·Clinical Research·

Femtosecond laser-assisted deep anterior lamellar keratoplasty in phototherapeutic keratectomy versus the big-bubble technique in keratoconus

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Abstract

● **AIM: To compare the functional and anatomic results of femtosecond laser (FSL)-assisted deep anterior lamellar keratoplasty (DALK) associated with phototherapeutic keratectomy (PTK) and FSL-assisted DALK performed using the big-bubble technique in keratoconus.**

● **METHODS: During the first phase of the study, an electron microscopy histopathology pilot study was conducted that included four unsuitable donor corneas divided into two groups: in FSL group, FSL lamellar cuts were performed on two corneas and in FSL+PTK group, PTK was performed at the stromal beds of two corneas after FSL lamellar cuts were made. During the second phase of the study, a randomized clinical trial was conducted that included two treatment groups of patients with keratoconus: group 1 (*n*=14 eyes) underwent FSL-assisted DALK associated with PTK and group 2 (*n*=12 eyes) underwent FSL-assisted DALK associated with the big-bubble technique. The main outcome measures were the postoperative visual acuity (VA) and optical coherence tomography (OCT) measurements, confocal microscopic findings, and contrast sensitivity.**

● **RESULTS: In the pilot study, histopathology showed a more regular stromal bed in the FSL+PTK group. In the clinical trial, group 1 had significantly worse best spectacle-corrected VA and contrast sensitivity (*P*<0.05 for both comparisons). The residual stromal bed measured by OCT was significantly (*P*<0.05) thicker in group 1. Confocal microscopy detected opacities only at the donor-receptor interface in group 1.**

● **CONCLUSION: Patients with keratoconus treated with FSL-assisted DALK performed using the big-bubble technique fare better than treated with FSL-assisted DALK associated with PTK.**

● **KEYWORDS:** deep anterior lamellar keratoplasty; phototherapeutic keratectomy; big-bubble technique; keratoconus

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INTRODUCTION

Keratoconus is the most common primary ectasia that leads to stromal thinning and low visual acuity (VA). The initial treatment includes glasses and contact lenses, however, corneal transplantation is indicated to treat advanced cases[1]. One important treatment option is corneal collagen cross-linking (CXL), which can stop or slow the progression of keratoconus, by using riboflavin and ultraviolet interaction that forms reactive oxygen species. CXL produces additional covalent bonds between collagen molecules, with subsequent biomechanical stiffening of the cornea[2]. Regarding corneal transplantation for keratoconus, deep anterior lamellar keratoplasty (DALK) is performed to remove the corneal tissue up to Descemet's membrane (DM). DALK maintains the recipient endothelium, thus eliminating the potential for endothelial rejection, which is considered one of the most important causes of failure associated with donated corneal tissue[3-4]. DALK can be performed using manual techniques or femtosecond laser (FSL)[5]. Good visual and refractive results have been reported in patients with keratoconus and corneal ectasia who underwent FSL-assisted DALK[6]. These results are possible because FSL incisions, such as those that are mushroom-shaped, allow better donor-receptor coaptation due to a bigger contact area, resulting in better healing and lower astigmatism[7].

The aim of the current study was to compare the VA, residual stromal bed thickness measured by optical coherence tomography (OCT), donor-recipient interface transparency observed by confocal microscopy, contrast sensitivity, and complication rates in patients who underwent corneal transplantation during FSL-assisted DALK associated with phototherapeutic keratectomy (PTK) and FSL-assisted DALK associated with manual dissection using the big-bubble technique.

SUBJECTS AND METHODS

The current study was conducted at the Federal University of São Paulo and the Sorocaba Eye Bank in 2011, after the Research Ethics Committee approved the study protocol [Universal trial number (UTN): U1111-1169-8770].

Electronic Microscopy Study A pilot study was performed initially using scanning electron microscopy (SEM) to evaluate the quality of the corneal stromal bed of four donor corneal buttons that had been rejected due to positive serology or for not being of sufficient quality for corneal transplantation. The corneal buttons were divided into two groups. In the FSL group, lamellar cuts were made in two corneal buttons using FSL (IntraLase FS laser™ 60 kHz, Abbott Medical Optics, Santa Ana, CA, USA) using the following parameters: keratoplasty module; diameter, 8.5 mm; incision, Z-shaped; energy, 1.8 µJ; spot separation, 5 µm; and depth, 300 µm. In the FSL+PTK group, lamellar cuts were made in two corneal buttons using FSL and the same parameters as in the FSL group, with subsequent PTK application to the residual stroma using 50-µm-thick and 7-mm diameter excimer laser applications (Technolas 217z™ Bausch & Lomb, Rochester, NY, USA) and a 2% methylcellulose mask.

Randomized Clinical Trial A randomized clinical trial was performed that included 26 eyes with keratoconus. One fully qualified and higly experienced surgeon performed all surgeries at the Sorocaba Eye Bank. The inclusion criteria were patients for whom DALK was indicated who had a VA below 20/60 corrected by contact lenses and a mean keratometry exceeding 55 diopters. All patients had a corneal thickness exceeding 280 µm and a clear cornea (no scarred cornea). Patients were excluded who had undergone previous surgeries with visual potential lower than 20/40 and a previous failed attempt at corneal grafting. All participants provided written informed consent before participating in the study.

The patients were divided into two groups. In group 1 (14 eyes), FSL-assisted DALK (IntraLase FS laser 60 kHz) was performed associated with PTK (Technolas 217z). PTK was performed on the residual stromal bed using a thickness of 50 µm and diameter of 7 mm and a 2% methylcellulose mask after the FSL cut was performed. In group 2 (12 eyes), FSL-assisted DALK was performed using manual dissection and the big-bubble technique. Manual delamination was performed using the big-bubble technique after the lamellar FSL cuts were made. The complication rates were evaluated in both groups.

Preparation of Donor Corneas in the Study Groups The FSL was programmed using lamellar keratoplasty module parameters, *i.e.* incisions, Z-shaped; diameter, 8.5 mm; energy, 1.8 µJ; and spot separation, 5 µm. The preparations for the FSL cuts of the donor cornea were identical in both groups. The donor cornea was removed from the Optisol GS solution (Optisol, College Station, TX, USA) and mounted on the

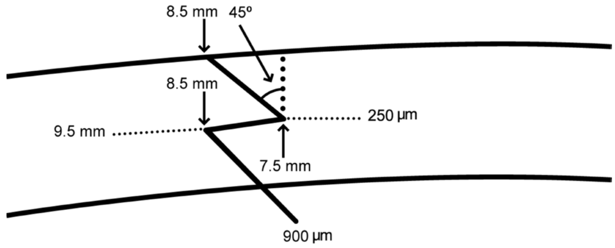

Figure 1 Z-shaped incision construction of the donor corneas in groups 1 and 2.

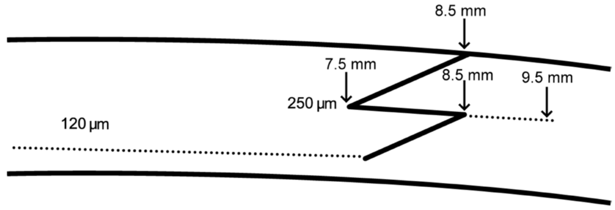

Figure 2 Z-shaped incision construction of the recipient corneas in groups 1 and 2.

artificial anterior chamber, which was filled with Optisol and viscoelastic substance, where it was centered and subjected to three FSL-modulated incisions in the following sequence (Figure 1). First, a Z-shaped posterior angled incision was created at an initial depth of 900 µm, which was 30 µm longer than the annular lamellar cut, and a diameter of 8.5 mm. Second, an annular lamellar cut was made in the stroma at a depth of 250 µm with internal and external diameters of 7.5 and 9.5 mm, respectively. Third, an anterior angled incision was made with an anterior external diameter standardized at 8.5 mm and 250 µm long.

The donor cornea then was removed from the scleral rim and placed in the preservation medium (Optisol GS); the endothelium was not removed. The donor corneas were sutured using 16 interrupted 10-0 mononylon sutures in both groups.

Preparation of Recipient Corneas of Patients in Group 1 The FSL was programmed using lamellar keratoplasty module parameters, *i.e.* incisions, Z-shaped; diameter, 8.5 mm; energy, 1.8 µJ; spot separation, 5 µm; and depth, calculated to allow a 120-µm residual stromal bed starting from the point at which the cornea was thinnest measured by the Orbscan Corneal Topographer (Bausch & Lomb). Thus, three cuts were made (Figure 2). First, a stromal lamellar cut was made with a raster pattern programmed to allow a 120-µm-thick residual stromal bed. Second, a posterior angled cut was standardized at 8.5 mm starting from the depth of the lamellar cut and going up to 250 µm from the anterior surface. Third, an anterior angled cut was standardized at 8.5 mm for the anterior external diameter. PTK was performed on the residual stromal bed using a thickness of 50 µm and a diameter of 7 mm, and a 2% methylcellulose mask was used.

Preparation of Recipient Corneas of the Patients in Group 2 The FSL was programmed same as group 1. After the FSL procedure, manual delamination was performed using the big-bubble technique. A 30-gauge needle coupled to a syringe and filled with 5 mL of air was inserted bevel down. The needle was positioned in the central or paracentral corneal region and the air was introduced to produce stromal emphysema (enabling the sense of depth), which was followed by a large bubble. A 15-degree blade was used to puncture the bubble, and a corneal scissors was used to remove the residual stroma up to DM through transparent tissue. The donated cornea then was sutured with 16 interrupted 10.0 mononylon sutures.

The best spectacle-corrected visual acuity (BSCVA) levels were expressed in logarithm of the minimum angle of resolution (logMAR) units and Snellen scale 3mo postoperatively. The 12-month postoperative results were used for statistical analyses. OCT was performed 4mo postoperatively using Visante OCT (Carl Zeiss AG, Oberkochen, Germany). Confocal microscopy using the Heidelberg Retina Tomograph Rostock Cornea Module (Heidelberg Engineering, Heidelberg, Germany) was performed at 3 and 6mo postoperatively. Contrast sensitivity was performed using the Functional Acuity Contrast Test (Stereo Optical, Chicago, IL, USA) at 12mo postoperatively. The same examiner measured the BSCVA and performed OCT, confocal microscopy, and contrast sensitivity testing.

Statistical Analysis All data were collected and analyzed using SPSS version 12 for Windows (IBM Corporation, Armonk, NY, USA). The normality test was performed through the Kolmogorov-Smirnov test. The Student's *t*-test was used for normally distributed data which shown as mean±standard deviation (SD); the Mann-Whitney test was used for nonparametric analysis. Spearman's correlation coefficient was calculated to assess the correlation between variables. Preoperatively, all participants were randomized in both groups in a 1:1 ratio, according to a computer-generated randomization table (Stata version 10, College Station, TX, USA). The surgeon was aware of the patient allocations at the time of surgery.

RESULTS

Electronic Microscopy Study Histopathologic analyses showed that the stromal surfaces of the corneal buttons were more regular in the FSL+PTK group. FSL group shows irregularity at the interface of the residual stromal bed (Figure 3A). Otherwise, FSL+PTK group shows a more regular stromal surface of the residual stromal bed (Figure 3B).

Randomized Clinical Trial The patient age, gender, and BSCVA (P=0.914 for the normality test, P=0.919 for the Student's *t*-test) did not differ significantly between the groups preoperatively. All patients were followed for 12mo.

Twelve months postoperatively, groups 1 and 2 had mean BSCVAs of 0.7 (20/100) and 0.35 (20/44), respectively. The BSCVA was significantly (P<0.001, by the normality test; P=0.009 by the Mann-Whitney test) worse in group 1 than in group 2. The mean residual stromal bed thicknesses measured by OCT in groups 1 and 2 were 198.2±37.8 and 62.1±20.8 µm, respectively, a difference that reached significance (P<0.001). No statistical correlation was detected between the residual stromal bed thicknesses and the BSCVAs in both groups (group 1, Spearman r=0.22, P=0.439; group 2, Spearman r=0.51, P=0.08). A big bubble was not achieved in four (33.3%) of 12 cases in group 2 during dissection.

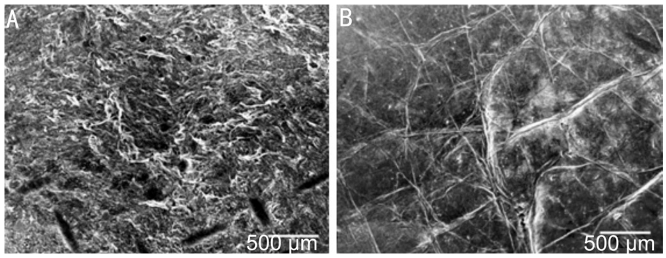

Figure 3 SEM image at the interface of the residual stromal bed A: FSL group shows irregularity at the stromal bed; B: FSL+PTK group shows a more regular stromal surface. Magnification: 500 µm.

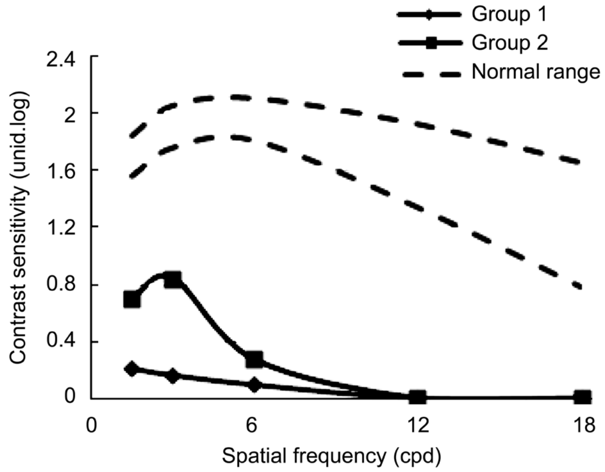

Figure 4 The contrast sensitivity with curve under daylight conditions (85 cd/m^2) between groups 1 and 2 cpd: Cycles per degree.

Confocal microscopy showed that the epithelial and endothelial layers were unchanged in all patients in both groups. Regarding the donor-recipient interface, confocal microscopy showed transparency in all patients in group 2 and small random opacities in all patients in group 1.

The contrast sensitivity in group 1 was significantly (P<0.001 by the normality test and Mann-Whitney test) lower than in group 2. The results for both groups were below the normal range (Figure 4).

Complications A perforation developed in two (14.3%) of 14 eyes and one of 12 (8.3%) eyes in groups 1 and 2, respectively. One eye with a microperforation remained in the study as a DALK case, and one eye with a macroperforation in group 1, which was converted to a penetrating keratoplasty (PK), was excluded from the study. One microperforation occurred in group 2 and remained in the study. Nine (69.2%) of the 13 eyes in group 1 underwent PK for visual rehabilitation due to a VA below 20/60 with contact lenses after 1y of follow-up. No eyes of rejection occurred during the follow-up period.

DISCUSSION

Although DALK is associated with higher survival rate and less rejection compared to PK, the risk of DM perforation arises and, thus, conversion to PK is a disadvantage[8]. To facilitate corneal dissection and avoid perforation in DALK, FSL can be used. But a FSL deep lamellar cut in DALK leads to an irregular dissection[9]. In contrast, deep ablations created using an excimer laser produced more regular stromal beds, but the vertical cut still produces inferior results similar to those created by manual trephination[10]. One study reported *in vitro* regularization of the residual stromal bed during PTK associated with FSL[11]. Based on those two studies, the ideal DALK technique would consist of lamellar and vertical cuts using FSL and stromal bed regularization by PTK. In our pilot study, SEM showed that corneas that underwent PTK application in FSL residual stromal bed had an interface that appeared more regular. Based on the pilot study, in the current study, we decided to perform DALK with FSL associated with PTK to increase the FSL-created stromal bed regularity by performing PTK in the residual stroma in patients in group 1. In group 2, we performed DALK associated with manual dissection using the big-bubble technique to provide more safety in relation to DM perforation.

In group 2, FSL DALK was performed in combination with manual dissection using the big-bubble technique to provide complete stromal removal and consequently a clear and regular interface. The literature shows that pre-DM dissection, as we used in the big-bubble technique, facilitates achieving the best VA results in DALK[12-13]. Thus, it was possible to compare our functional (BSCVA and contrast sensitivity) and anatomic results (residual stromal bed thickness by OCT and interface transparency by confocal microscopy) with those in the literature. Regarding the current visual results, 28.5% of patients in group 1 had a BSCVA of 20/40 or better, and in group 2, 83.3% of patients achieved a BSCVA of 20/40 or better. The results in group 2 were comparable to those reported previously, *i.e.* 77.8%[14], 87%[8], 92.3%[15], and 100%[16] of cases with a BSCVA of 20/40 or higher. In the current study, FSL lamelar cuts near DM produced a big-bubble rate of 66% in group 2, which was comparable to a study that reported 65.6%[17].

Achieving success with the DALK technique is associated with a long learning curve, which is why the surgeon in the current study was highly experienced with corneal transplant and had performed numerous DALK surgeries. The ramdomization made it possible that an experienced surgeon performed all surgeries in both groups, thus avoiding worse results and more complications. Even in group 1, in which PTK was performed, an inexperienced surgeon could have caused worse results because of a lack of experience in performing corneal surgeries.

In group 1, the thicker residual stromal bed probably led to interface irregularity and opacity detected by confocal microscopy in all patients, resulting in the worse visual results of the two groups. Nine (69.2%) of the 13 cases in group 1 underwent a second PK for visual rehabilitation because of low VA. Despite stromal surface regularization by PTK observed in the pilot and main studies, there was insufficient clinical reproducibility to improve the VA. In the pilot study, it is important to consider that the use of 2% methylcelulose as a masking agent to regularize the stromal surface could lead to irregular maintenance as the result of FSL in eye bank corneas. Another important factor is that these eye bank corneas were obtained from patients who did not have keratoconus, and because of this, the cellular architecture differed, which can explain these results. For comparison purposes, a published laboratory study used SEM to show that eye bank corneas that were dissected using a FSL associated with PTK had more regular and smoother residual stromal beds compared to those dissected using an excimer laser. The authors also reported more regular residual stromal beds compared to corneas dissected manually in association with PTK[11]. The study also found that at the macroscopic level, dissection using FSL created large and concentric depressions in the residual stromal bed that persisted despite PTK. However, at the microscopic level, the residual stromal bed dissected using FSL had irregularities despite PTK[11].

Considering the relation between the OCT stromal bed measurements and VA, group 2 had a significantly higher BSCVA and thinner stromal bed values than group 1. These results were corroborated by an important literature review study of DALK, which found that complete stromal removal is associated with better visual outcomes[18]. The BSCVA was comparable to PK in DALK cases, in which the residual stromal bed thickness was between 25 and 65 µm. However, whenever the residual stromal bed thickness exceeded 65 µm, the interface became opaque and there were folds in DM, which decreased the VA[18]. Another study reported that the VA in DALK was similar to that in PK whenever the residual stromal bed was thinner than 20 µm and that the BSCVA in the DALK group significantly decreased due to the increased residual stromal bed thickness, which resulted from interface opacity and irregularity and reduced optical quality[19].

A keratoconus study of 16 eyes that underwent manual dissection reported that the residual stromal bed measured by OCT was 67.1±24.3 µm, which was not correlated with the BSCVA[20]. These results were comparable to those in the current group 2. In another retrospective study of DALK performed to treat keratoconus, OCT was performed intraoperatively to measure the thickness between the cannula insertion depth (related to big-bubble formation) and DM, which resulted in a 104.3±34.1 μm thickness[21]. That study also reported that in the big-bubble formation group, the residual stromal bed thickness was 90.4±27.7 μm, which was significantly lower compared to the dissection group, in which the residual stromal bed thickness was 136.7±24.2 μm. These results were comparable to the current study, in which group 2 had a mean residual stromal thickness of 62.1±20.8 μm, which was significantly less than that of group 1 (198.2±37.8 μm).

Some authors have suggested that removing the endothelium of the donor corneal graft results in an improved interface[22-23]. However, another study suggested that removing the donor endothelium might cause mechanical trauma to the donor button, resulting in a significant reduction in the keratocyte density and interface irregularities and debris[24]. The Sorocaba Eye Bank surgical protocol for DALK, which was used in the current study, maintains the donor graft endothelium. The endothelium was not removed from the donor grafts based on comparative studies that found no significant differences after DALK in the VA and contrast sensibility, irrespective of whether the donor endothelium was removed or left attached[14,22-23,25].

The current study used confocal microscopy to assess the donor-recipient interface and detected interface opacities only in the group that underwent PTK application to the residual stromal bed (group 1). However, group 2 had a transparent interface. This was comparable to the results of a DALK study that proved that cases in which the stroma was removed completely had less interface scarring and opacity and achieved better visual outcomes compared with cases of DALK in which the stroma was not removed completely[22]. In addition, regarding confocal microscopy in DALK, a study of manual dissection of a stromal disease found a significant negative correlation between the interface reflectivity and BSCVA, to the extent that the reflectivity decrease was correlated with the BSCVA improvement after 6mo of follow-up[12,26-27]. Another confocal microscopy study reported that the inflammatory process increased immediately after FSL-assisted DALK, as a result of the increase in dendritic cells and keratocytic activation, but the inflammation decreased significantly until 12mo of follow-up[28].

The current study found that the contrast sensitivity in group 1 was significantly lower than in group 2, suggesting that the residual stromal bed thickness affected visual quality. One study supported this theory with the findings of lower contrast sensitivity in DALK cases with a residual stromal bed that was less than 80 μm thick and contrast sensitivity results comparable to PK in cases with a residual bed thinner than 20 μm[19]. These data suggested that the BSCVA and contrast sensitivity after DALK with complete stromal removal are higher than after manual DALK as the result of greater interface transparency[29]. This was supported by a study that reported that patients treated with the Anwar technique had contrast sensitivity that was significantly higher than the group treated with Melles technique[30].

In the current study, the incidence rates of DM rupture in groups 1 and 2 were 14.2% and 8.3%, respectively, and the PK conversion rates were 7.1% and 0, respectively. These data were consistent with those in the literature in which DM perforation rates ranged from 4% to 39.2% and PK conversion rates ranged from 0 to 14%[12,14-15,31-35]. In the current study, there were no cases of epithelial or stromal rejection in either group during the follow-up period. Previous studies have reported incidence rates of stromal rejections between 2% and 12% after DALK in patients with keratoconus[12,36].

The comparison between corneal transplantation techniques in patients with keratoconus showed that regarding the VA, FSL-assisted DALK associated with manual dissection using the big-bubble technique (group 2) had better visual outcomes than the FSL-assisted DALK associated with PTK (group 1); group 1 had worse results for residual stromal bed thickness, interface transparency, and contrast sensitivity and a higher complication rate. Interestingly, nine (69.2%) of the 13 eyes in group 1 underwent a second PK for visual rehabilitation because of low VA.

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