Electron microscopic comparison of the donor cut edges using femtosecond laser-assisted keratoplasty versus conventional keratoplasty

Mohamed Y. Tolba[1], Iman M. A. Zaki[2], Karim A. Raafat[3], Amr A. Al Dib[4], Taher Eleiwa[5], Collin Chase[6], Ibrahim M. Taher[4]

Access this article online

Quick Response Code:

Website: www.saudijophthalmol.org

DOI: 10.4103/1319-4534.325785

Abstract:

PURPOSE: To describe and compare the histological changes in the cut edges of the remaining donor corneal rim using femtosecond laser-assisted keratoplasty (FAK) versus conventional penetrating keratoplasty (PK) via light and transmission electron microscopic examination.

METHODS: This was a prospective observational study of 10 eyes; 5 FAK (top-hat technique) and 5 conventional PK. Main outcomes were histological findings at the cut edge of the donor corneal rim (at 3, 6, 9, and 12 o'clock).

RESULTS: Cellular and ultra-cellular changes in the form of stromal edema, disorganized collagen fibers, and nuclear changes were more prominent in the FAK eyes as compared to the conventional PK ones.

CONCLUSION: FAK induces more collateral damage in the cut edge of corneal donor graft at cellular and ultra-cellular levels, compared to conventional trephination. Further studies are required to investigate the clinical ramifications of this observation.

Keywords:

Electron microscopic study of the cornea, femtoassisted keratoplasty, penetrating keratoplasty

[1]Ophthalmology Department, Eye Specialty Hospital, Medical Military Compound, Koubri Elkouba, Cairo, Egypt, [2]Pathology Department, Research Institute of Ophthalmology, Cairo University, Cairo, Egypt, [3]Ophthalmology Department, Faculty of Medicine, Cairo University, Cairo, Egypt, [4]Ophthalmology Department, National Institute For LASER Science , Cairo University, Cairo, Egypt, [5]Ophthalmology Department, Faculty of Medicine, Benha University, Benha, Egypt, [6]Morsani College of Medicine, University of South Florida, Tampa, FL, USA

Address for correspondence:
Dr. Mohamed Y. Tolba, Eye Speciality Hospital, Medical Military Compound, Koubri Elkouba, Cairo, Egypt.
E-mail: m.y.elminiawy@gmail.com

Submitted: 14-Aug-2020
Revised: 10-Sep-2020
Accepted: 13-Dec-2020
Published: 09-Sep-2021

Introduction

Penetrating keratoplasty (PK) is the most common solid-organ transplant surgery worldwide.[1] Despite several complications of PK such as graft rejections, infection, unpredictable astigmatism, healing complications, and prolonged recovery period, it is still considered one of the most successful human organ transplant surgeries.[2]

Femtosecond laser (FS) technology development has improved the outcomes of several corneal surgeries.[3] When applied to the cornea, the FS pulse leads to the formation of cavitation bubbles that hastens customized corneal dissection for PK,[4-6] deep anterior lamellar keratoplasty,[7] intrastromal corneal ring segments,[8] and small microincisional lenticular extraction.[9]

In PK, FS laser-assisted keratoplasty (FAK) has been reported to have many advantages over conventional mechanical trephination.[10,11] The superiority of the FAK method was attributed to the improvised postoperative corneal biomechanical stability.[12] Several studies investigated the advantages of using FS in different corneal surgeries and reported perfect wound apposition, faster wound healing, better visual outcomes, and quicker suture removal.[10,13-15]

The purpose of our study is to evaluate the microstructural changes at the cut edges of the remaining donor corneal rim in FAK as compared to conventional PK.

Methods

This is an observational study involving 10 eyes of 10 patients who were prospectively recruited and were randomly allotted to FS trephination

How to cite this article: Tolba MY, Zaki IM, Raafat KA, Dib AA, Eleiwa T, Chase C, *et al*. Electron microscopic comparison of the donor cut edges using femtosecond laser-assisted keratoplasty versus conventional keratoplasty. Saudi J Ophthalmol 2021;35:47-51.

or conventional trephination; 5 eyes by FAK and 5 eyes via conventional PK. The present study was approved by the Cairo University Institutional Review Board. The study was conducted according to the ethical standards set in the 1964 Declaration of Helsinki, as revised in 2000.

Ten human corneal grafts suitable for transplantation (Eye Bank of Canada, Ontario Division, Toronto, Canada) were used in this study. All the grafts were from adult donors aged 18–40 years and were used within 10 days of the time of death, with an endothelial cell count of 2500 cells at least. We subdivided the 10 eyes into two groups; A - FAK, and B - conventional PK.

Surgical techniques

Femtosecond-assisted keratoplasty

All trephined corneas were performed by the same expert corneal surgeon. The corneal buttons were removed from the storage medium (Optisol; Bausch and Lomb Surgical, Irvine, California, USA) and mounted on an artificial anterior chamber (Automated Corneal Shaper; Chiron Inc, Irvine, USA). Then, the tight seal of the mounted container was confirmed. The used FS was "Intra Lase FS 60 kHz;" (Intra Lase Corp, Irvine, California, USA), with the standard parameters as described elsewhere.[16]

The remaining unused corneoscleral rim of the donor graft was prepared for histopathological evaluation as described later.

Conventional penetrating keratoplasty

Five human corneal grafts were trephined manually using the Barron Marking corneal donor punch.

Histological preparation

The remaining corneoscleral rims were labeled and immediately fixed in 2.5% buffered glutaraldehyde for 6 h and then phosphate buffer (pH 7.3). Then, the specimens were placed in propylene oxide for 10 min. At last, they were embedded in Araldite Cy212.[17]

Semi-thin sections of 1.2 μm were obtained using (Laboratoire Kastler Brossel, LKB) ultratome and then stained by toluidine blue.[18] Then, the prepared sections were examined by light microscopy (LM). Furthermore, the ultra-thin sections (50–80 nm) were obtained using a diamond knife, then double-stained with uranyl acetate and lead citrate, and examined by transmission electron microscopy (TEM). The data obtained from LM and TEM examinations were analyzed and documented by photographs.

Results

Histopathological evaluation of the Group A specimens

The LM examination showed unremarkable changes in the epithelium, Bowman's layer, Descemet's membrane, and the endothelium. However, the stroma demonstrated focally disrupted collagen fibers with loss of normal architecture in different sections, mainly in the anterior part [Figure 1a-d].

The TEM examination illustrated interlamellar and interfibrillar stromal edema. The keratocyte cytoplasm showed vacuolations, and the collagen fibers arrangement was disrupted in longitudinal and oblique directions. A band-like configuration made of the disrupted collagen fibers and cellular debris was found extending from the anterior to the posterior corneal stroma [Figure 2a-e]. No changes in other corneal layers were found.

Histopathological evaluation of Group B specimens

The LM examination of Group B showed no changes in the epithelial and endothelial layers, with intact Bowman's layer and Descemet's membrane. The corneal stroma showed moderate edema as compared to Group A. Arrangement of corneal collagen fibers was not affected [Figure 3a-c].

The TEM examination confirmed the LM results [Figure 4a-d]. Less stromal edema was evident compared to Group A. No cytoplasmic vacuolation was found in the keratocytes with intact nuclei. No disruption of collagen fibers or cellular debris were visualized.

Discussion

Visual outcomes of FAK compared to conventional PK have been reported in the literature. For instance, Buratto *et al*. reported a 6/9 best-corrected visual acuity 3 months after top-hat FAK in keratoconus.[19] FS-assisted keratoplasty allowed precise dissection of donor and recipient corneas even when significant opacities were existing as in herpetic corneal scarring, pseudophakic bullous keratopathy, and Fuchs' endothelial dystrophy.[20]

Regarding wound healing, more efficient wound healing post-FAK was reported compared to conventional PK using the water pressure leakage method.[21] Bahar *et al*. reported that water leakage occurred at much higher-pressure levels on the eyes with grafts trephined with FS than eyes trephined by the conventional technique.[21]

For histological evaluation, Stojkovic *et al*. reported the effect of using Q-switched erbium YAG laser (very short nanosecond pulsed laser) on corneal trephination. They reported the thermal effect in the form of band of carbonization and a coagulation zone associated with collagen and cellular damage.[22] Jones *et al*. reported a smooth surface architecture of FS-assisted trephined corneas using scanning electron microscopic.[23] In our study, we used TEM to evaluate the ultracellular structures, while scanning electron microscopy only helped to show the surface topography of the specimen. We used a higher energy level (up to 3–4 times more than the energy used in FS-LASIK)[24] and revealed the presence of a band formed of disrupted collagen fibers and cellular fragments, extending from the posterior to anterior parts of the corneal stroma. A temperature rise occurs and remains confined in the focal volume because the thermal diffusion is too slow to dissipate the laser energy during the pulse duration, even with the higher pulse energies required.[25-27] This band was thicker anteriorly, in agreement with Nuzzo *et al*., who reported the same finding using an Intra Lase FS60 system. However, they conducted their study using six swollen corneas from eye bank eyes that were unsuitable for transplantation. In

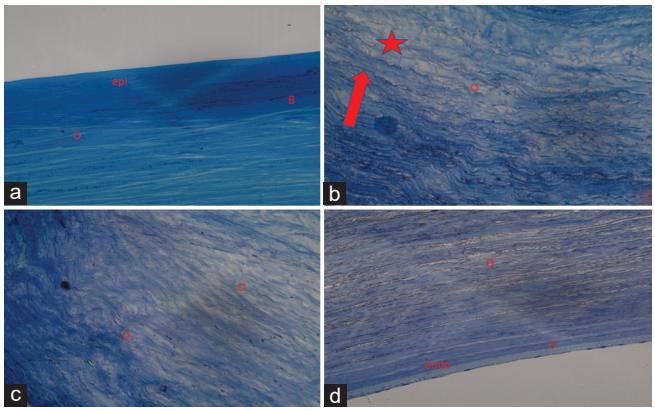

Figure 1: (a) Light photomicrograph of semi-thin section of corneal tissue from Group A showing epithelium (epi), Bowman's layer (B), and underlying stroma exhibiting interlamellar edema (o) (TB stain, ×500). (b) Light photomicrograph of semi-thin corneal tissue from Group A showing prominent stromal interlamellar edema (o) (Tb stain, ×500). (c) Light photomicrograph of semi-thin section of corneal tissue from Group A showing prominent stromal interlamellar edema (o) with evident fragmentation (star), and disarray of collagen bundles (arrow) (TB stain, ×500). (d) Light photomicrograph of semi-thin of corneal tissue from Group A showing prominent stromal interlamellar edema (o), Descemet's membrane (D), and well-arranged endothelial cells (end) (TB stain, ×500)

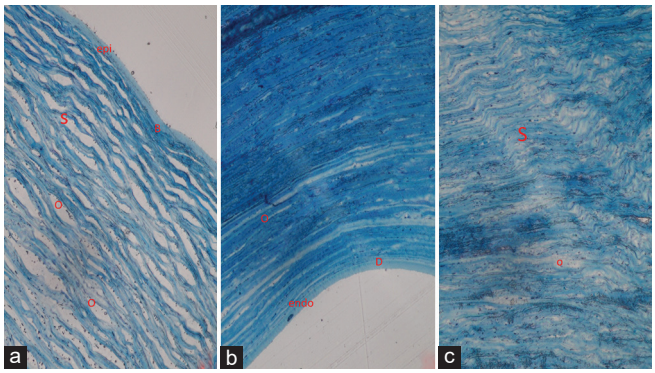

Figure 3: (a) Light photomicrograph of the corneal tissue from Group B showing normal epithelium (epi), Bowman's layer, stroma (s), and moderate stromal (s) interlamellar edema (o) (TB stain, ×500). (b) Light photomicrograph of corneal tissue from Group B showing moderate stromal (s) interlamellar edema (o), Descemet's membrane (D), and endothelial cell (end) (TB stain, ×500). (c) Light photomicrograph of corneal tissue from Group B showing moderate posterior stromal (s) interlamellar edema (o) (TB stain, ×500)

our study, we used healthy donor tissues that were trephined before PK. Besides, we compared FS effect to conventional mechanical trephination.[28] The anterior part of the assumed debris layer thickness within the anterior corneal stroma may be indicative that the energy used was excessive, leading to the formation of more debris compared to that amount of debris in the posterior corneal stroma. The cellular and the disrupted collagen fibril debris deposited on the edge of the incision might be due to tissue photodisruption as a result of material ejection and tissue decomposition.[29,30] The higher the energy level used for tissue dissection resulted in a larger volume of the breakdown

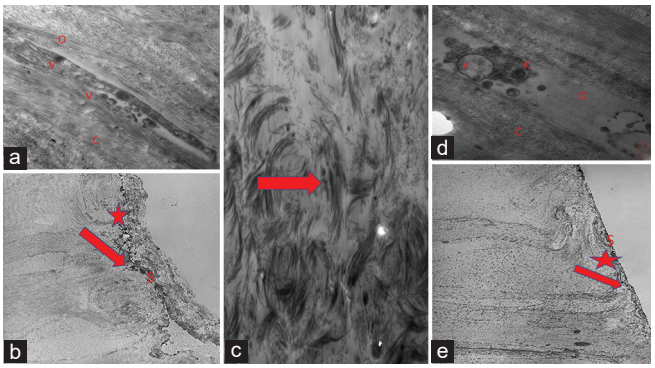

Figure 2: (a) Electron photomicrograph of corneal tissue from Group A showing stroma with keratocyte exhibiting intracytoplasmic vacuoles (v) together with collagen fibrils (c) cut longitudinal and oblique with evident inter-fibrillar edema (o) (×4000). (b) Electron photomicrograph of corneal tissue from Group A showing marked disruption of stromal collagen fibrils (arrow) (×2000). (c) Electron photomicrograph of corneal tissue from Group A showing stromal keratocyte (k) with cytoplasmic edema (o) with large internuclear vacuole (v), the surrounding collagen fibrils (c) cut partially longitudinal and partial oblique (×5000). (d) Electron photomicrograph of corneal tissue from Group A showing disrupted collagen fibrils (arrow) in the anterior middle stroma with the debris (star) forming the suggested semi-membrane (s) (×2500). (e) Electron photomicrograph of corneal tissue from Group A showing disrupted collagen fibrils (arrow) in the posterior stroma with the debris (star) forming the suggested semi-membrane (s) (×3000)

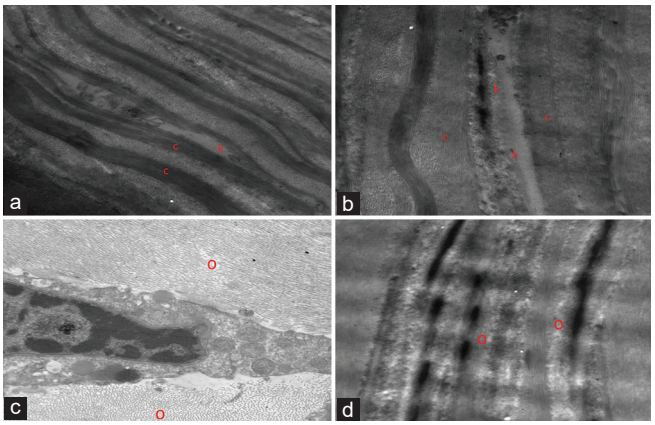

Figure 4: (a) Electron photomicrograph of the corneal stromal tissue from Group B showing keratocytes (K) with surrounding regularly arranged collagen fibrils (c) (×2500). (b) Electron photomicrograph of corneal stromal tissue from Group B showing well-arranged collagen fibrils (c) intermingling keratocyte (k) (×3000). (c) Electron photomicrograph of the corneal stromal tissue from Group B with higher magnification showing edema (o) between the collagen fibrils (×5000). (d) Electron photomicrograph of corneal stromal tissue from Group B showing moderate edema (o) between the collagen fibrils (×3000)

region.[31] Presumably, if we can use low pulse energies to avoid the formation of the thick debris layer in the anterior stroma, it may be possible to produce a quality of an incision similar to that obtained in the posterior stroma.

Previous studies confirmed that the usage of conventional blade trephines generates mechanical forces which squeeze

the corneal tissue and induces vertical tipping and horizontal torsion.[32,33] A prospective study by Seitz *et al*. confirmed that trephination with excimer laser as nonmechanical laser trephination trial showed an improved refractive outcome after suture removal, in comparison with the results after conventional PK with a hand-held motor trephine.[34] Another study by the same author analyzed 1000 cases with PK over 12 years, to confirm that using conventional blade trephination causing marked vertical tipping and horizontal torsion of the transplant and using nonmechanical methods (excimer laser) did not show these effects.[35] In our study, the LM examination of semi-thin sections of the conventional mechanical trephination group revealed almost normal histologic pattern, except for stromal edema in the posterior stroma. The EM examination of the ultrathin sections of the conventional mechanical trephination group revealed less edema, no cellular or collagen debris. This disruptive effect of FS could be contributing to the faster wound healing after FAK, as compared to conventional PK.

Our study is not without limitations. First, we did not include *in vivo* studies to analyze wound healing and clinical outcomes after FAK. Second, we only used high-energy FS pulses to trephine the corneal tissue. Finally, our results stem from a limited number of samples; however, we have sufficient evidence that collateral damage is more prominent in the FS group. Therefore, future studies with larger dataset and comparing the microstructural changes using different FS pulse energies are warranted.

Conclusion

The microscopic evaluation of the cut donor edges showed less structural damage of the corneal tissue in the conventional PK versus the FAK. Further studies are required to evaluate the FS induced *in vivo* ultrastructural changes and correlate the changes in the corneal tissue status with the clinical outcomes at the levels of healing and biomechanical stability.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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