Annals of Surgery and Perioperative Care

Open Access

Austin Publishing Group

Special Article – Ocular Surgery

Corneal Graft Preparation Using Femtosecond Laser Cut from the Endothelial Side for Endothelial Keratoplasty

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Received: March 13, 2017; **Accepted:** April 05, 2017; **Published:** April 10, 2017

Abstract

Purpose: To evaluate the quality of human corneal endothelial grafts and the difficulty of tissue separation prepared by low pulse energy femtosecond laser cut using endothelial side approached technique.

Methods: Sixteen human corneas were used in the study. Each cornea was mounted on an artificial anterior chamber with the endothelial side up, coated with 1.8% sodium hyaluronate. Low pulse energy type femtosecond laser was set up to create a corneal lenticule of 110 micron thickness with 8.5mm in diameter. Quality of corneal endothelial graft was determined by specular microscopy to measure endothelial cell density and Trypan blue/Alizarin red staining to demonstrate cell viability. Difficulty of tissue separation was also determined.

Results: The mean endothelial cell density was 2,569 ± 505 cells/mm^2 and 2,340 ± 441 cells/mm^2, before and after femtosecond laser cut respectively. This represented 8.9% (range from 0.8 to 24.7%) of endothelial cell loss after the procedure. Mean area of endothelial cell damage staining with vital dye was 4.6% (range from 0.2 to 9.9%). Four out of sixteen tissues (25%) required scissors to excise rim tags. Mild resistance during lamellar separation was found in 25% of the tissues, the rest showed minimal to no resistance.

Conclusion: The technique of corneal graft preparation using femtosecond laser cut from the endothelial side with viscoelastic coating produced good quality of endothelial cells. Tissue separation both rim and lamellar plain were uncomplicated. However, modification of laser setting may require increasing the simplicity of tissue cutting and separation.

Keywords: Corneal transplantation; Corneal graft preparation; Endothelial side cut; DSEAK; Endothelial keratoplasty

Abbreviations

EK: Endothelial Keratoplasty; DSAEK: Descemet Stripping Automated Endothelial Keratoplasty; LASIK: Laser-Assisted In Situ Keratomileusis

Introduction

Endothelial keratoplasty (EK) has currently become the standard treatment for Fuchs corneal dystrophy and other causes of corneal endothelial dysfunction instead of a full thickness cornea transplantation or penetrating keratoplasty (PKP) [1]. EK provided faster visual recovery, corneal integrity and lower surface problem comparing to PKP [2,3]. Descemet stripping automated endothelial keratoplasty (DSAEK) is a technique which surgeon strip and remove patient's diseased endothelium. Then the donor cornea graft composed of endothelium and a thin layer of posterior stroma was replaced.

Generally, donor corneal preparation for DSAEK is routinely performed by an experienced technician using microkeratome cut from the epithelial side [4]. However, it produces varies in thickness and causes a meniscus shape of the corneal graft or lenticule, i.e. thicker at the periphery and thinner at the center of the lenticule. The negative optical power induced by this lenticular shape caused hyperopic shift of refractive power for recipient [5]. Additionally, corneal lenticule thickness is another important concerned since it affects the visual outcome. Many studies showed that the thinner corneal lenticule demonstrated better visual and refractive outcome and suggested that the studies showed that a 100-150 micron corneal graft is a desirable thickness [6-8]. This preferred thickness often requires a second microkeratome cut in order to reach the desirable depth, which may result in a higher rate of corneal perforation and/or endothelial cell damages [6,7,9,10].

Therefore, the idea of using femtosecond laser to make a cut and create a corneal graft is introduced to overcome these obstacles [11,12]. In general, femtosecond laser created a very precise cut, but the cut of this laser from front corneal surface produced irregular interface which may be the result of deep focusing of the laser beam, causing in homogeneity of the laser energy [13]. Significantly, this irregularity affects visual outcome [14].

Lasik flap creation using femtosecond laser was a well-known procedure. The laser cut produced a very precise flap thickness and smooth interface. The surface damage or epithelial injury is very minimal and also can be prevented with coating agents.

Citation: Reinprayoon U, Bhoopat T and Kasetsuwan NG. Corneal Graft Preparation Using Femtosecond Laser Cut from the Endothelial Side for Endothelial Keratoplasty. Ann Surg Perioper Care. 2017; 2(2): 1025.

Some published experimental and clinical data showed promising outcomes [15-17]. For these reasons, we propose an experiment trial to prepare a corneal graft using femtosecond laser approached from the endothelial side which will cause shallower of the focusing beam, expecting in less irregularity of the lenticule. Quality of the corneal endothelial graft was determined by endothelial cell density and viability. The difficulty of this technique to separate the tissue was also assessed.

Materials and Methods

Donor corneal tissue

Sixteen human donor corneas unsuitable for clinical transplantation provided by Thai Red Cross Eye Bank were used in the study. The corneas were stored in Optisol-GS medium (Bausch & Lomb Inc, Irvine, CA, USA) at 4 degree celcius. The mean donor age was 70.6 year old (range 48 to 93 year). The mean death to corneal preservation time was 3.25 hours (range 1 to 4 hours). The mean death to femtosecond laser cut time was 4 days (range 1-9 days).

Femtosecond laser setting

The femtosecond laser machine was Femto LDV (Femto LDV Z6 Power Plus, Zimmer, Switzerland), which generated a low pulse energy. The laser setting was 110 micron depth with 8.5mm in diameter and 90 degree vertical side cut. The pattern of laser firing started with raster pattern on the lamellar cut and vertical side cut for the lenticule edge.

Cutting technique

The cornea was mounted on the anterior artificial chamber with endothelial side up. The cover was assembled and tightened while the connector was closed. The pressure was gently increased to let the cornea slowly turned to reversed position. Few amount of sodium hyaluronate (IAL-F, TRB Chemedica, Geneva, Switzerland) was instilled on the corneal surface in order to coat the endothelium. The column of artificial chamber was set at the lowest position to prevent accidentally touching the femtosecond laser intershield. The laser headpiece was placed in the position. The column was lifted up slowly until water mark was observed and continued lifting until the mark reached the edge of the intershield. Femtosecond laser was then activated. The column was lower slowly after the completed cut. The connector was closed to maintain the stable pressure during disassembling the cover. The cornea was removed and flipped back to normal shape before keeping in the Optisol-GS medium chamber.

Endothelial cell count and staining

Central endothelial cell density was evaluated pre and post cutting with keratoanalyzer (Konan Medical, Inc., Irvine, CA, USA) by experienced eye bank technicians. The mean endothelial cell density before laser cutting was 2,569 cell/mm^2 (range 1,414 to 3.106 cell/mm^2).

Corneal viability was evaluated using a live/dead cell staining with 0.3% Trypan blue/0.25% Alizarin red staining. The tissue was stained with 0.3% Trypan blue in phosphate-buffered saline for 90 seconds, after which it was rinsed with phosphate-buffered saline. The tissue was subsequently stained with 0.25% Alizarin red for 90 seconds and rinsed again with phosphate-buffered saline [18]. Trypan blue stained damaged cells, while Alizarin red stained border of the cell. Photographs were taken under a stereo type microscope, Nikon SMZ800. The dead cell area demonstrated by Trypan blue stained cells was calculated and compared to total cell area by using Image-Pro Plus and Image-Pro Analyzer software version 7.0.1.

Tissue separation

Sinskey hook and 0.12 forceps were used to separate the tissue. Difficulty of tissue separation at the side cut and stromal bed peeling were evaluated after finishing endothelial cell count using subjective grading scores, range from 1 to 4, which previously mentioned by Jodhbir SM et al [19]. Grade 1 represents more tissue resistance during separation than grade 4.

Results

The mean central endothelial cell density was 2,569 ± 505 cell/mm^2 before cutting and 2,340 ± 441 cell/mm^2 after the femtosecond laser cut. The average endothelial cell loss at central area was 8.9% (range 0.8% to 24.7%). Four out of sixteen eyes had air bubble located in the central area which interfered the cell counting process. Technician had to delay about two hours to re-count the cell density.

The mean area of endothelial cell damage assessed by Trypan blue/Alizarin red staining was 4.57% and the ranged was 0.23 to 9.85%. The Trypan blue positive staining area was frequently found along the laser cut edge.

Regarding the difficulty of tissue separation, the rim separation was grade 1 in four tissues, grade 2 in 8 tissues and grade 3 in 4 tissues. To complete the graft cut, we used vannus scissor to excise tissue tags. However, bed separation was classified in grade 2 in 4 tissues and grade 3 in 12 tissues.

Discussion

This novel technique to prepare donor graft using femtosecond laser making a cut from the endothelial side was first described by Sikder et al in 2006 [15]. The long tracking experience of lasik flap cut using femtosecond laser during decade had shown advantages of these kind of laser. The high precision of the flap and the smooth surface of lamellar bed might overcome these problems [20-22]. However, damage to endothelial cells was a very important issue to be concerned.

In this present study, we addressed many factors and procedures during the protocol in order to preserve the endothelial cells. Firstly, we used LDV Z6 plus femtosecond laser system which has a lowest pulse energy (100 nJ) compared to other femtosecond lasers in the market. The advantage of using this low pulse energy femtosecond laser is the uniform thickness of corneal endothelial graft which had been reported by Singh et al [23]. Secondary, the donor cornea was flipped smoothly and slowly outward by controlling the water pressure system in the anterior chamber maintainer. This was to prevent suddenly turned the cornea into outward position which may cause avulsion of the cell junction, in the other hand, slowly increased stress may distribute a tensile strength all over the area. Since crushing the cornea can cause serious injury to the endothelium, we paid attention on this particular step by carefully and slowly lifted the cornea to gently touch the applanating interface. The fourth factor was a coating agent. We used sodium hyaluronate which can easily spread over the contact surface as it is a dispersive ophthalmic viscoelastic device.

The viability of endothelial cell in term of endothelial cell loss was 8.9% in our study. Although this loss was in an acceptable range, there was a limitation in using specular microscope counting the cells since this instrument can measure cell density only at the center or the cornea. Thus, it may not represent the total cell loss. Moreover, bubble generated from the laser or opaque bubble layer (OBL) which we found in 4 eyes was also obscured view for technicians to assess the cell. However, this problem can be managed easily as we experienced the OBL when performing LASIK flap creation by adjusting the balance of laser repetitive rate and energy setting. According to a live/dead vital staining, the mean area of endothelial cell damages was 4.57% which was comparable to the study of Sikder S et.al. and Liu YC et.al. [15,16]. We observed that area of damaged cells frequently found along the laser cut edge. To solve this problem, we recommend making a larger graft diameter and recut it with a trephine to the exact diameter by surgeon.

Corneal lamellar separation and handling may be a challenging step for surgeons. The mechanism of femtosecond laser interacted to the tissue is photo disruption which creates the cavitations and causes tissue bridges in the cornea stroma. Many previous studies showed that the more energy power is used, the less tissue resistance occurs. However, it may cause more bubble or OBL in the interface. This factor has to be balanced between endothelial cells damages from the laser power directly and the simplicity to separate the lamellar plain. Importantly more tissue manipulation may also damage endothelial cells. We decided to use femtosecond laser: LDV in this study because it is a femtosecond laser that has the lowest pulse energy compared to the others. Moreover, the pattern of laser beam firing is overlapping, which causes less tissue bridges, resulting in less resistance to tissue separation. Laser adjustment may also require minimizing rim tags problem.

Conclusion

In conclusion, a technique to prepare DSAEK donor graft using low pulse energy femtosecond laser making a cut from the endothelial side was promising. The advantages of this technique are minimally invasive technique or bladeless procedure. Femtosecond laser creating graft makes it more accurate, precise, regularity and uniform thickness, no risk of blade chatter or abrasion compared to microkeratome created graft. However, the disadvantages are higher cost, increased time used more technical training and supported. A further clinical study is necessary to evaluate both visual and refractive outcomes. We suggest that eye banks or tertiary eye centers should integrate this novel effective and safe technique to prepare a pre-cut donor cornea and distribute to local surgeons for better outcome in endothelial keratoplasty.

Acknowledgement

We would like to thank Ms. Supaporn Kramchantuk for her help in endothelial cell staining process. We further thank Thai Red Cross Eye Bank staffs for endothelial cell counting process.

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