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Research Article

Influence of Corneal Wound Healing Process on Graft Biomechanics Following Primary Versus Repeat Descemet Stripping Automated Endothelial Keratoplasty

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Received: September 23, 2019; **Published:** October 21, 2019

Abstract

Purpose: To study the influence of wound healing process at the graft margin and lamellar interface on corneal biomechanical properties after successful primary versus repeat Descemet stripping automated endothelial keratoplasty (DSAEK).

Methods: This retrospective comparative study included 86 eyes: 33 eyes of 33 patients had primary DSAEK, 35 eyes of 35 patients underwent repeat DSAEK, and 18 eyes of 18 normal subjects served as the control group. Ocular response analyzer was used to measure corneal hysteresis (CH) and corneal resistance factor (CRF), Goldmann- correlated intraocular pressure (IOPg) and cornea-compensated intraocular pressure (IOPcc) among the study groups at least 6 months postoperatively. In vivo confocal microscopy was used to study the corneal wound healing process at the graft margin and graft-host interface in the study groups and to correlate these findings with the corneal biomechanics.

Results: CH and CRF were significantly lower in the primary DSAEK group (6.9 ± 1 and 6.7 ± 1.1 mmHg, respectively) as compared to the repeat DSAEK group (9.9 ± 1.1 and 9.4 ± 1.2 mmHg, respectively) ($P < 0.0001$). The biomechanical properties in the repeat DSAEK group showed comparable results to normal subjects (10.3 ± 1.06 and 9.6 ± 1.07 mmHg, respectively). In the repeat DSAEK group, confocal microscopy revealed highly reflective and activated keratocytes at both graft margin and graft-host interface as compared to the primary DSAEK group.

Conclusion: Repeat DSAEK can yield corneal biomechanics comparable to those in normal corneas, perhaps because of the increased healing response at both graft margin and graft-host interface.

Keywords: *Corneal Biomechanics; Corneal Hysteresis; Corneal Resistance Factor; DSAEK; Ocular Response Analyzer; Confocal Microscopy*

Introduction

Penetrating keratoplasty (PK) is not the best choice for most corneal pathologies. Targeting the diseased corneal layer is the procedure of choice in modern corneal surgeries. Recently, deep anterior lamellar keratoplasty (DALK) is preferably performed for corneal diseases not affecting the endothelium and Descemet membrane (DM) [1,2] and Descemet stripping automated endothelial keratoplasty (DSAEK)

is performed for corneas with only endothelial dysfunction [3]. The advantage of this technique is to decrease complications that might occur after full thickness PK. The incidence of traumatic wound dehiscence and high postoperative astigmatism are reduced by this new technique. The procedure is used to treat corneal endothelial dysfunction mainly pseudophakic bullous keratopathy and Fuchs endothelial dystrophy [4-7]. DSAEK maintains the structural integrity of the eye and has rapidly become the surgical procedure of choice for managing corneal endothelial dysfunctions due to its smaller incision size, faster visual recovery, smaller rejection rate and increased refractive predictability [3,8]. There is also a risk of graft dislocation following surgery [9].

Biomechanical properties of the cornea can affect the outcomes of different ocular procedures. In DSAEK, some healing responses occur at the graft-host interface which can entirely affect the biomechanical properties of DSAEK grafts. The Ocular Response Analyzer (ORA; Reichert Ophthalmic Instruments, Depew, NY, USA) is used to measure the biomechanical properties of the human cornea in both health and disease [10]. Corneal hysteresis (CH) is a measure of the viscoelastic properties of the cornea. Corneal resistance factor (CRF) indicates overall resistance of the cornea [11]. In vivo confocal microscopy (IVCM) permits noninvasive, high- resolution, high-magnification imaging of the microstructures of the cornea. In the present study, the confocal microscopy was used to study the corneal wound healing response at both the graft-host interface and graft margin after primary and repeat DSAEK. In confocal microscopy, keratocyte nuclei appear as bright oval or bean-shaped objects in the resting stage against a dark background. Cellular processes are not visible in the quiescent state [12,13]. During confocal microscopy, the activated keratocyte appears to have greater corneal light backscattering than the inactive keratocyte and their cytoplasmic processes are often visible [14-17]. The repair fibrocytes has a rough endoplasmic reticulum that is not visible in the quiescent keratocyte, indicating active protein synthesis [18,19].

To my knowledge, there are no published results yet addressing the influence of wound healing process at the graft margin and lamellar interface on the biomechanical properties of the cornea after primary and repeat DSAEK. The aim of this study was to evaluate and compare the influence of wound healing process at the graft margin and lamellar interface on corneal biomechanics after primary and repeat DSAEK using the Reichert ORA and the confocal microscope.

Patients and Methods

Informed consent was obtained from all subjects. Study procedures followed the Declaration of Helsinki. This retrospective comparative study included 86 eyes: 33 eyes of 33 patients had primary DSAEK, 35 eyes of 35 patients underwent repeat DSAEK, and 18 eyes of 18 normal subjects served as the control group. Primary DSAEK and repeat DSAEK groups were matched in age, graft diameter (8 mm in all eyes) and in indications to avoid gross variations in the biomechanical properties of the recipient corneal bed. Indications for primary DSAEK included pseudophakic bullous keratopathy (n = 18), and Fuchs endothelial dystrophy (n = 15). Indications for repeat DSAEK were slowly progressive endothelial cell loss (n = 15) and endothelial rejection (n = 20). The primary corneal pathology in repeat DSAEK group was pseudophakic bullous keratopathy (n = 20), and Fuchs endothelial dystrophy (n = 15). The time elapsed between DSAEK surgeries in both groups and the start of corneal evaluation was matched. It was 7.97 ± 1.2 (range 6 – 12 months) and 8.11 ± 1.3 (range 7 - 12 months) in DSAEK group and repeat DSAEK group, respectively (P = 0.63). None of the eyes had glaucoma or had a history of systemic disease. All sutures were removed at least 3 months prior to entering the study. Exclusion criteria were the presence of ocular disorders other than the indication for keratoplasty, systemic disorders such as diabetes mellitus and history of any previous refractive surgeries. All donor corneas met the criteria of the Eye Bank Association of America for donor quality. The eye bank standard for pre-cut tissue thickness was ≤ 150 μm.

Common surgical technique

A marked circular ring of the planned donor size was placed on the surface of the cornea to outline where to strip DM and place the donor lenticule. A 5-mm superior scleral tunnel incision was performed. Air was then injected to fill the anterior chamber and a reverse Terry- Sinskey hook was used to cut DM in a circular fashion guided by the area of epithelial marking. Descemet stripper was used to

strip DM and endothelium. The donor cornea was then trephined from the endothelial side to the desired diameter using 8.0 mm Barron corneal punch (Katena Products, Inc, NJ, USA). The size of the graft was same for both the groups. A small amount of dispersive viscoelastic material was placed on the endothelial surface, and the pre-cut disc was folded into a taco-fold and introduced into the anterior chamber using forceps. Air bubble was used to attach the unfolded donor lenticule to the host corneal stroma. In repeat DSAEK, the failed donor lenticule was stripped using a Descemet stripper. The donor lenticule was then introduced and the surgery completed as before. Patients were instructed to maintain a strict supine position right after completing the procedure till postoperative day 1.

Ocular response analyzer

The Reichert ORA was used to measure corneal biomechanical parameters for all eyes at least 6 months postoperatively. The patient was seated comfortably on a chair and asked to fixate on the red blinking light in the device before the device was activated. The mean of three good-quality readings for each eye was taken. CCT was measured using a hand-held ultrasonic pachymeter (DGH-550 Pachette II; DGH Technologies, Exton, PA, USA). The probe was placed perpendicular to the midpupillary axis, and the mean of three measurements for each eye was taken. All examinations were performed by a single examiner.

In vivo confocal microscopy

In vivo confocal microscopy (IVCM) was used to study the corneal wound healing process at the graft margin and graft-host interface in the study groups. IVCM was performed using advanced scanning confocal microscope (ASL-1000; Advanced scanning Ltd., USA). An optically clear gel was placed on the front surface of the microscope lens as well as on the ocular surface as a coupling agent. The area observed was approximately 500 x 500 µm at approximately 400x magnifications. Scanning the corneas of the control group was performed using the same microscope to differentiate between unoperated corneas with quiescent keratocytes and operated corneas with activated keratocytes postoperatively. The confocal microscope had a modified specular objective lens. Light source was provided by a remote xenon lamp. All scans were evaluated by a single reviewer who was masked as to timing of scans.

Statistical analysis

Statistical analysis was performed using the Student's t test and a p value of less than 0.05 was considered statistically significant. Data were expressed as mean ± standard deviation (SD). Calculations were performed using the Statistical Package for the Social Sciences (SPSS) version 18.0 system for personal computers (SPSS Inc., Chicago, IL).

Results

Demographic data and corneal biomechanical metrics of the study groups are listed in table 1. Mean patient age (years) was 65.3 ± 7.2 (range 50 - 77) in the control group, 65.7 ± 11.8 (range 50 - 88) in the primary DSAEK group and 69.6 ± 8.8 (range 55 - 90) in repeat DSAEK group. Mean age was comparable in all groups ($P > 0.09$). Both groups were matched in terms of graft diameter which was 8.0 mm in all operated eyes. CH and CRF were significantly lower in the primary DSAEK group (6.9 ± 1 and 6.7 ± 1.1 mmHg, respectively) as compared to the repeat DSAEK group (9.9 ± 1.1 and 9.4 ± 1.2 mmHg, respectively) ($P < 0.0001$). The repeat DSAEK group demonstrated biomechanical parameters comparable to normal subjects (10.3 ± 1.06 and 9.6 ± 1.07 mmHg, respectively). Central corneal thickness (CCT) was 551.1 ± 14 in control group, 610.2 ± 29.5 in primary DSAEK group, and 622.1 ± 44.2 in repeat DSAEK group. CCT was significantly thicker in the DSAEK groups as compared to control group (P values < 0.001). No statistically significant difference was found among the DSAEK groups in terms of mean CCT ($P = 0.2$). No statistically significant difference was found among the three groups in terms of mean IOPg and IOPcc ($P > 0.09$).

In vivo confocal microscopy

In the repeat DSAEK group, many highly reflective activated keratocytes were evident at both the peripheral stroma at the graft margin and at the graft-host lamellar interface. The brightness and reflectivity of activated keratocytes at both the graft margin and at the graft-host interface were more intense in the repeat DSAEK group compared with the primary DSAEK group (Figure 1A and 1C). Some of the highly reflective keratocytes appeared considerably larger than others. In primary DSAEK group, the healing in the central graft and at graft margin was very minimal which resulted in less bright and less reflective activated keratocytes (Figure 1B and 1D). Although the activated keratocytes were more intense at the graft-host lamellar interface in the repeat DSAEK group, no clinical interface haze was reported in any of the eyes of the repeat DSAEK group. In the control group of normal subjects, keratocytes nuclei looked quiescent and appeared as bright, oval or bean-shaped objects against a dark background with not prominent cellular processes. Their Cellular processes were not evident in this quiescent resting state (Figure 2).

Discussion

The present study has shown that CH and CRF after repeat DSAEK were comparable to normal subjects. In sharp contrast, these metrics were significantly lower after primary DSAEK as compared to repeat DSAEK and normal age- matched control subjects. This observation coincides with the results of a study by John, et al. [20] who reported that corneal biomechanics were significantly lower following descemetorhexis with endokeratoplasty as compared to a normal age-matched group. This observation was attributed to the removal of the host DM which may act as a posterior pillar and contribute to the biomechanical properties of the normal cornea. The strengths of this study include in vivo assessments of corneal biomechanical properties and their correlates with healing process at the graft circumference and graft-host interface after primary and single repeat DSAEKs. The ORA was used in this study to evaluate the biomechanical properties of the cornea. To my knowledge, it is the first study assessing the graft margin and graft-host interface healing

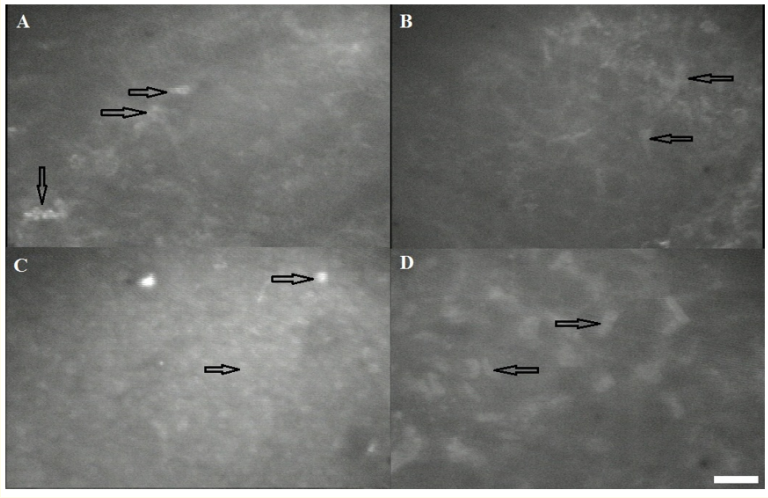

Figure 1: *Confocal micrographs of graft margin of both DSAEK and repeat DSAEK groups (A, B) and graft-host interface (C, D). Repeat DSAEK group (A): More bright, dense and more reflective activated keratocytes with elongated cell processes populating the graft margin (black arrows). Primary DSAEK group (B): Less bright and less reflective activated keratocytes (black arrows). Repeat DSAEK group (C): More bright, dense and more reflective activated keratocytes populating the interface (black arrows). Primary DSAEK group (D): Less bright and less reflective activated keratocytes (black arrows). Scale bar measures approximately 100 µm.*

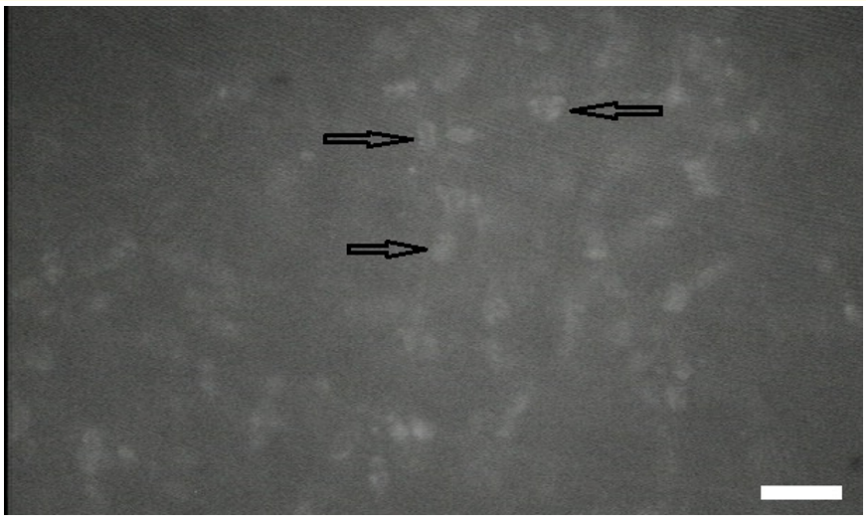

Figure 2: *Confocal images of the cornea of control group. Quiescent keratocytes (black arrows) appeared as bright oval or bean-shaped objects against a dark background. Cellular processes are not evident. Scale bar measures approximately 100 µm.*

Parameters	Control group (n = 18)	Primary DSAEK group (n = 33)	Repeat DSAEK group (n = 35)	P		
				DSAEK vs. Repeat DSAEK	DSAEK vs. Control	Repeat DSAEK vs. Control
Age [mean (SD) (range) years]	65.3 ± 7.2 (range 50 - 77)	65.7 ± 11.8 (range 50 - 88)	69.6 ± 8.8 (range 55 - 90)	0.1	0.9	0.1
Male/Female ratio	12/6	12/21	12/23	-	-	-
OD/OS	20/0	15/18	21/14	-	-	-
Central corneal thickness	551.1 ± 14	610.2 ± 29.5	622.1 ± 44.2	0.2	< 0.001	< 0.001
Corneal Hysteresis (mmHg)	10.3 ± 1.06	6.9 ± 1	9.9 ± 1.1	< 0.0001	< 0.0001	0.14
Corneal resistance factor (mmHg)	9.6 ± 1.07	6.7 ± 1.1	9.4 ± 1.2	< 0.0001	< 0.0001	0.5
IOPg (mmHg)	15 ± 1.9	14.8 ± 1.8	15.5 ± 1.6	0.1	0.6	0.4
IOPcc (mmHg)	15.5 ± 1.7	15.1 ± 3.5	16.1 ± 1.2	0.2	0.6	0.2
Time elapsed between surgery and measurement [mean (SD)(range) months]	-	7.9 ± 1.2 (6-12)	8.2 ± 1.3 (6-14)	0.4	-	-

Table 1: *Demographic data and corneal biomechanical metrics of normal eyes and eyes undergoing primary DSAEK, repeat DSAEK.*

process and keratocyte status after primary and repeat DSAEK that correlates these events with the corneal biomechanics recorded postoperatively using ORA. Three factors may contribute to changes in CH and CRF after any type of corneal transplantation, including biomechanical characteristics of both transplanted corneal button and the residual host cornea and, the healing response between donor and host corneas. Histopathologic findings in corneas with bullous keratopathy have revealed the accumulation of extracellular matrix proteins including collagen and fibrillin-1 in the anterior stroma below the epithelium [21,22], as well as abnormal intrastromal deposits which were shown to be reduced significantly after DSAEK [23-25]. In the repeat DSAEK group, many highly reflective activated keratocytes were evident at both the peripheral stroma at the graft margin and at the graft-host lamellar interface. The reflectivity of activated keratocytes at both the graft margin and at the graft-host interface were more intense in the repeat DSAEK group compared with the primary DSAEK group. Activated keratocytes produce the repair extracellular matrix which reinforces the wound inducing a stronger wound healing reaction and hence superior corneal biomechanics that were observed in the repeat DSAEK group. The explanation for the observed lower parameters after primary DSAEK can be attributed to the absence of the ring scar between donor and recipient corneas. The repeated surgery may induce a foreign body reaction at the circumferential graft margin and graft-host interface between donor and recipient tissues stimulating an influx of inflammatory cells, a transformation of myofibroblasts and a production of new ground stromal substance [26].

Limitation of the Study

One limitation of the current study was that corneal biomechanics were not measured before keratoplasty. Thus, it could have been determined the limit to which each technique of transplantation changes corneal biomechanics.

Conclusion

In conclusion, repeat DSAEK can yield corneal biomechanics comparable to those in normal corneas, perhaps because of the increased healing response at both circumferential graft margin and graft-host lamellar interface.

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