Chapter 10

CURRENT PROGRESS IN FEMTOSECOND LASER-ASSISTED ENDOTHELIAL KERATOPLASTY

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ABSTRACT

The introduction of femtosecond laser has been a revolution in corneal refractive surgery. The femtosecond laser is able to deliver precise intrastromal cuts and offer a wide range of applications in corneal refractive surgery, including endothelial keratoplasty (EK). Femtosecond lasers offer more consistency and flexibility in cutting depth than conventional microkeratomes in dissecting donor posterior lamellar disc (PLD) for EK surgeries. However, there is variability in the smoothness of cut interface when using femtosecond laser to prepare the donor tissue, which has prevented it from achieving on-par clinical results with microkeratome-assisted EK. Therefore, many studies have focused on optimizing the laser settings and refining the technique. In this chapter, we summarize the current progress in femtosecond laser-assisted EK.

1. INTRODUCTION

The cornea is a highly organized, avascular and transparent tissue. In addition to serving as a protective barrier for intraocular tissues (e.g., the retina, iris and lens), it is also responsible for refracting approximately two-thirds of the incoming light to focus on the retina. The human cornea comprises five distinct layers. Anteriorly, the cornea is composed of a stratified, squamous and non-keratinized epithelium that overlies Bowman’s membrane,

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acting as the main protective barrier [1]. The underlying stroma comprises the bulk of the cornea and is constructed of highly organized collagen fibrils [2,3]. The stroma is separated from the monolayer of endothelial cells by the Descemet’s membrane (DM), which acts as a basement membrane for the endothelial cells. Each of the five layers has distinct functions and collectively, they function to maintain the homeostasis of the whole cornea. For the purpose of this chapter, we will focus on the function of the corneal endothelium, corneal endothelial defects, and the application of femtosecond laser in endothelial keratoplasty (EK), a corneal surgical procedure that allows selective replacement of diseased endothelium.

2. PHYSIOLOGY OF CORNEAL ENDOTHELIUM

The corneal endothelium is a monolayer of cells, lining the posterior surface of the cornea. The function of the cells is unique in that they allow leakage of solutes and nutrients from the aqueous humor into the more superficial layers of the cornea to provide nutrition for epithelial cells and stromal keratocytes, while at the same time actively pumping fluid out of the stroma into the anterior chamber of the eye [4,5]. The presence of abundant glycosaminoglycans in the stroma absorbs large amounts of fluid, inducing an edematous and opaque cornea that can lead to the loss of vision [6]. Therefore, the metabolically active-endothelial cells are an important barrier for excessive fluid moving into the stroma.

The normal corneal endothelial cells are predominantly hexagonal in shape (Figure 1). The honeycomb-like shape provides the greatest efficiency, in terms of total perimeter and cell packing [7,8]. The cells are post-mitotic and do not proliferate in the post-natal human cornea [9,10]. Unlike corneal epithelial cells, which have renewal capability after trauma or injury, apoptosis of the corneal endothelium prompts healing of the monolayer by physiological spreading and enlargement of adjacent endothelial cells in order to maintain functional integrity, corneal deturgescence and corneal transparency [10,11]. The loss of endothelial cell density is, therefore, accompanied by increases in variability of cell size (polymegathism) and cell shape (polymorphism).

Figure 1. En-face image of corneal endothelium under in vivo confocal microscope.
Because of the non-proliferative state of corneal endothelial cells, there is an inverse relationship between age and cell density [11-14]. The cell density of 2-month-old infant has been reported to be as high as 5624 cells/mm$^2$, with an average of 4252 cells/mm$^2$ within the first year from birth [15]. The cell density reduces rapidly during early childhood and is associated with the increase in corneal size due to normal eye growth [11]. At 5 years of age, cell density of $3591 \pm 399$ cells/mm$^2$ decreases to $2697 \pm 246$ cells/mm$^2$ by 10 years of age [12]. The rapid changes of the cell density decelerate to a more gradual decline throughout adulthood, to approximately 0.6% per year [11,13,16]. Although the corneal endothelial cell density declines with age, its average reserve is sufficient in maintaining the critical barrier and pump function throughout a human’s lifetime [17].

The function of the endothelium is compromised once the cell density falls under the critical threshold of 500-1000 cells/mm$^2$ [18,19]. Accelerated or acute endothelial cell loss can be caused by inadvertent surgical trauma [20], endothelial dystrophy [21-23], or from prior intraocular surgery [24,25]. These patients usually present with stromal edema, corneal opacity, degrading visual acuity, and in severe cases, total corneal blindness. Currently, the only effective treatment strategy to restore the vision of these patients is to replace the defective endothelium with healthy, functional donor corneal endothelium by corneal transplantation [26].

**3. EVOLUTION OF CORNEAL TRANSPLANTATION**

Corneas are the most commonly transplanted tissue worldwide, and the indications for transplantation include a wide range of diseases, such as corneal ectasias, corneal dystrophies and endothelial disorders [26,27]. In the United States, 42,642 corneal transplantations were performed in 2010 compared with a total of 12,623 solid-organ transplantations in 2008, which included kidney, liver, lung, pancreas, heart, and intestine replacements [26].

The first successful human full-thickness corneal transplant was performed by Zirm [28]. The technique of replacing the full-thickness diseased corneal tissue with full-thickness donor cornea is known as penetrating keratoplasty (PK). Over the last half of the 20th century, PK was the standard of surgical correction for most corneal diseases [29-31].

With advancements in surgical instrumentation and technology over the last decade, there has been an increased popularity in lamellar keratoplasty (LK). LK through a posterior approach is now widely known as EK and has become the preferred technique over PK for treating endothelial disorders. There is growing evidence that supports the advantages of EK over PK [32,33], which include: 1) reduced rates of severe complications, such as suprachoroidal hemorrhage; 2) sutureless surgery that minimizes induced astigmatism; 3) preservation of corneal innervations; 4) better tectonic strength; and 5) improved visual recovery.

**4. ENDOTHELIAL KERATOPLASTY**

The concept of selective replacement of dysfunctional endothelium was first described by Tillet in a case of posterior lamellar keratoplasty (PLK) [34]. However, the current success of
EK is attributed to Melles et al. [35], who described a PLK technique that utilized air injection, instead of sutures to affix the graft to the recipient cornea. The procedure has since undergone many refinements since its introduction. The initial procedure described by Melles et al. [35] involved the creation of large incision where the recipient and donor tissue were manually dissected. Terry and Ousley [36] then refined the procedure and renamed it deep lamellar endothelial keratoplasty (DLEK). They reported good visual and refractive outcomes, comparable to those of PK [37]. The technique was further refined by Melles et al. [38], to a small-incision procedure in which the recipient cornea was prepared by stripping the DM and endothelium and replaced with a thin layer of donor stromal tissue, DM and endothelium. The technique was popularized by Price et al. [39], who termed it Descemet’s stripping endothelial keratoplasty (DSEK). In brief, recipient’s diseased Descemet’s membrane (DM) and endothelium are first stripped and removed (Figure 2A). Donor corneal graft is then prepared by stripping a thin layer of stromal tissue, DM and endothelium, which is then transplanted into the recipient’s bare stromal bed (Figure 2B).

Recently, a further refinement of EK was made by Melles et al. [40] in which the stromal portion of the donor was completely eliminated so that the graft consists purely of DM and endothelium. DMEK (Figure 2A and C) essentially replaces the same portion that is stripped off the recipient cornea and thus perfectly replicates pre-operative corneal anatomy, unlike other procedures.

The procedures described above, however, relied on manual lamellar dissection of the donor cornea. The resulting depth of manual dissection was often inconsistent, which caused donor-host interface irregularities, reduced post-operative visual acuities and occasionally, perforation of the donor tissue [41]. Gorovoy [42] was first to introduce the use of microkeratome-assisted lamellar technology in which the donor tissue dissection was performed using an automated lamellar therapeutic keratoplasty (ALTK) unit. This variant of DSEK was termed Descemet’s stripping automated endothelial keratoplasty (DSAEK) and is currently the most popular form of EK.

Figure 2. Illustration of Descemet’s stripping endothelial keratoplasty (DSEK) (A and B) and Descemet’s membrane endothelial keratoplasty (DMEK) (A and C) surgical procedures.
Automated microkeratomes can produce a smoother surface than manual dissection both for superficial and deeper dissections [43], but there are still variations in the quality of the cut surfaces [44]. The usual complications of microkeratome-laser in situ keratomileusis (LASIK), for example incomplete flaps, buttonholes and perforations may also occur. The precision of a microkeratome cut is often affected by the pressure of artificial anterior chamber where the donor cornea is mounted on for dissection [45], and the translation/oscillation speeds of the blade [46]. Although multiple heads are available in the ALTK set, surgical customization is often restricted to the imposed increments in head size. To resolve the shortcomings of automated microkeratomes, femtosecond laser has been extensively studied and optimized to produce posterior donor lenticules for EK.

5. **MODE OF ACTION OF FEMTOSECOND LASER**

The physical mechanism of laser-tissue interaction is called laser-induced optical breakdown. The mechanism relies on the non-linear absorption in the target achieved when the tissue specific radiant exposure is exceeded. When the laser pulse is compressed in time (in femtosecond range) and space (highly focused), the threshold for plasma formation diminishes, which minimizes thermal and mechanical effects of the laser, as well as collateral damage to the surrounding tissue [47].

The currently available femtosecond laser platforms for corneal refractive surgery demonstrate the optical breakdown at the focal plane of the laser beam as the micrometer-sized laser spots are fired, leading to tissue photodisruption (Figure 3). The high field intensities at the focal region of close to $10^{13}$ W/cm$^2$ lead to generation and accelerations of free electrons, forming plasma [48]. At such intensities, the corneal tissue becomes ionized by multiphoton absorption [49]. The thermal relaxation of the electron gas with the surrounding media increases the temperature rapidly [50]. Consequently, the heated media expands explosively and creates a shock wave, leading to formation of a cavitation bubble. The formation of the cavitation bubbles, confined to a layer at the focal plane of the laser, is the main driving force in creating a plane of dissection within the corneal stroma.

6. **FEMTOSECOND LASER IN ENDOTHELIAL KERATOPLASTY**

The introduction of femtosecond laser has been a major advancement in corneal refractive surgery. The femtosecond laser is able to deliver precise intrastromal cuts and offer a wide range of applications in corneal refractive surgery. It has been most widely adapted for the creation of LASIK flap due to its improved safety, consistency and planar flap thickness [51-53]. Other applications of femtosecond laser include creation of intrastromal pocket for insertion of intrastromal corneal ring segments [54,55], arcuate keratotomy and/or wedge resection for correction of high astigmatism [56], and creation of intrastromal lenticule to correct myopia and astigmatism in refractive lenticule extraction (ReLEx) [57,58]. Femtosecond lasers have been used in the field of corneal transplantation for PK [59,60], as well as anterior LK [61]. A natural extension of the use of femtosecond laser in corneal transplantation would therefore be to perform EK. In the literature, two approaches have been
studied to dissect the donor posterior lamellar disc (PLD) using femtosecond laser: epithelial approach and endothelial approach.

Figure 3. Illustration of photodisruption process in corneal stroma.

Figure 4. Visualization of corneal endothelium on posterior lamellar disc dissected at 3 different depths (A). Correlation between graft thickness and residual endothelial cell density (B).
6.1. Epithelial Approach

In the literature, the majority of studies have documented the preparation of donor lenticules using femtosecond laser from an epithelial approach. There are two parameters that have been used to determine the quality of donor PLDs: corneal endothelial density and viability, and smoothness of lenticular interface. The reported outcomes of the quality of the donor lenticular interface have varied [62,63]. It is now understood that smoothness of the lenticular interface made by femtosecond laser can be determined by the type of laser or laser pattern used, the frequency or energy of the laser, and the type of applanation glass, i.e. flat or curved [61,64-67]. The preservation of corneal endothelial density and viability, however, has been consistent and similar between the donor tissues prepared using femtosecond laser and microkeratome [68,69]. Figure 4, kindly reproduced with permission from Dr. Holger Lubatschowski, contains anterior segment-optical coherence tomography (AS-OCT) images showing the variability of the graft thickness and the corresponding endothelial cell density viewed under the scanning electron microscope (SEM) after laser cutting. The residual endothelial cell density was reduced when the graft prepared had a thickness of 36 µm, but not 89 and 106 µm (Figure 4A). The bar graph shows that the residual endothelial cell density was not affected when the graft thickness was maintained at 50 µm and above, which is sufficiently thin for use as an ultrathin DSAEK donor tissue (Figure 4B).

When using the FEMTEC system (20/10 Perfect Vision, Heidelberg, Germany) with a repetition rate of 12.5 kHz and pulse energy of <10 µJ in both porcine and human eyes, Seitz et al. [62] reported smooth cut surfaces and rectangular corners with minor remaining tissue bridges. In 2005, Soong et al. [63] utilized the IntraLase system (IntraLase Corp., Irvine, CA) to prepare PLDs. They set the firing rate at 15 kHz and energy level at 8.7 µJ, and were able to produce an excellent side-cut quality and a smooth lamellar interface. However, the measured disc thickness was 55 ± 61 µm thicker than the intended thickness, which might be caused by hyperosmotic corneal deturgescence during tissue cutting and exacerbated by optical aberrations during deep treatment in opaque corneas [70]. Indeed, when using a higher frequency and lower energy setting laser (e.g., 30 kHz and 60 kHz IntraLase and 40 kHz FEMTEC), the measured thickness of the PLDs became similar to the intended thickness [64-66]. However, it has been a common problem in that the deeper into the corneal stroma the lamellar cut, the rougher the interface created. The difference in lamellar architecture across the cornea can explain this phenomenon. The anterior stroma is constructed of large bundles of collagen fibers [3] and dense interlamellar branching [71], which prevents the collagen lamellae from excessive distortion during suction and anterior lamellar dissection. The posterior stromal lamellae are less interweaved and randomly distributed [3], which easily distorts during suction and posterior lamellar cut, and impairs the regularity of the resulting cut interface. Using a 500 kHz VisuMax femtosecond laser system (Carl Zeiss Meditec, Jena, Germany), which has considerably lower suction [57] and use energy significantly lower than previous femtosecond laser platforms (IntraLase and FEMTEC), the resulting donor PLD interface was however, still similarly rough [72]. Using the same femtosecond laser platform, we also found that the PLD interface was rough under SEM and the quality was not as good as a microkeratome created stromal bed (Figure 5A), although the accuracy of the cuts was superior (Figure 5B and C). There was a strong correlation between the achieved thickness and attempted thickness of the PLDs prepared using the femtosecond laser system ($r^2 = 0.70; p = 0.001$) (Figure 5B). On the other hand, the correlation between the achieved
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thickness and attempted thickness of PLDs prepared using microkeratome was poor ($r^2 = 0.24; p = 0.001$) (Figure 5C).

Studies on spiral cutting patterns of corneal posterior stroma using IntraLase and FEMTEC have shown the appearance of concentric rings and a more stucco-like texture of the stromal bed [63,73]. However, our group did not document similar findings using the later generation FEMTEC system, which had higher frequency laser setting [66]. In another study comparing raster cutting pattern and conventional spiral cutting pattern, Sarayba et al. [61] reported that the former produced a smoother stromal bed than the latter in deep stromal ablation.

The irregular corneal graft can potentially lead to persistent DM folds reported in several patients after femtosecond laser-assisted DSAEK in a case study [74]. The irregularities and folds of PLDs have been the primary cause for the limited improvement in corrected visual acuity [74,75]. Several studies have been conducted to overcome the rough cut surface of the PLDs. Mehta et al. [66,76] proposed the use of double-pass ablation to produce a smooth donor lenticular interface. The second-pass ablation at the same depth as the first-pass ablation ensures the remaining tissue bridges are disrupted and easy separation of the lamellar disc from the whole donor cornea. Using SEM, the authors showed qualitatively smoother stromal beds than those after single-pass ablation. Recently, Rousseau et al. [77] introduced a double-layer cutting technique, in which two-thirds of the anterior stroma was first removed and followed by creation of the lenticule, which was only 150 μm away from the zero referential point of the laser at this point. Although the cut was still performed in the less compact layer of posterior stroma, but with a thickness of 150 μm only, the light diffraction and optical aberrations no longer affect the quality of the lamellar cut. On SEM, the authors showed a smoother lenticular interface than that created by the conventional technique using the same femtosecond laser system (60 kHz IntraLase). However, it’s paramount to find an optimal artificial anterior chamber pressure and laser settings before the second-layer ablation. The residual mid-stroma, thinner at this point, can bend easily and affect the quality of the stromal cut.

Another possible technique to smoothen the femtosecond laser-created irregular lenticular surface is by an excimer laser. A recent study by Cleary et al. [78] demonstrated the excimer laser passes were able to improve the countour and smoothness of DSAEK grafts without damaging donor endothelial cells. However, the use of high energy excimer laser can induce more cellular damage and inflammation in the corneal stroma compared to femtosecond laser [79], which may induce interface haze and delay the visual recovery after transplantation.

There are only a few clinical results of femtosecond laser-assisted EK reported in the literature. In 2008, Cheng et al. [75] reported an improvement of best spectacle-corrected visual acuity (BSCVA) from 20/110±4 lines pre-operatively to 20/57±1 line 6 months post-transplantation in 11 eyes. The mean endothelial cell density at 6 months was 1368±425 cells/mm². In 2009, the same group of clinicians showed at 12 months, the mean BSCVA was 20/70±2 lines and 20/44±2 lines (p<0.001) after femtosecond laser assisted-EK and PK, respectively [80]. The endothelial cell loss was significantly higher after femto-EK than PK (65±12% vs 23±15%; p<0.001). These two studies utilized a 30-kHz IntraLase system to prepare the donor PLDs. Recently, utilizing a 500-kHz VisuMax femtosecond laser system, Vetter et al. [74] compared the clinical results of microkeratome-DSAEK and femto-DSAEK, and found that the BSCVA was poorer in the latter group (0.48±0.20 vs 0.3±0.11; p=0.038).
The post-operative endothelial cell density was not reported by the authors. One common issue of femto-EK raised by these three studies was the irregular donor PLD interface that attributed to the poor BSCVA.

6.2. Endothelial Approach

Current laser technology only allows the calculation of depth of the horizontal lamellar cut from the anterior surface of the donor cornea. This limitation produces a meniscus-shaped donor lenticule that is thinner in the center and thicker at the edges, because the human cornea is anatomically thicker in the periphery than in the center [81]. A meniscus-shaped lenticule can produce a mild hyperopic shift, as has been shown in previous studies [41,82]. To address this problem, Sikder and Snyder [83] reported the preparation of the donor cornea from endothelial approach using a viscoelastic material as “cushion” to protect the endothelium during the applanation and laser delivery. The regularity of the stromal bed and graft thickness were not assessed in the study, but the authors showed the use of hydroxypropylmethyl-cellulose viscoelastic material resulted in 6% endothelial cell loss compared to 18% cell loss using BSS.

Figure 5. Quality of posterior lamellar disc (PLD) dissection using 500-kHz VisuMax femtosecond laser system (A). Correlation between the achieved thickness and attempted thickness of the PLDs prepared using femtosecond laser (B) and mechanical microkeratome (C).
Using a 500kHz VisuMax femtosecond laser system, Hjortdal et al. [84] were able to produce planar and thin grafts, which were subsequently transplanted in 10 patients with Fuchs’ endothelial dystrophy. Although only a thin layer of organ culture medium was applied between the applanation glass and donor endothelial layer, the authors reported endothelial cell loss and corrected visual acuity similar to after DSAEK with microkeratome prepared grafts one year post-operatively [85].

CONCLUSION

EK surgeries are constantly evolving with improvements being made to improve visual recovery rate, endothelial cell counts and graft survival rates. One of the improvements is the utilization of femtosecond laser to help improve the precision of donor graft dissection. The problems, that hinder the femtosecond laser-assisted EK in achieving similar clinical outcomes as microkeratome-assisted EK, are mainly associated with the irregularity of the donor stromal bed and meniscus-shaped lamellar disc. To solve the problem, attempts have been made to optimize the laser settings and to modify the donor preparation technique. Preparation of donor tissue from endothelial approach using the femtosecond laser with the help of viscoelastic “cushion”, may have addressed the aforementioned problems and the technique warrants a clinical trial.

Recent reports have also suggested DMEK to be the next iteration of EK, and it has been shown to produce superior post-operative visual acuity and visual recovery compared to DSAEK [86,87]. However, by using femtosecond laser, it is possible to create ultra-thin posterior donor tissues with consistent thickness and minimal endothelial cell loss [88], and thus enables us to perform ultra-thin DSAEK consistently. Clinical results have shown similar post-operative visual acuity and visual recovery between ultra-thin DSAEK and DMEK [89]. The future of EK may lie on ultra-thin DSAEK, which maintains the advantages of both DSAEK, with respect to ease of tissue handling and good post-operative cell counts, and DMEK, with respect to good post-operative visual acuity.

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