagues [34]. Patients who have undergone either femtosecond-assisted or microkeratome-assisted LASIK revealed no attrition in the endothelial cell count at 5 years postsurgery, suggesting that those who have undergone this refractive procedure may be suitable candidates for future donation of tissue [34]. Another important laser-related complication is an incomplete incision pattern. Failure to complete a wound once the laser sequence has been initiated, for instance, if suction breaks or there is excessive movement, can result in this problem. Price and colleagues determined that when creating a Femto-PK wound configuration an incomplete cut did not affect the tensile strength to complete surgery [48]. The consequences in lamellar cuts, especially those close to the endothelium, are potentially more serious, and further investigation is warranted.

Many units will likely consider whether they wish to invest in a platform for refractive work, corneal transplantation, and/or cataract surgery. At present, the choices are limited in achieving this with the same docking procedure and with the same machine. This is likely to have changed by the time of publication and may encourage greater uptake and drive down costs further. Publicly funded healthcare systems may therefore lag behind in the introduction of these systems, in particular when refractive applications are not widely available in these settings and can be incorporated more easily for keratoplasty.

Future Developments

Femtosecond laser technology has advanced considerably in the short time it has been available. The "holy grail" of course is a cost-effective platform that is easy to use, has a low side-effect profile and high-quality and expedient outcomes.

The advent of liquid-based interfaces or even the absence of applanation may prove to be a milestone in safety and laser delivery as manipulation of the cornea, in particular with flat applanation, could potentially become obsolete. Real-time tracking of the corneal profile is also a critical step in ensuring an optimal interface is achieved. In particular in ectatic corneas, small movements may result in disaster and systems that can abrogate these problems, for example, by linking the femtosecond laser cutting to real-time imaging platforms such as spectral-domain OCT or topography will no doubt influence its uptake. Suffice it to say larger-scale clinical trials are needed to evaluate this technology in particular with regard to lamellar surgery and comparing platforms. Clearer, well-constructed RCTs will also have an important bearing on the trajectory and uptake of femtosecond laser keratoplasty.

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Limbal Stem-Cell Expansion and Transplantation

16

Paolo Rama, Stanislav Matuska, and Graziella Pellegrini

Abstract

Limbal stem-cell transplantation is indicated for treating a corneal epithelial stem-cell disorder known as limbal stem-cell deficiency (LSCD). Lamellar and/or penetrating keratoplasty cannot be used successfully in these cases as donor corneal epithelium is replaced by that of the recipient within months. In the presence of corneal epithelial stem-cell compartment deficiency, donor graft reepithelialization will not take place, with subsequent epithelial defects and the ultimate recurrence of conjunctivalization and the risk of rejection and failure. Unilateral limbal stem-cell deficiency has been successfully treated for years by directly grafting a portion of the healthy limbal tissue taken from the contralateral eye, but some concerns exist regarding potential donor eye risks. To overcome risks for the donor eye, a technique to reduce biopsy dimension using cell expansion in culture has been developed. Autologous cultivated limbal stem-cell transplantation is an effective and safe procedure to treat limbal stem-cell deficiency when there is an undamaged, even small, portion of the limbus that will provide donor cells to be expanded in vitro. Ex vivo limbal grafts have several advantages compared with the previously used technique of directly grafting limbal tissue, including fewer risks for the donor eye, possibility to treat bilateral LSCD, and possibility of regraft after failure. Unilateral and partial bilateral limbal deficiency can thus be successfully treated with long-term survival and without the need for systemic immunosuppression.

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J. Hjortdal (ed.), *Corneal Transplantation*, DOI 10.1007/978-3-319-24052-7_16

Keywords
Cornea • Limbus • Limbal stem cells • Corneal epithelial stem cells • Limbal stem-cell deficiency • Limbal stem-cell transplantation • Cultivated limbal stem-cell transplantation • Ex vivo expansion

Introduction

Limbal stem-cell transplantation is indicated for treating a corneal epithelial stem-cell disorder known as limbal stem-cell deficiency (LSCD) [8, 36].

LSCD includes a group of heterogeneous diseases involving failure of the corneal epithelial stem cells caused by congenital abnormalities, acquired diseases such as chemical and thermal injuries, immunological diseases, toxicity, and infections [8, 36]. Such diseases may not only damage the limbus but also the eyelids, conjunctiva, corneal nerves, stroma, and lacrimal system. Ocular surface disease is the most appropriate term for such a complex disorder.

Impairment of the limbal stem-cell compartment causes corneal epithelial turnover breakdown, resulting in damage to the corneal epithelium, which will ultimately repair due to conjunctiva migration on to the cornea [8, 36]. Conjunctival migration, or "conjunctivalization," is a compensatory repair mechanism that protects the cornea from infection, stromal ulceration, melting, and perforation. While it provides a stable and protective superficial layer to the cornea, it is often accompanied by persistent inflammation, severe visual impairment, and other symptoms.

Lamellar and/or penetrating keratoplasty cannot be used successfully in these cases as donor corneal epithelium is replaced by that of the recipient within months. In the presence of corneal epithelial stem-cell compartment deficiency, donor graft reepithelialization will not take place, with subsequent epithelial defects and the ultimate recurrence of conjunctivalization and the risk of rejection and failure.

Limbal stem-cell transplantation (LSCT) is a step in the reconstruction of the ocular surface, while lamellar or penetrating corneal graft will finally restore corneal transparency, leading to the recovery of visual capacity.

The Need for Stem-Cell Expansion

Unilateral limbal stem-cell deficiency has been successfully treated for years by directly grafting a portion of the healthy limbal tissue taken from the contralateral eye [12, 15, 19]. Some concerns exist regarding potential donor eye risks [1] although few reports have shown consequences related to harvesting [17], patients are often unenthusiastic about having the "good" eye touched, together with the great responsibility felt by surgeons. Moreover, further harvesting of the limbus following possible failure is not advisable.

To overcome risks for the donor eye, much effort has been made to develop a technique to reduce biopsy dimension using cell expansion in culture. The pioneering work of Rheinwald and Green showed that it was possible to obtain a layer of stratified squamous epithelium from a single cell after serial cultivation of human epidermal epithelial cells (keratinocytes) on a layer of lethally irradiated murine fibroblasts (3 T3 cells) [34]. Some years later, cultivated skin grafts were successfully used to treat severe-burn patients [13]. In 1997, Pellegrini et al., using the Rheinwald and Green protocol, showed that autologous grafts of cultivated cells obtained from a 1 mm^2 limbal biopsy restored the corneal surface in two patients with complete loss of the corneal-limbus epithelium [27]. The culture procedure was then standardized [26], and to date more than 270 grafts have been transplanted in various centers throughout Italy, with long-term stability reported in more than 150 patients and with a success rate in 70–80 % of cases [29, 32].

Ex vivo limbal grafts might have several advantages compared with the previously used technique of directly grafting limbal tissue: (i) fewer risks for the donor eye; (ii) possibility to treat bilateral LSCD should there be a spared part of the limbus, albeit small; (iii) possibility of

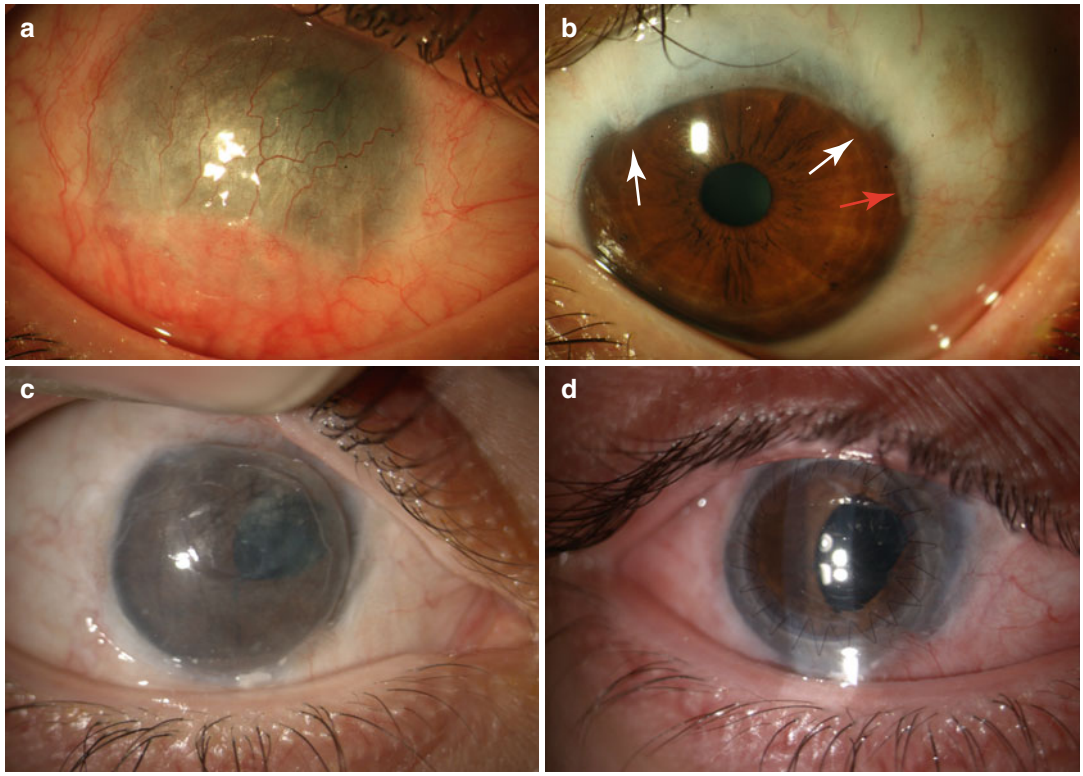

Fig. 16.1 Failure of autologous limbal transplantation with recurrence of corneal conjunctivalization (**a**). Contralateral donor eye with signs of a large previous limbal harvesting for limbal transplantation (*white arrows*) and the small biopsy for ex vivo expansion done after failure of the first graft (*red arrow*) (**b**). One year after successful cultivated limbal stem-cell transplantation with transparent, avascular, and stable epithelium (**c**). The same patient after penetrating keratoplasty combined with cataract extraction, lens implantation, and pupilloplasty (**d**)

regraft after failure (Fig. 16.1); (iv) cells can be frozen and stored, allowing additional transplantation or banking if required; (v) association with gene therapy; and (vi) proof of concepts to use another cell source to treat total bilateral disease.

Autologous Versus Allogeneic Limbal Grafts

In unilateral LSCD, or in bilateral LSCD, where a small portion of healthy limbus can be used as donor tissue for ex vivo expansion, autologous limbal grafts are advised [30, 32]. On the contrary, in total LSCD when the limbus is completely destroyed in both eyes, limbal tissue taken from a deceased donor or from a living relative can be used. In the literature, contrasting results have been reported on the use of allogeneic keratolimbal grafts, with an overall success rate of 73 % [1]. Both clinical successes and failures have been observed in the presence of systemic immunosuppressive therapy [6, 16, 23], while positive clinical results have been reported in the absence of immunosuppression [20, 33] and/or in the absence of allogeneic cell survival [2, 14]. In most cases, however, the interpretation of results has been hampered, either by the lack of a proper genetic evaluation of the presumptive long-term engraftment of allogeneic limbal grafts or by the inadequate length of follow-up. In the absence of demonstrated surviving donor cells, a possible explanation for clinical success is that patients with non-total limbal stem-cell deficiency have been included, and the grafted allogeneic limbal cells might have induced modification of the

microenvironment and promoted proliferation of the patient's own dormant stem cells, whose progeny gradually replaces donor cells. While remaining in situ in the injured eye, these limbal cells are evidently unable to generate corneal epithelium, either because of the lack of a suitable microenvironment for multiplication or because of fibrotic obstruction to their migration over the cornea. This would explain the mixed population of donor and recipient corneal cells observed at short-term follow-up. These findings are consistent with reports showing that clinical improvement observed following allogeneic keratolimbal grafts does not necessarily correlate with the long-term survival of donor cells [2, 14]. Similarly, cultured allogeneic epidermal keratinocytes do not engraft permanently, but provoke epidermal regeneration in partial-thickness skin burns, presumably by stimulating residual hair follicle stem cells [3].

Indications and Contraindications

Limbal stem-cell grafting is indicated to treat limbal stem-cell deficiency (LSCD) [8, 36]. As said above, LSCD includes heterogeneous diseases where the limbus has been damaged. The eyelids, conjunctiva, corneal stroma, nerves and endothelium, and immune and lacrimal systems can also be involved. Scrupulous step-by-step reconstruction should be planned, treating the structures involved separately, to prepare the best recipient bed for the cultivated cells. Eyelid malposition and malocclusion should first be treated. Conjunctival symblepharon should be then addressed using the appropriate procedures. Once the eyelids and conjunctiva have been treated, tear film and inflammation should be carefully evaluated. The minimum of tear film and the maximum inflammation allowing the successful long-term survival of the grafted stem cells are not clear. In our previous clinical trials [31, 32], we excluded patients with Schirmer's test below 5 mm/5 min, but this was arbitrarily chosen, and one might suggest that the quality of tears might be even more important than the quantity. Unfortunately, at present there is still no valid method for its assessment. We do not include in our clinical protocol for limbal transplantation patients showing severe active inflammation. As for tear film, we are still far from having reproducible clinical assessment and inflammation grading, with the exception of redness scoring.

Diagnosis of LSCD

LSCD diagnosis is based on the evidence of a previous insult (cause) and peculiar clinical features (signs) and is eventually confirmed by instrumental tests [8, 36]. The causes of LSCD are shown in Table 16.1.

Clinical Features

Symptoms

The acute phase is characterized by pain, photophobia, and blurred vision with severity based on the extension of the damage. The chronic phase presents with milder photophobia, foreign body sensation, and pain in the presence of recurrent epithelial erosion. Varying visual acuity decrease

Table 16.1 Causes of LSCD

Congenital	Acquired
Aniridia	Chemical/thermal injuries
Dyskeratosis congenita	Radiation
Autoimmune polyglandular syndrome	Contact lens abuse
Ectrodactyly ectodermal	Drug induced
Dysplasia-clefting syndrome	Extensive limbal surgery
Endocrine deficiency	Extensive corneolimbal infections
Xeroderma pigmentosum	Stevens-Johnson syndrome
	Mucous membrane pemphigoid
	Atopic keratoconjunctivitis
	Graft vs. host disease

depends on the extension of the damage. However, when the visual axis is not involved, there is no reason to propose surgical treatment. Conservative treatment for symptom relief, such as preservative-free artificial tears, autologous serum eyedrops, therapeutic soft or scleral contact lenses, and short courses of low-dose topical steroids, is therefore indicated.

Signs

Corneal signs can be, depending on the severity of the damage, the loss of normal limbal anatomy with disappearance of the palisades of Vogt, irregular epithelium with fluorescein uptake, recurrent or persistent epithelial defects, superficial neovascularization ("*conjunctivalization*") and/or fibrovascular pannus formation, deep stromal neovascularization, and chronic inflammation (Figs. 16.1a and 16.3a) [8, 36].

Supplementary Tests

Impression Cytology

Cytokeratins (CKs) are intermediate filaments present in almost all epithelial cells [11]. Epithelia from different parts of the body express keratins, which are unique for each location: this specificity can be thus used to differentiate genotypically different cell types [24]. The cornea expresses the cytokeratins K3 and K12, but not K19, while the conjunctiva specifically expresses K19, but not K3 and K12 [10, 35].

We previously showed that immunocytochemistry carried out on corneal impression cytology specimens allows us to distinguish between corneal and conjunctival epithelial cells with positive/negative staining of K3/K12 (cornea) and K19 (conjunctiva) [7]. We also showed that impression cytology can be used to grade limbal stem-cell deficiency and assess the final results after limbal stem-cell grafting [31] However, it is an invasive procedure that, despite its simplicity, may cause painful epithelial defects that might be difficult to treat due to the underlying limbal problem. Therefore, impression cytology should only be implemented in those cases where there is a specific question needing to be answered.

Confocal Microscopy

Confocal microscopy is a noninvasive procedure that can distinguish between corneal and conjunctival epithelial cells and is therefore useful in the diagnosis of limbal stem-cell deficiency (Fig. 16.2) [9, 21, 37]. In a recent study by Nubile et al., confocal microscopy was compared with impression cytology in patients with limbal disorders with concordance in 90 % of cases [25]. Confocal microscopy is therefore a useful noninvasive method to confirm limbal stem-cell deficiency. We should remember, however, that confocal microscopy evaluates cell morphology but cannot recognize their true phenotype: without specific markers we might mistake transient morphological changes of corneal epithelial cells for conjunctival cells.

Procedure

Various protocols for the cultivation of limbal stem cells for transplantation have been proposed and recently reviewed by Shortt et al. and Joe and Yeung, including methods to extract cells from the biopsy (mechanical disruption or enzymatic dissociation), substrates and carriers (fibrin sheet, amniotic membrane, polymers, contact lenses, collagen), or mediums with animal-derived components or xeno-free [18, 36]. Although good clinical outcomes have been reported with all of these different culture procedures, few studies have evaluated the clonal characteristics of the cultivated cells and their proliferative potential. When dealing with stem-cell-based therapies for diseases involving cell-renewing tissue, it should be mandatory to demonstrate the presence, survival, and concentration of stem cells in culture and in the graft and validate the procedure under GMP conditions [4, 28].

We previously showed, analyzing the proliferative potential and cloning characteristics,

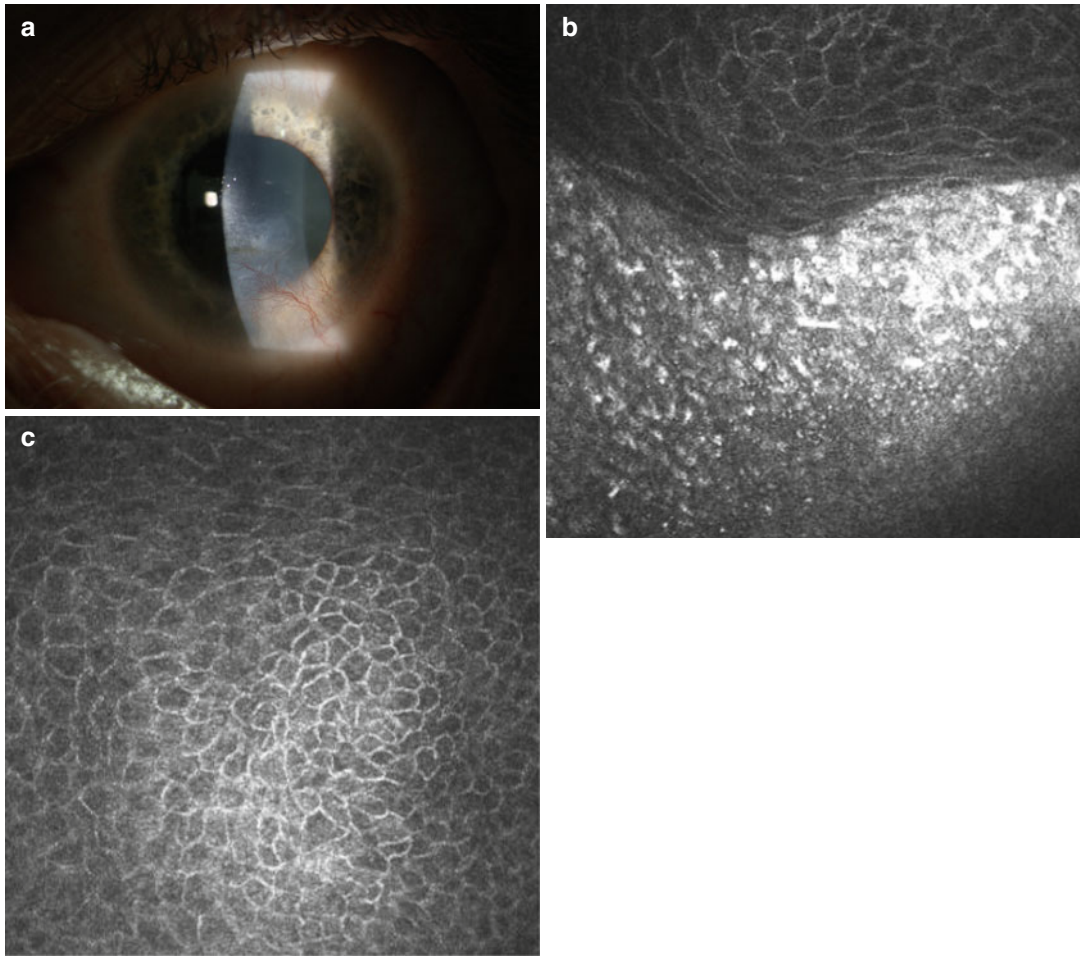

Fig. 16.2 Cornea with partial limbal stem-cell deficiency (**a**). Although in some cases the detection of conjunctival pannus is obvious with confocal microscopy (**b**), in some other cases the presence of conjunctival epithelium may be proposed, but not so obvious (**c**). In these cases, further confirmation with clinical findings and/or standard impression cytology techniques with staining for specific markers may clarify the diagnosis

that corneal stem cells are segregated in the limbus, while conjunctival stem cells are uniformly distributed in the bulbar and forniceal conjunctiva. Moreover, conjunctival epithelial cells and goblet cells derive from a common bipotent progenitor [26]. We also showed that autologous limbal stem cells, cultivated on fibrin and 3 T3 feeder layer, maintain their properties and are able to restore corneal integrity in severe limbal stem-cell deficiency [31]. We later confirmed the long-term stability of the results, up to 10 years, and validated the procedure, comparing clinical results with the level of expression of ΔNp63α in culture [5, 29, 32]. Clinical success was statistically associated with the percentage of p63-positive cells in culture. Cultures in which p63-*bright* cells made up more than 3 % were associated with successful transplantation rate close to 80 %. In contrast, cultures with less than 3 % were associated with poor results, with successful transplantation in only 10 % of patients. On the basis of these data, only cultures that contain more than 3 % ΔNp63α cells are now grafted on patients.

We hereafter report our protocol: (i) biopsy, (ii) stem-cell expansion in culture, (iii) grafting, and (iv) postoperative management.

Biopsy

A 1–2 mm^2 wide, approximately 100 μm deep, limbal biopsy is taken from the contralateral eye (Fig. 16.1b) or from an unaffected portion of the limbus in partial bilateral cases. The procedure can be carried out under topical anesthesia with oxybuprocaine or para/retrobulbar anesthesia with Carbocaine or Marcaine without adrenaline depending on patient collaboration. The use of topical lidocaine should be avoided due to its toxicity. Limbal tissue is normally harvested in the superior quadrant. Meyer-Blazejewska et al. found that stem-cell isolation is highest when using biopsies from the superior limbus and also that harvesting in the superior quadrant keeps it less exposed [22], although harvesting can be carried out from any quadrant if necessary. We previously showed that there are no differences in the efficacy of stem-cell isolation and growth comparing different areas of the limbus [26]. The biopsy specimen is then inserted into a sterile tube containing the transport medium and immediately sent to the laboratory where it will be processed within 24 h. Sutures are not required, but we use two 10/0 nylon stitches to bring the conjunctiva over the area of the corneal biopsy to reduce risks and symptoms. Bandaging is generally not required.

Stem-Cell Expansion in Culture

Cells are enzymatically dissociated, characterized, and expanded in vitro on a feeding layer of lethally irradiated 3 T3-J2 cells to a size of approximately 2.2 cm^2 [26, 31, 32]. Limbal biopsies are processed within 24 h of withdrawal.

Following dissociation with a solution of trypsin and EDTA, one aliquot of the cell suspension (10 %) is plated on a lethally irradiated layer of 3 T3-J2 cells for colony-forming efficiency analysis, while the remaining volume of the cell suspension (90 %) is plated at high density on lethally irradiated layer of 3 T3-J2 cells. When the culture reaches sub-confluence, cells are again dissociated using trypsin, divided into two aliquots, and cryopreserved.

Once surgery is planned, one aliquot of cells is thawed and plated on a layer of lethally irradiated 3 T3-J2 cells on a supportive fibrin layer. The fibrin disk carrying cultivated cells, 2.2 cm^2 in dimension, is packed in sterile stainless steel containers with 4 ml of transport medium, placed in a sterile Petri dish, and inserted into a polystyrene box for transport. Once packaged, the graft has a shelf life of 36 h.

The second aliquot of frozen limbal cells cultivated from the original biopsy, when available after having prepared the graft, is kept cryopreserved to be used for a second application, if required.

Grafting

The anesthesia can be para/retrobulbar, using a long-lasting drug such as naropine to prolong the blocking of eye movement after surgery. When a general anesthesia is used, an associated para/retrobulbar injection will help prevent eye movement after surgery. Lidocaine and adrenaline must not be used due to their toxic effects on the cultivated cells.

The surgical procedure is as follows:

1. Limbal peritomy a few millimeters outside the limbus, with proper coagulation. A 4–5 mm pocket in the bulbar conjunctiva is created into which the fibrin-cultured epithelial sheet is inserted.
2. Pannectomy: removal of corneal fibrovascular layer of conjunctival origin; try to find the cleavage level between the pannus and the cornea to avoid, when possible, keratectomy.
3. Lavage with BSS while checking for an absence of consistent blood loss that could form blood collections (“sacks”) under the epithelial graft to be applied.
4. Transfer of the stem-cell graft on fibrin from the transport container to a suitable dish. It is best to use the protective film of the adhesive tab from surgical gowns, which is to be kept sterile; under the microscope it is possible to recognize the fibrin “nude” side (smooth and translucent) from the cell-seeded side (rough).

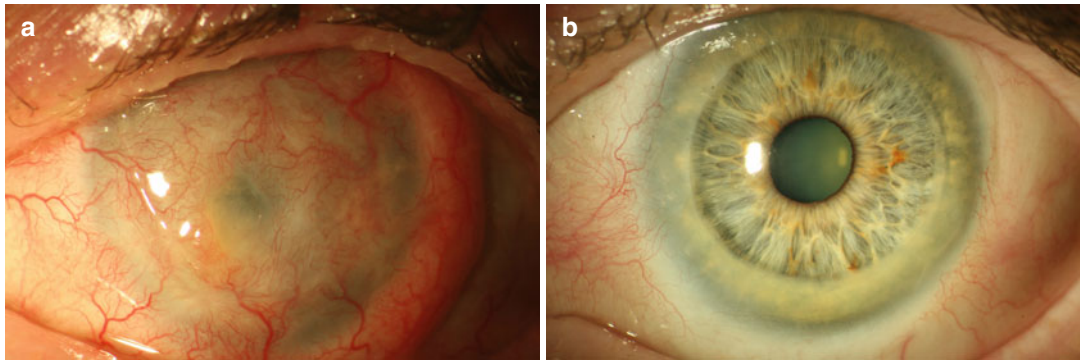

Fig. 16.3 Total limbal stem-cell deficiency after alkali burn (**a**). Ten years after successful cultivated limbal stem-cell transplantation and penetrating keratoplasty with transparent, avascular, and stable epithelium (**b**)

It is absolutely crucial to place the fibrin sheet with the cultivated cells outside and not upside down. The fibrin sheet is allowed to slide onto the recipient's prepared graft area, using BSS and slight traction with tweezers at the edge of the graft as required.

5. The excess of the fibrin sheet is trimmed, and the edge is covered with the conjunctiva applying 2 or 3 stitches of Vicryl or silk 8/0.
6. Close the eyelids with Steri-Strips.

Postoperative Management

We prefer systemic treatment for the first 2 weeks to avoid inadvertent trauma and local toxicity: oral doxycycline 100 mg (or, if allergic, amoxicillin 500 mg) twice a day for 2 weeks and oral prednisone 0.5 mg/kg/day for 2 weeks, tapering the dose after that to 0.25 mg/kg/day for 1 week and 0.125 mg/kg/day for 1 week and then stopped.

After 2 weeks, topical treatment is started: topical preservative-free dexamethasone 0.1 % three times per day for 2 weeks, then reduced to 1 drop twice daily for 1 week and 1 drop once daily for a further week and then stopped. The topical corticosteroid can be continued in the presence of persistent ocular inflammation. Topical preservative-free antibiotics are used only in the presence of epithelial defects.

Eyedrops containing benzalkonium chloride should be avoided. Benzalkonium chloride (as well as other quaternary ammonium compounds) is cytotoxic, and eyedrops containing this preservative might damage the newly regenerated corneal epithelium.

Treatment of Residual Corneal Opacity

Injuries that cause limbal stem-cell deficiency often affect the deep layers of the cornea, causing stromal opacity that, in most cases, requires lamellar or penetrating keratoplasty (Fig. 16.3). Although it is possible to combine limbal transplantation with keratoplasties, we suggest planning it for a different time. The halftime of corneal reepithelialization is 9 weeks, and complete corneal epithelium replacement requires 9–12 months [38] 6 months might be a sufficient period of time to assess the survival and function of the grafted stem cells. If the epithelium is stable after 6 months, it probably means that the regenerated limbus can support the physiological turnover and will thus be able to replace the donor epithelium of the corneal graft. Even though in our previous studies we waited 12 months before planning keratoplasty [31, 32], we now believe that it can be carried out from month six, if necessary.

Conclusions

Autologous cultivated limbal stem-cell transplantation is an effective and safe procedure to treat limbal stem-cell deficiency when there is an undamaged, even small, portion (1–2 mm^2 is sufficient) of the limbus that will provide

donor cells to be expanded in vitro. Unilateral and partial bilateral limbal deficiency can thus be successfully treated with long-term survival and without the need for systemic immunosuppression.

Limbal stem-cell deficiency is part of the complex disorder known as ocular surface disease, and scrupulous step-by-step reconstruction should be planned, treating the structures involved separately, to prepare the best recipient bed for the cultivated cells.

The procedure of ex vivo stem-cell expansion is crucial and mandatory to demonstrate the presence, survival, and concentration of stem cells in culture and in the graft and validate the procedure under GMP conditions. We are still dependent on the presence of animal-derived products, such as 3 T3 feeder layer and fetal calf serum. Even though all these ingredients have been proven to be safe, and have been approved for human use by regulatory agencies, we hope to find a way to be free of them in the future.

We still lack a valid solution for total limbal stem-cell deficiency cases. Contrasting results have been reported on the use of allogeneic keratolimbal grafts, and in the absence of allogeneic cell survival we cannot rely on this treatment for long-term success in total bilateral diseases.

Future perspectives include: (i) finding other sources of autologous stem cells able to function like the corneal epithelium to treat bilateral limbal stem-cell deficiency; (ii) preparation of a "composite" graft with stem cells seeded with other cells, such as keratinocytes, fibroblasts, melanocytes, and/or other cells, on a 3D scaffold that might reproduce the "niche" where stem cells normally reside; (iii) improvement of tear substitutes and/or tissue engineering of the lacrimal gland to treat severe dry eye; and (iv) more accurate modulation of the inflammatory response before and after grafting.

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Decision-Making in Keratoplasty

17

Anders Ivarsen and Jesper Hjortdal

Abstract

Within the last 10–15 years, dramatic improvements have occurred within the field of corneal transplantation. The advent of sutureless posterior lamellar keratoplasty has revolutionized the treatment of endothelial disease. Similarly, developments in surgical technique and technology have improved the outcome of anterior lamellar procedures. Despite the many improvements, however, keratoplasty is not without complications, and patients with one or more risk factors for graft failure still pose significant challenges. Thus, although modern-day surgeons have several treatment modalities available, any given corneal condition needs careful consideration to decide whether or not to graft and to choose which procedure is most beneficial for the patient as seen in context of the supply of donor tissue and local organization.

In the present chapter, the various treatment modalities are outlined including their indications and which treatment to consider under given circumstances.

Keywords

Decision-making • Keratoplasty • Corneal transplantation • DSAEK • DMEK • DALK • Patient information

Introduction

For more than 50 years, keratoplasty has been the mainstay of treating corneal blindness. During this period, microsurgical technique has improved, corticosteroid treatment has been developed, the importance of the corneal endothelium for maintaining corneal clarity has been realized, and the understanding of immunological reactions has increased. Already at an early

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J. Hjortdal (ed.), *Corneal Transplantation*, DOI 10.1007/978-3-319-24052-7_17

point in history, a lamellar approach to corneal transplantation was attempted, but results were generally unsatisfactory [8, 103]. Thus, for decades, penetrating keratoplasty (PK) remained the general surgical approach, irrespective of the underlying corneal pathology. Within the last 10–15 years, however, surgical developments have allowed a more differentiated view, enabling the surgeon to choose from a variety of treatment modalities. Of these developments, the recognition that a posterior lamellar graft can stick to the recipient cornea has proven one of the most important advances in corneal surgery for years [68–70]. The technique was refined through the contribution from several groups all over the world [36, 81, 82, 100], and today Descemet's stripping automated endothelial keratoplasty (DSAEK) is the most frequently performed surgical procedure for endothelial dysfunction [27, 52]. Descemet's membrane endothelial keratoplasty (DMEK) represents an newer and more refined posterior lamellar approach that has been reported to offer an even better postoperative outcome than DSAEK [37, 38, 104]. Irrespective of the surgical technique, however, the posterior lamellar approach allows the surgeon to avoid several of the major challenges after PK including slow visual recovery, high postoperative astigmatism and reduced mechanical strength of the globe.

Another major challenge after PK is endothelial rejection, why anterior lamellar keratoplasty (ALK) has long been suggested as the obvious approach in patients with a functioning endothelium [8, 41]. In early attempts on ALK, visual outcome was disappointing [86], but over the last one to two decades, more sophisticated surgical approaches, microkeratomes and femtosecond lasers (FS lasers) have renewed the interest in ALK. Despite these technological developments, superficial anterior lamellar procedures still have inferior visual outcome in comparison with PK [4]. In contrast, the surgically more challenging deep anterior lamellar keratoplasty (DALK) nearly rivals PK in terms of postoperative visual acuity [55, 74, 90, 93, 116].

The variety of new surgical modalities in keratoplasty has placed an increasing demand on the surgeon to decide which procedure to choose, both with respect to the individual patient and with respect to the supply of donor corneas and the organization at the specific place of practice. This chapter aims to discuss some of the considerations that may affect the choice of surgical approach in various cases.

Basic Considerations

The decision to perform a keratoplasty is multifaceted and requires an extensive evaluation of the eye including eye history, intraocular pressure and evaluation of the retinal function. An intact corneal surface is mandatory to obtain a functioning, clear graft, and any abnormalities of the eyelids, changes of the ocular surface or dry eye disease should be actively treated before keratoplasty is considered in order to ensure optimal protection of the eye and graft.

Although the cornea is generally considered to be immune privileged, the introduction of allogeneic tissue may elicit an immunological response. The most frequent immunological rejection occurs against donor endothelium [2, 66, 83], although rejection of epithelial or stromal cells may occur as well [73, 83, 89]. Several host factors increase the risk of rejection and subsequent graft failure (Table. 17.1).

Vascularization of the recipient stroma is recognized as one of the most significant risk factors for graft rejection [2, 66]. In many cases, the vascularization includes both blood and lymph vessels [24, 62], causing the immune privilege to be compromised.

Patients with inflammatory conditions of the eye or ocular surface are also at increased risk of

Table 17.1 Risk factors in keratoplasty

Vascularization of the recipient stroma
Anterior synechiae
Previous graft failure due to immunological rejection
Previous or ongoing anterior segment inflammation
Uncontrolled glaucoma or glaucoma surgery
Herpes simplex keratitis
Ocular surface disease

graft failure due to rejection. Ocular surface morbidity represents a significant challenge and often have poor prognosis after keratoplasty, even if the underlying inflammatory condition appears to be under control. Similarly, long-term graft survival may be reduced in patients with insufficiently treated intraocular inflammation [14, 61] and in patients with herpes simplex virus, where recurrence of the infection may lead to scarring or rejection of the graft [26, 35, 92]. Uncontrolled glaucoma or need of subsequent glaucoma surgery also may lead to graft failure [115]. Also, previous rejection and graft failure are significant indicators for renewed failure after regrafting [118].

Thus, the indication for performing elective keratoplasty in patients with one or more risk factors should be carefully considered.

Surgical Approaches

Penetrating Keratoplasty

In PK a full-thickness cornea with clear stroma and viable endothelium is transplanted. There are several variations upon the surgical technique, but clinical outcomes are generally comparable [32].

Although successful in many cases, PK has several disadvantages. The surgery causes significant structural changes in the cornea and causes the cornea to be permanently weakened. Thus, even years after surgery, minor blunt trauma may lead to devastating wound dehiscence and globe rupture.

Due to the slow corneal wound healing, sutures have to remain in place for at least 1 year after PK. Although, optical properties tend to stabilize during this period, large refractive changes may occur after suture removal [49, 59, 103]. Thus, visual recovery after PK is slow and typically extends for 1.5–2 years after surgery, since unexpected postoperative ametropia or high astigmatism may require further surgical interventions. In a large registry study of more than 1100 eyes, an average astigmatism of 4.56 dioptres was reported [21]. Various attempts have been made to try to control the postoperative refractive outcome, including suture adjustments or selective removal of single sutures in the postoperative period. However, in most reports, the effect of these approaches is limited and with considerable variation [29, 31, 42, 110]. In addition, suture regularity has been found to have only little influence on the postoperative astigmatism [45].

New technological developments such as femtosecond laser (FS) penetrating keratoplasty allow precise and identical cuts to be made in donor and recipient. FS laser penetrating keratoplasty allows sutures to be removed earlier than after PK. However, FS laser-based approaches were hoped to improve the postoperative refractive outcome, but has so far disappointed. Thus, in several studies, the average astigmatism after FS laser PK is reported to be of the same magnitude or only marginally better than that of conventional surgery [13, 34].

In most cases, the unpredictable refractive outcome after PK can be relieved with glasses or rigid contact lenses; however, additional surgical interventions may be needed including arcuate keratotomy or laser keratorefractive surgery [58, 117]. In some patients, development of cataract may allow postoperative ametropia to be corrected during subsequent cataract surgery.

Immunological rejection is a major complication after penetrating keratoplasty. In most cases the immunological response is raised towards the endothelial cells leading to acute cell loss and imminent graft failure. The patient typically complains of slight ocular irritation or inflammation and reduced visual acuity and clinically presents with endothelial precipitates, sometimes in a Khodadoust line, and overlying stromal oedema [51]. If the condition is treated promptly, the inflammation may be controlled leading to gradual resolution. However, untreated or late-treated endothelial rejection will eventually lead to failure of the graft. An immunological response towards stromal cells is much more rare but should also be aggressively treated, since an untreated stromal rejection may lead to clouding of the graft [75]. The risk of endothelial rejection after PK varies with the pathology that led to corneal transplantation [25]. In keratoconus patients, the risk of endothelial rejection is reported from

5.8 to 6.8 % within the first 5 years [3, 20], whereas in high-risk corneas (Table. 17.1), the risk of rejection may be more than 50 % [2]. In a recent graft registry study, the 10-year graft survival was reported to be 89 % in keratoconus patients, 73 % in patients with endothelial dystrophy, 66 % in corneal scars, 59 % in herpetic scars, 42 % in secondary endothelial failure and 37 % in regrafts [118]. Thus, in patients with one or more risk factors, the indication for PK should be carefully considered, and the expected outcome thoroughly discussed with the patient.

Over the last years, it has been recognized that penetrating keratoplasty is followed by an accelerated loss of endothelial cells that fits a bi-exponential decay [9]. Thus, an initial rapid loss during the first approximately 4 years is followed by a slower but abnormal cell loss. Since endothelial cells are required to maintain corneal hydration and clarity, the accelerated loss of cells eventually leads to graft failure. The underlying pathology that led to keratoplasty, however, influences the rate of late endothelial changes. Thus, one study found that patients with bullous keratopathy or herpetic uveitis had a higher cell loss than patients with keratoconus [61]. In another study, the incidence of endothelial failure 15 years after PK was 8 % in keratoconus patients and 33 % in patients with bullous keratopathy [12], and it was hypothesized that a reservoir of viable endothelial cells in the recipient cornea reduce the overall cell loss, thus explaining the better outcome in patients without underlying endothelial pathology.

Indications for Penetrating Keratoplasty

Since most corneal pathologies tend to affect either the endothelium or the stroma, it is desirable to try to selectively treat the diseased part of the cornea, reducing the risk of some of the complications after PK. In patients with isolated stromal disease, an anterior lamellar approach may be preferred to PK in order to avoid the risk of failure due to endothelial cell loss or rejection. In contrast, patients with endothelial dystrophy or secondary bullous keratopathy usually have limited stromal changes until in very late stages of the disease. Thus, after the advent of EK, indications have been shifting, and PK is no longer the first choice in patients with endothelial failure. Today the main indications for PK are combined endothelial and stromal disease or deep scars extending to the most posterior layers of the stroma, which significantly reduces the possibility for successful deep anterior lamellar keratoplasty (DALK). Other indications for PK include regrafting in patients with failed previous PK, uncontrolled infectious or immunological keratitis or patients with failure during attempted DALK.

Anterior Lamellar Keratoplasty

As detailed above, two of the major reasons for graft failure after PK are loss of endothelial cells and risk of endothelial rejection [2, 9, 12]. Thus, it makes sense to conserve the endothelial cell layer in patients with isolated stromal conditions such as scars, stromal dystrophies or ectatic disease. Depending on the extent of the stromal changes and the employed equipment, different types of ALK can be performed.

Superficial Anterior Lamellar Keratoplasty

In patients with superficial stromal changes, ALK may be performed as an automated lamellar therapeutic keratoplasty (ALTK). In ALTK, a microkeratome is used to create a lamellar graft and to similarly remove the anterior part of the recipient cornea. Depending on the thickness of the microkeratome cut, a bandage contact lens may be sufficient to protect the graft for the first period after surgery [96]. Thus, with thin grafts, sutures may not be necessary giving ALTK a considerable advantage over deeper grafts or PK, where sutures may contribute to the unpredictable postoperative astigmatism [21].

In contrast to manual dissection, the microkeratome creates a very smooth interface, and visual outcome has improved with the automated approach. Still, many patients do not achieve as good a visual acuity with ALTK as with PK [77, 95]. Development of haze at the interface and

variations in graft thickness may be some of the factors that contribute to the suboptimal postoperative visual performance. A specific challenge with ALTK is risk of epithelial ingrowth into the interface that may be detrimental to the final postoperative outcome [97].

The recent development of femtosecond lasers (FS lasers) has led to new possibilities in ALK surgery (FS-ALK). FS lasers allow the formation of a planar graft with precisely defined diameter and edge. By performing a similar cut in the recipient cornea, a near-perfect match between the graft and the recipient can be obtained. Studies are few, but the visual outcome has been disappointing with only half of the patients obtaining a best-corrected visual acuity of 20/30 or better [15, 91]. At present, clinically controlled studies are needed to determine whether FS-ALK offers any significant advantage over traditional ALTK when it comes to postoperative outcome.

Deep Anterior Lamellar Keratoplasty

In patients with deep stromal changes or ectatic disease, superficial ALK is insufficient and deeper stromal dissection required. Traditionally, pre-Descemetic ALK has been performed by manual dissection, in which up to 10 % of the most posterior recipient stroma is left [8]. However, even with a meticulous surgical technique, it is difficult to obtain a smooth interface, and the visual outcome is often mediocre. A newer approach, deep anterior lamellar keratoplasty (DALK), allows the surgeon to obtain stromal separation at, or very close to, Descemet's membrane producing a smooth interface while leaving the endothelium intact. The various techniques to obtain the deep stromal separation are discussed in detail elsewhere in the book.

With DALK, it is possible to obtain a visual outcome rivalling that of PK [5, 22, 74, 90]. Unfortunately the technique is difficult to master, and rupture of the thin Descemet's membrane during surgery or insufficient separation of corneal layers is frequent complications that may require conversion to conventional PK in a high percentage of cases. When successful, however, DALK allows the recipient endothelium to remain untouched, eliminating both the risk of endothelial rejection and the accelerated postoperative endothelial cell loss that occurs after PK. Although rare, stromal rejection may still occur, which requires prompt reaction and treatment [73, 89]. The most frequent postoperative complication after DALK is unpredictable postoperative astigmatism of the same magnitude as after PK [5, 90].

Tectonic Keratoplasty

A tectonic lamellar keratoplasty is a therapeutic intervention performed to reinforce the cornea or replace tissue lost due to inflammatory ulceration or non-inflammatory thinning disorders. Thus, tectonic procedures are used in patients where ALK or PK is not possible or preferable. The techniques for performing tectonic grafts are multiple and depend on the specific condition being treated. The donor tissue is fashioned to match the defect in the recipient cornea and may include annular, horseshoe shaped, crescent shaped or oval grafts [18, 39, 40, 108]. Depending on the location of the graft and the underlying ocular pathology, the visual outcome after tectonic grafting may be very poor, and a penetrating keratoplasty may be needed to restore the patient's visual performance after the eye has quieted down.

Indications for Anterior Lamellar Keratoplasty

An anterior lamellar approach is indicated in corneas with isolated stromal changes. The decision between ALTK and DALK depends primarily on the depth of the stromal changes, but in very superficial cases, other treatment modalities such as excimer laser ablation may be considered if available. Superficial ALK may have the advantage of being sutureless with thin grafts, reducing the postoperative astigmatism; however, the visual outcome is often inferior in comparison with DALK or PK. Since most patients expect a good visual outcome after surgery, it is important that the patient is thoroughly informed about the benefits and disadvantages of a superficial anterior lamellar procedure.

In patients with deep stromal changes or ectatic disease, DALK will often be the most

obvious choice since it spares the recipient endothelium. However, the surgical procedure is difficult and more time consuming than PK. Inadvertent perforation of the thin Descemet's membrane or inability to obtain separation of the corneal layers occurs in a high percentage of cases. In patients with very deep stromal scars after hydrops or keratitis, the risk of perforating Descemet's membrane is high, and DALK with hydrodissection or big-bubble technique is unlikely to succeed. In these patients, PK may be considered the first choice, although pre-Descemetic ALK with manual dissection might be attempted to reduce the risk of endothelial rejection and graft failure [72].

Endothelial Keratoplasty

Descemet's Stripping Automated Endothelial Keratoplasty

In DSAEK a microkeratome is used to prepare a stromal-endothelial graft that is introduced into the recipient eye, positioned and kept in place with an air bubble. Several surgical approaches have been described, but the specific approach seems to have little influence on the clinical outcome, and overall DSAEK is quick to perform and can be mastered with relative ease. The various techniques are described in detail elsewhere in this book.

Since only the two most posterior corneal layers are affected by DSAEK, it offers several advantages over PK, including a more stable eye, less induced astigmatism and faster visual recovery. Thus, there is no major risk of globe rupture or wound dehiscence with minor blunt trauma after DSAEK. Furthermore, the surgery induces only little astigmatism, and the initial visual recovery is fast, allowing most patients to function normally within a few weeks after surgery. Nevertheless, even though visual acuity improves for more than one year after DSAEK, it still tends to be poorer than after PK [6, 19, 76]. There has been much debate on the underlying cause for the reduced visual acuity after DSAEK including graft thickness, irregularities or haze at the donor-recipient interface, lamellar orientation or changes in the recipient extracellular matrix [16, 17, 43, 50, 101]. Most probably several factors are at play, but the importance of each of these factors remains to be elucidated.

A unique complication related to endothelial keratoplasty is the risk of graft detachment within the first few days after surgery. The reported risk of detachment after DSAEK varies considerably, but in eyes with a normal anterior segment, it is generally in the range of 5–15 % [7, 60]. The underlying reason remains obscure, but detachment occurs more frequently in eyes where the amount of air in the anterior chamber after surgery may have been insufficient. This tends to be eyes with other ocular pathology including previous vitrectomy, iris defects, aphakia and previous glaucoma surgery. Graft detachments can usually be managed by re-centration of the graft and repeated air injection (termed rebubbling).

In DSAEK, the manipulation of the graft leads to a significant loss of endothelial cells during surgery; however, in recent studies, the endothelial cell density after 2 and 3 years has been found to be comparable to that of PK [80, 99].

In contrast, the rejection rate appears to be lower after DSAEK than after PK and has been reported to be 5–9 % after DSAEK versus 15–20 % after PK [44, 78, 80] with no major difference between surgery for primary or secondary endothelial failure.

Descemet's Membrane Endothelial Keratoplasty

The slightly disappointing outcome in terms of visual acuity after DSAEK has led to development of another surgical approach termed Descemet's membrane endothelial keratoplasty (DMEK) [69–71]. In DMEK, Descemet's membrane with endothelium is carefully harvested from the donor cornea and subsequently introduced into the recipient. Several techniques have been described and are detailed elsewhere. In contrast to DSAEK, DMEK is surgically much more challenging since the thin Descemet's membrane scrolls up with the endothelium facing outwards. Thus, the surgeon has to unscroll the tissue while at the same time ensuring proper centration and introducing and air bubble to keep

the graft in place. In addition to being a more difficult procedure, the risk of graft detachment after DMEK is higher than after DSAEK. Thus, graft detachments have recently been reported to occur in as much as 33–78 % of eyes within the first 4 days after DMEK requiring rebubbling in 7–30 % [105]. Furthermore, eyes with total graft detachment after DMEK represent a considerable surgical challenge since the thin graft will curl up again.

In comparison with PK, DMEK has the same favourable advantages as DSAEK when it comes to globe stability, induced astigmatism and visual recovery; however, in terms of postoperative visual outcome, DMEK seems to fare better than DSAEK [37, 38, 104]. Although the initial loss of endothelial cells may be higher after DMEK, the long-term cell loss has been reported to be similar to that of DSAEK and PK [30]. Furthermore, the risk of endothelial rejection has been reported to be much lower after DMEK as compared to DSAEK and PK [6].

Indications for Endothelial Keratoplasty

Due to the favourable outcome of EK in comparison with PK, all endothelial pathologies should, in principle, be treated with endothelial keratoplasty. Most secondary stromal changes due to endothelial pathology are reversible or may be addressed during surgery. Thus, patients with long-standing endothelial failure may have deposition of sub-epithelial fibrotic tissue that can be scraped or peeled off during surgery without compromising Bowman's layer. Full-thickness transplantation should only be considered in cases where other significant stromal changes such as keratoconus or stromal scars are considered to influence upon the postoperative visual outcome.

When it comes to choosing between DMEK and DSAEK, the decision may be more difficult. Overall, DMEK appears to be more favourable than DSAEK due to a better visual outcome and lower rejection risk. However, DMEK is surgically more challenging, which should be taken into account. First, harvesting the thin graft for DMEK requires considerable skill and may cause loss of tissue, which needs be considered in countries with shortage of donors. Second, the time required for successful DMEK surgery is more variable than for DSAEK, and even when the tissue has been prepared, DMEK may take considerably longer than DSAEK. Besides, many cornea banks are able to deliver pre-cut tissue for DSAEK, whereas only few banks as of yet are able to deliver pre-dissected DMEK grafts. Finally, patients with anterior chamber abnormalities including anterior chamber IOLs, aphakia, large iridectomies, partial aniridia, previous filtering surgery, glaucoma tubes or previous vitrectomy are generally not good candidates for DMEK, whereas DSAEK may be attempted.

Taken together, there are several factors to consider when choosing the optimal surgical approach for endothelial failure, where local circumstances may play a significant role, including availability of donor tissue and local logistics. Thus, although DMEK represents the state-of-the-art approach, DSAEK may still be the more obvious choice at many institutions.

To Graft or Not to Graft

Keratoplasty surgery has been rapidly evolving during the last 15 years, and indications have been changing. Where corneal transplantation used to be considered primarily in patients with severe visual reduction and bilateral affection, there has been an increasing tendency towards earlier intervention as well as treatment in cases with unilateral disease and a normal contralateral eye. However, the basic premise for doing surgery remains an estimation of the expected outcome in any given case. In other words, what are the odds that the patient will benefit from the surgical intervention? In most corneal transplantations, the main concerns will be the patient's postoperative visual acuity, the refraction and the expected risk of graft failure. To address these concerns, the surgeon needs to have specific knowledge of the possible surgical approaches as well as the postoperative treatments. In addition, the patient needs to be thoroughly informed about the procedure and the

expected outcome, as well as the potential risk for failure after surgery. Below, some of the most common pathologies leading to corneal transplantation are considered.

Endothelial Failure

In patients with endothelial failure due to Fuchs dystrophy or secondary bullous keratopathy, endothelial keratoplasty as either DSAEK or DMEK is the obvious surgical approach. The refractive outcome is generally excellent in both procedures, although a minor hypermetropic shift may occur [48]. However, DMEK usually offers better visual acuity than DSAEK, as well as a lower rejection rate, but the surgery is more complicated and with higher risk of perioperative tissue loss and postoperative graft detachment. Thus, local organization and tissue availability may make DSAEK the preferred approach. Furthermore, DMEK is usually not recommended in patients with anterior segment abnormalities, whereas DSAEK may be attempted in these cases.

Both DMEK and DSAEK can be performed as triple procedures with concurrent cataract surgery. However, in patients with limited changes due to endothelial dystrophy, it may be appropriate to do cataract surgery alone and postpone keratoplasty [109].

Keratoconus

Before keratoplasty is considered in patients with keratoconus, an attempt to correct the refraction with rigid, gas-permeable contact lenses should have been performed.

Other surgical interventions including implantation of intrastromal corneal ring segments should also have been considered [23]. However, if these approaches are found insufficient to help the patient, keratoplasty may be attempted.

During recent years, there has been a gradual shift in the preferred treatment of keratoconus at some institutions. Where PK used to be the preferred procedure of many surgeons, the use of DALK is gradually becoming more widespread. The main complication of both procedures is an unpredictable refractive outcome; yet, the best-corrected visual acuity is usually good. DALK offers the advantage of eliminating the risk of endothelial rejection; however, graft survival after PK in keratoconus is usually excellent, and a 10-year survival of 89 % has been reported [118]. Two recent studies found similar long-term graft survival after DALK or PK in keratoconus patients, with marginally better visual outcome after PK, but fewer postoperative complications after DALK [65, 122]. In this context, both procedures may still be considered as acceptable approaches when keratoplasty is needed in keratoconus patients.

Stromal Dystrophies

Keratoplasty for stromal dystrophies other than keratoconus constitute only a fraction of the total number of corneal transplantations. The surgical approach to stromal dystrophies varies according to the location of the stromal changes. In patients with predominantly superficial changes such as early granular dystrophy, treatment with excimer laser photoablation or superficial ALK may be performed. With deeper stromal changes, DALK or PK may be considered. Overall, the outcome of transplantation in stromal dystrophies is good with respect to the risk of rejection episodes. Unfortunately, several dystrophies show a high tendency towards recurrence, which severely may limit the outcome after transplantation, as detailed elsewhere in this book. Thus, knowledge of the tendency towards recurrence of the various dystrophies is of high importance in order to decide whether keratoplasty should be performed.

Stromal Non-herpetic Scars

Scars after infectious keratitis or corneal trauma may vary considerably in their extent. Furthermore, stromal scars tend to diminish over time due to slow corneal remodelling, why keratoplasty eventually may not be needed [67].

Thus, in most cases the surgeon should wait for several months, before surgery is considered. The specific surgical approach may be varied according to the depth of the stromal changes. In very superficial scars, excimer laser photoablation may be attempted or superficial ALK considered. With deeper scarring, DALK or PK may be preferred, but in patients with previous corneal perforation, DALK is unlikely to succeed.

A 10-year graft survival of 66 % has been reported after PK in patients with stromal scars, whereas only 47 % of grafts after traumatic injury were clear after 10 years [118]. In one report of DALK for infectious scars or trauma, a six-month graft survival of 94 % was reported [112]; unfortunately long-term studies are lacking.

It should be noted that stromal scars due to infection or trauma represent a very diverse group due to varying degree of accompanying risk factors. In many patients with stromal scarring, vascularization or glaucoma may be present, which will negatively affect the outcome of subsequent keratoplasty. Thus, in each case, the presence of risk factors should be noted and incorporated into the preoperative assessment.

Herpetic Scars

Stromal scarring secondary to herpetic eye disease may lead to severely compromised visual acuity and photophobia. However, performing a keratoplasty in herpetic eye disease may represent a significant challenge. First, vascularization of the stroma is often present which increases the risk of graft rejection significantly. Furthermore, even if the underlying infection has been quiet for years, the surgical insult and the subsequent steroid treatment may lead to herpetic recurrence [26, 63]. Viral reactivation will also lead to accompanying inflammation within the eye, further increasing the risk of a rejection episode [46, 102]. To reduce the possibility of viral reactivation, prophylactic antiviral medication should be administered for a long time after surgery [10, 33, 111]. Overall, there is considerable risk of graft failure, but with prophylaxis, a 2-year failure rate of 14 % was reported as compared to 56 % in patients with no prophylactic treatment [10]. Since the endothelium may be assumed to be unaffected in most patients, DALK may reduce the risk of a rejection episode and was recently reported to have better postoperative outcome than PK [119], whereas another study reported of a high percentage of postoperative complications including rejection and graft failure [64]. Thus, both PK and DALK may be attempted, but the indication for performing keratoplasty in patients with herpetic eye disease should be carefully considered, and the patient thoroughly informed about the potential outcome of the surgery.

In active herpetic eye disease, stromal melting and corneal perforation may occur. In these cases, high-dose antiviral medication should be instituted and emergency repair performed in order to preserve the eye. Emergency repairs may include amniotic membrane transplantation, tectonic keratoplasty or penetrating keratoplasty; however, due to considerable inflammation and ongoing viral replication, the long-term prognosis is usually poor.

Limbal Stem-Cell Deficiency

In patients with deficiency of the limbal stem-cell population, regeneration of the corneal epithelium is deficient, leading to development of pannus formation, corneal neovascularization and persistent epithelial defects or conjunctivalization [88]. Several conditions may be accompanied by limbal stem-cell deficiency including aniridia, Stevens-Johnson syndrome, ocular cicatricial pemphigoid and alkali injury. Irrespective of the underlying cause, these conditions represent a major challenge in keratoplasty surgery, since an intact and smooth epithelium is necessary for functioning corneal graft.

The ultimate treatment of epithelial stem-cell deficiency is limbal grafting. In patients with unilateral disease, conjunctival-limbal autografting with harvesting of tissue from the unaffected eye may be considered [53]. With this method, postoperative immunosuppression is not needed, and the survival rates for the transplanted tissue are good.

Following a successful limbal graft, subsequent DALK or PK will often be needed to obtain a clear stroma. Unfortunately, the amount of tissue that can be harvested for limbal autografting is limited and incurs a risk for the unaffected eye, which may reduce the expectations in patients with severe limbal stem-cell disease. Ex vivo expansion of autologous cells may increase the amount of available tissue [57, 79]. Unfortunately, culturing of limbal stem cells is at present only performed in few laboratories over the world.

When tissue for autografting is not available, transplantation of tissue from living-related donors or cadaveric eyes may be attempted. However, allogeneic conjunctival-limbal grafts are at considerable risk of rejection, although a 77 % success rate has been reported with extensive systemic immunosuppression for 1–2 years [47]. In addition to the guarded prognosis, the use of systemic immunosuppressants incurs a short- and long-term risk for development of malignant tumours. Thus, the indication for limbal grafting with allograft tissue should be extensively discussed with the patient.

In patients where systemic immunosuppression is contraindicated or unwanted, pre-Descemetic ALK or DALK as an isolated procedure (without prior limbal grafting) may be attempted to obtain a short- or medium-term improvement in the patient's visual performance. However, the patient should be carefully informed that the prognosis usually is poor with expected recurrence of symptoms.

Dry Eye Disease

Patients with severe dry eye due to primary or secondary Sjögren's syndrome or graft-versus-host disease have problems maintaining an intact epithelium, and persistent epithelial defects represent a risk for secondary stromal melting [94]. Thus, emergency corneal repair with amniotic membrane transplantation, tectonic grafting or PK may be needed. A temporary or permanent partial tarsorrhaphy may promote postoperative epithelial repair, in combination with frequent lubrication or serum eye drops. Still, despite intensive treatment, severe dry eye disease has a poor prognosis with a very high risk of graft failure after keratoplasty.

Corneal Emergencies

In corneal emergencies, keratoplasty may be performed in order to preserve the eye. Thus, corneal perforation or near perforation due to uncontrolled infectious keratitis or severe immunological disease may call for an emergency keratoplasty. Underlying causes may include melting due to herpes infection, peripheral ulcerative keratitis or Mooren's ulcer. In most instances, emergency keratoplasty may be performed as amniotic membrane transplantation, a lamellar tectonic (repair) procedure or a penetrating keratoplasty. The purpose of the acute surgical intervention is preservation of the eye, giving time for control of the underlying infectious or inflammatory condition. In tectonic procedures, later penetrating keratoplasty may be needed to restore the patient's visual performance. Similarly, in emergency penetrating keratoplasty, the heightened immunological response will often reduce the long-term graft survival, and a 32 % 10-year graft survival has been reported [118]. Thus, regrafting may be needed to improve the patient's visual acuity at a later time point, preferably after the eye has been quiet for a long time.

High-Risk Grafts

In patients with one or more risk factors for graft failure (Table. 17.1), the indication for keratoplasty needs careful consideration. If regrafting is performed, one or more approaches may be attempted to reduce the postoperative failure rate.

In corneas with stromal vascularization, attempts to reduce the amount of vessels, either before or during grafting, may include fine-needle diathermy and anti-VEGF injections [28, 56, 107], but clinically controlled studies are lacking.

In some studies, HLA matching has been found to reduce the risk of rejection. Thus, a survival rate of 92 % in HLA class I and II matched donors as

compared to 66 % in mismatched donors in normal-risk PK has been reported [84]. Similarly, better survival has been reported in grafts with few HLA-A or HLA-B mismatches in high-risk PK [11, 87, 113]. However, although several studies suggest a beneficial effect in tissue matching, clinically controlled studies are lacking.

Systemic immunosuppression in high-risk keratoplasty has been reported with various drugs including cyclosporin A, mycophenolate mofetil or tacrolimus [85, 114]. However, although these drugs may reduce the risk of graft failure in high-risk cases, there is a substantial need for clinically controlled studies to determine when to use which drugs and for how long. Still, systemic immunosuppressive therapy may be considered as a means of reducing the failure rate. However, the therapy may induce secondary malignancies, and the potential benefit for the patient should be carefully weighed against the risk.

Keratoprosthesis surgery represents another approach in patients with recurrent failure due to one or more risk factors. At present, the Boston type 1 K-Pro is the most frequently used keratoprosthesis, and successful outcomes have been reported in high-risk transplantations due to multiple failed grafts, herpetic eye disease and aniridia [1, 54, 120, 121]. On the other hand, results in patients with ocular surface disease or severe dry eye are discouraging, and these patients are usually poor candidates for Boston K-Pro implantation. In these patients, an osteo-odonto-keratoprosthesis may be considered [98]. Regardless of the surgical approach, all keratoprosthesis procedures require frequent and lifelong control and have significant risk of complications including glaucoma, endophthalmitis, retro-prosthetic membranes or retinal detachment [98, 121]. Thus, keratoprosthesis surgery should only be considered in highly motivated patients that accept the potential short- and long-term complications.

Summary

The field of corneal transplantation has been rapidly evolving during the last decades with selective lamellar approaches gradually replacing full-thickness transplantation in a number of conditions. The corneal surgeon can no longer rely on a single technique for treating corneal blindness but needs to be proficient with several different approaches, some of which are technically demanding. For each patient, the surgeon has to decide whether to graft and which procedure to choose, a decision that requires intimate knowledge of the strengths and weaknesses of the various approaches. First and foremost, however, the surgeon should consider the patient's needs and evaluate whether a surgical intervention has a fair chance of improving the patient's quality of life that remains the overall purpose of keratoplasty surgery.

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Index

J. Hjortdal (ed.), *Corneal Transplantation*, DOI 10.1007/978-3-319-24052-7