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

Mohamed Y. Tolba¹, Iman M. A. Zaki², Karim A. Raafat³, Amr A. Al Dib⁴, Taher Eleiwa⁵, Collin Chase⁶, Ibrahim M. Taher⁴

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

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.¹ When applied to the cornea, the FS pulse leads to the formation of cavitation bubbles that hastens customized corneal dissection for PK,³⁴ deep anterior lamellar keratoplasty,⁵ intrastromal corneal ring segments,⁶ and small incisional lenticular extraction.⁷

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.
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
our study, we used healthy donor tissues that were trephined before PK. Besides, we compared FS effect to conventional mechanical trephination. 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. The higher the energy level used for tissue dissection resulted in a larger volume of the breakdown region. 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
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.

**References**

1. Gain P, Julienne R, He Z, Aldossary M, Acquart S, Cognasse F, et al. Global survey of corneal transplantation and eye banking. JAMA Ophthalmol 2016;134:167-73.

2. Tan DT, Dart JK, Holland EJ, Kinoshita S. Corneal transplantation. Lancet 2012;379:1749-61.

3. Kymionis GD, Kankariya VP, Plaka AD, Reinstein DZ. Femtosecond laser technology in cornal refractive surgery: A review. J Refract Surg 2012;28:912-20.

4. Bashir ZS, Ali MH, Anwar A, Ayub MH, Butt NH. Femtosecond and PRK: The recent innovation in laser assisted refractive surgery. J Pak Med Assoc 2017;67:609-15.

5. Plamann K, Aptel F, Arnold C, Courjaud A, Crotti C, Deloison F, et al. Ultrashort pulse laser surgery of the cornea and the sclera. J. Opt. 2010;12:084002.

6. Soong HK, Malta JB. Femtosecond lasers in ophthalmology. Am J Ophthalmol 2009;147:189-97.e2.

7. Price FW Jr., Price MO, Grandin JC, Kwon RJ, Surgery R. Deep anterior lamellar keratoplasty with femtosecond-laser zigzag incisions. J Cataract Refract Surg 2009;35:804-8.

8. Monteiro T, Alfonso JF, Franqueira N, Faría-Correia F, Ambrosio R, Madrid-Costa DJ. Predictability of tunnel depth for intrastromal corneal ring segments implantation between manual and femtosecond laser techniques. J Refract Surg 2018;34:188-94.

9. Wu Z, Wang Y, Zhang J, Chan TC, Ng ALK, Cheng GPM, et al. Comparison of corneal biomechanics after microincision lenticule extraction and small incision lenticule extraction. Br J Ophthalmol 2017;101:650-4.

10. Tóth G, Szentmáry N, Langenbucher A, Akhmedova E, El-Husseiny M, Seitz B. Comparison of excimer laser versus femtosecond laser assisted trephination in penetrating keratoplasty: A retrospective study. Adv Ther 2019;36:3471-82.

11. Meltendorf C, Schroeter J, Bug R, Kohnen T, Deller T. Corneal trephination with the femtosecond laser. Cornea 2006;25:1090-2.

12. Proust H, Baeteman C, Matonti F, Conrath J, Ridings B, Hoffart L. Femto‑LASIK: The femtosecond laser-assisted sutureless anterior lamellar keratoplasty. Am J Ophthalmol 2011;151:29-34.

13. Cheng YY, Schotten JS, Tajib NG, Wijdh RJ, Pels E, van Cleytenbreugel H, et al. Efficacy and safety of femtosecond laser-assisted corneal endothelial keratoplasty: A randomized multicenter clinical trial. Tranplantation 2009;88:1294-302.

14. Yoo SH, Kymionis GD, Koresishi A, Ide T, Goldman D, Karp CL, et al. Femtosecond laser-assisted sutureless anterior lamellar keratoplasty. Ophthalmology 2008;115:1303-7.e1.

15. Angunawela RI, Riau A, Chaurasia SS, Tan DT, Mehta JS. Manual suction versus femtosecond laser trephination for penetrating keratoplasty: Intraocular pressure, endothelial cell damage, incision geometry, and wound healing responses. Invest Ophthal Ophthalmol Vis Sci 2012;53:2571-9.

16. Kook D, Derhartunian V, Bug R, Kohnen T. Top‑hat shaped corneal trephination for penetrating keratoplasty using the femtosecond laser: A histomorphological study. Cornea 2009;28:795-800.

17. Glauert AM. The fixation and embedding of biological specimens. D.H. Kay (Ed.), Techniques for Electron Microscopy, Davis, Philadelphia (1965), pp. 166-212.

18. Meek DW, Nicpon PE, Meek VI. Mixed thiocyanate bonding in palladium (II) complexes of bidentate ligands. Journal of the American Chemical Society 1970;92:5351-9.

19. Buratto L, Böhm E. The use of the femtosecond laser in penetrating keratoplasty. Am J Ophthalmol 2007;143:737-42.e1.

20. Price FW Jr., Price MO. Femtosecond laser shaped penetrating keratoplasty: One-year results utilizing a top-hat configuration. Am J Ophthalmol 2008;145:210-4.e2.

21. Bahar I, Kaiserman I, McAllum P, Rootman D. Femtosecond laser-assisted penetrating keratoplasty: Stability evaluation of different wound configurations. Cornea 2008;27:209-11.

22. Stojkovic M, Küchle M, Seitz B, Langenbucher A, Viestenz A, Viestenz A, et al. Nonmechanical q-switched erbium: Yag laser trephination for penetrating keratoplasty: Experimental study on human donor corneas. Arch Ophthalmol 2003;121:1415-22.

23. Jones YJ, Gvais KM, Sutphin JE, Mullins R, Skeie JM. Comparison of the femtosecond laser (IntraLase) versus manual microkerate (Moria Altk) in dissection of the donor in endothelial keratoplasty: Initial study in eye bank eyes. Cornea 2008;27:88-93.

24. Nuzzo V, Plamann K, Savoldelli M, Merano M, Donate D, Albert O, et al. In situ monitoring of second-harmonic generation in human corneas to compensate for femtosecond laser pulse attenuation in keratoplasty. J Biomed Optics 2007;12:064032.

25. Steinert RF, Ignacio TS, Sarayba MA. “Top hat” – Shaped penetrating keratoplasty. Am J Ophthalmol 2007;143:689-91.

26. Buratto L, Böhm E. The use of the femtosecond laser in penetrating keratoplasty. Cornea 2006;25:1090-2.

27. Holzer MP, Rabitscher TM, Auffarth GU. Penetrating keratoplasty using femtosecond laser. Am J Ophthalmol 2007;143:524-6.

28. Nuzzo V, Aptel F, Savoldelli M, Plamann K, Peyrot D, Deloison F, et al. Histologic and ultrastructural characterization of corneal femtosecond
laser trephination. Cornea 2009;28:908-13.
29. de Medeiros FW, Kaur H, Agrawal V, Chaurasia SS, Hammel J, Dupps WJ Jr., et al. Effect of femtosecond laser energy level on corneal stromal cell death and inflammation. J Refract Surg 2009;25:869-74.
30. Choi SK, Kim JH, Lee D. The effect of femtosecond laser lamellar dissection at various depths on corneal endothelium in the recipient bed of the porcine eye. Ophthalmic Surg Lasers Imaging 2010;41:255-60.
31. Vogel A, Venugopalan V. Mechanisms of pulsed laser ablation of biological tissues. Chem Rev 2003;103:577-644.
32. Seitz B, Langenbucher A, Naumann G. Astigmatismus Bei Keratoplastik. Enke, Stuttgart: Refraktive Chirurgie; 2000. p. 197-252.
33. Naumann GO. The bowman lecture. Eye 1995;9:395-421.
34. Seitz B, Langenbucher A, Kus MM, Küchle M, Naumann GO. Nonmechanical corneal trephination with the excimer laser improves outcome after penetrating keratoplasty. Ophthalmology 1999;106:1156-65.
35. Seitz B, Langenbucher A, Nguyen N, Kus M, Küchle M, Naumann G. Results of the first 1,000 consecutive elective nonmechanical keratoplasties using the excimer laser. A prospective study over more than 12 years. Ophthalmologe 2004;101:478-88.