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ORIGINAL PAPER

Small-incision lenticule addition in ex vivo model of ectatic human corneas

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Abstract

Purpose To investigate the feasibility of intrastromal lenticule insertion to restore corneal shape in a model of ectatic human cornea.

Methods For this experimental ex vivo study on 34 human corneas unsuitable for transplantation, 17 corneas were thinned by decentralized posterior excimer laser ablation to 200 µm thickness and 6.5 mm diameter and then inflated up to 100 mm Hg to expose the ectasias (recipient corneas). Pachimetry and topography were obtained. Stromal lenticules of the same diameter and thickness as the ectasias were shaped with a femtosecond laser from the remaining 17 donor corneas. An intrastromal pocket was created with femtosecond laser within the ectatic recipient corneas and the donor lenticule was inserted inside it. Changes in corneal architecture and profile were evaluated by means of corneal topography and anterior segment optical coherence tomography.

Results All stromal lenticules were successfully implanted. Tomography confirmed regularity of the lenticule profile within the stromal pocket. Corneal thickness was significantly increased after the procedure ($P < 0.0001$). Maximal posterior elevation from the best-fitted toric ellipsoid was significantly reduced ($P < 0.0001$). Significant flattening of posterior K1 and K2 was also obtained ($P = 0.041$ and $P = 0.004$, respectively). Anterior and posterior astigmatism, anterior and posterior asphericity, and spherical aberration did not differ significantly after the procedure.

Conclusions Femtosecond laser-assisted stromal lenticule addition is feasible for restoring corneal thickness to an ectatic area and for regularizing posterior corneal elevation. The technique opens new perspectives for the treatment of corneal ectasias.

Keywords Additive keratoplasty · Ex vivo model · Ectasia · Femtosecond laser-assisted surgery

Introduction

Corneal ectatic disorders such as keratoconus (KC) develop with progressive corneal thinning that causes irregular astigmatism and refractive instability with decreasing visual acuity. A variety of surgical and non-surgical techniques is available; treatment is increasingly customized to the individual patient by clinical and topographic staging of the condition and the expected outcome [1].

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The newest options are techniques that use femtosecond laser (FSL): minimally invasive corneal surgery in which the laser produces precise, clear cuts in the intrastromal lamellae. A recent study described the use of stromal lenticule addition keratoplasty (SLAK) to flatten the anterior corneal surface by inserting a negative meniscus-shaped lenticule, thinner at the center and thicker at the periphery, into a stromal pocket in the recipient cornea in order to obtain an effect similar to intrastromal ring insertion [2].

However, since one of the earliest alterations in KC is posterior ectasia, which progresses in the anterior stroma concurrent with apical thinning of the cone, we hypothesized that restoration of normal architecture should primarily target corneal thickness at the apex and flattening of the posterior ectasia. The aim of the present study was to create an ex vivo model of ectatic corneas following excimer laser ablation and to demonstrate restoration of corneal thickness and flattening of the posterior ectasia by the FSL-assisted small-incision lenticule addition (SMILA) technique.

Materials and methods

For this ex vivo experimental study, 34 clear human donor corneas unsuitable for transplantation were provided by the Fondazione Banca degli Occhi del Veneto Onlus (Venice, Italy) following signed informed consent obtained from the donor's next of kin for research purposes. The corneas were maintained at room temperature in a dextran-containing medium and randomly assigned to two groups: 17 as the source of stromal lenticules (donor corneas [DC]) and 17 as the host for the lenticules after being shaped to exhibit artificial ectasia (recipient corneas, [RC]).

Stromal ectasia obtained by excimer laser ablation

The RC were thoroughly washed with balanced salt solution (BSS) and mounted on an artificial anterior chamber (ACC, Network Medical Products Ltd; Coronet House, UK), with the endothelium facing outward. Intracameral pressure was maintained at 37 mm Hg by positioning the BSS bottle connected to the ACC at a height of 50 cm.

To obtain a corneal ectasia simulating KC, two sequential ablations to 97 µm thickness in the infero-temporal corneal quadrant were performed using a Technolas Teneo® 317 excimer laser (Technolas Perfect Vision GmbH, Munich, GE, a Company of Bausch & Lomb Incorporated). The settings are presented in Table 1.

After the ectasia was shaped, the endothelial side was marked with a dermographic pen, and the RC was remounted on the AAC with the endothelium facing internally. To expose the ectatic apex, the intracameral pressure was increased to 100 mm Hg (136 cm H_20) and then brought back to a 25 mm Hg (34 cm H_20) for the remainder of the procedures.

To ensure precise and constant orientation of the cone during evaluation, the AAC was marked on the plastic ring. To protect the RC surface, Ial24® gel (Bausch & Lomb Incorporated, IT) was applied at the end of each procedure. Each RC was thoroughly washed with BSS before examination.

Intrastromal pocket shaped by femtosecond laser

To create the intrastromal pocket, each ectatic RC was shaped by FSL 80 kHz ablation (VICTUS®, Technolas Perfect Vision GmbH,). The settings are presented in Table 2.

The intrastromal pocket was centered on the KC apex and the depth of the dissection pocket was based on the anterior segment optical coherence tomography (AS-OCT) parameters, leaving 200 µm of stroma from the endothelial side.

Preparation and implantation of donor lenticules

Each DC was assembled on the AAC with the endothelium facing internally, so that the center of

Table 1 Excimer laser settings for preparing the donor corneas

Subjective refraction, sph	− 13.5 D
Correction refraction, sph	− 13.5 D
K/Q values	+ 43.3 D/ − 0.20
Normogram	100%
Optical zone	4.5 mm
Max ablation	97 µm
Treatment diameter	6.5 mm × 6.5 mm
Total pulses	5285

Table 2 Femtosecond laser settings for preparing the recipient corneas

Pocket depth	200 μm (from endothelium)
Pocket diameter	9.00 mm
Side cut angle	120°
Hinge width	2.10 mm

Table 3 Femtosecond laser settings for preparing the lenticules in the donor corneas

Lenticule thickness	200 μm
Lenticule diameter	6.5 mm
Shape	biconvex
Side cut	120°
Hinge width	2.1 mm
FSL spacing between lines	2 μm
FSL spacing between spots	4 μm
FSL spot energy	1.8 nJ

the anterior chamber matched that of the cornea. The intracameral pressure was maintained up to 37 mm Hg by positioning the BSS bottle at a height of 50 cm. The intrastromal lenticule cuts were centered on the corneal central zone using the AAC ''cross-target'' and the co-axial laser microscope intraoperative target. The stromal lenticules were created by FSL to obtain a biconvex shape with the same curvature radius. The settings are presented in Table 3.

The stromal lenticules were extracted from the DC using Buratto forceps (J2186, Jannach, Como, IT) after accurately separating the residual tissue with a delamination spatula (J2404, Jannach), and the center was marked with a dermographic pen.

The lenticules were folded using Buratto forceps, inserted into the stromal pocket, and then unfolded with bimanual maneuvers using a delamination spatula until the apex of ectasia and the center of lenticule coincided, while maintaining its original orientation (anterior surface directed upward). The corneal surface was carefully hydrated using BSS throughout the procedure.

Instrumental examination

The RCs were examined following excimer laser treatment and lenticule implantation by means of topography (Atlas® Zeiss 9000; Carl Zeiss Meditec, Jena, GE) and anterior segment optical coherence tomography ([AS-OCT] Visante®; Carl Zeiss Meditec) to obtain a comprehensive map generated by the inbuilt software (Visante Omni). Based on the Holladay report of the Visante Omni, we analyzed anterior and posterior curvatures, asphericity, spherical aberration, posterior elevation from the best-fitted toric ellipsoid (BFTE), and the global pachimetry map.

Statistical analysis

We assessed parameters using the Kolmogorov–Smirnov test and compared the differences between pre- and post-implantation with a paired t test. All comparisons were two-tailed, and a $P < 0.05$ was considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA).

Results

We successfully obtained ectatic corneas by excimer laser and stromal pockets and lenticules by FSL in all cases. Surgical insertion of the lenticules was effective in all but one cornea due to perforation (96% success rate). The AS-OCT evaluation showed the obtained ectasia (Fig. 1a) and the precise positioning and appropriate distention of the lenticules in the recipient pockets, with regular interfaces, profiles, and the absence of folds (Fig. 1b). Table 4 presents the mean tomographic values following excimer ablation and after SMILA and Fig. 2 represents the Holladay report of the ectatic cornea after excimer laser ablation (Fig. 2a) and after SMILA (Fig. 2b).

The mean values of central corneal thickness (CCT) and of thinnest corneal thickness (TCT) were significantly increased ($P < 0.001$; paired t test) after lenticule implantation, as expected. The difference between the expected increase (lenticule thickness) and the increase in corneal thickness was also statistically significant ($P < 0.001$; paired t test).

The mean curvatures of the anterior surface (SimK1 and SimK2) were increased after lenticule insertion, albeit without statistical significance. In addition, mean astigmatism of the anterior surface and the mean anterior asphericity Q (both at 4.5 mm and at 8.0 mm) did not vary significantly. All these

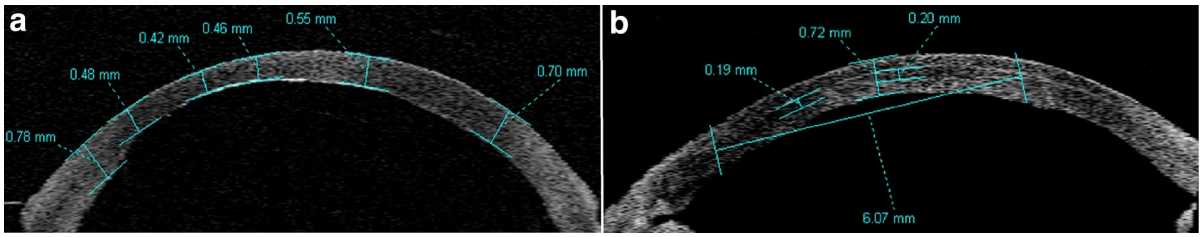

Fig. 1 Results of anterior segment optical coherence tomography examination. **a** The ectatic profile obtained after excimer laser ablation and following high pressure inflation of the artificial anterior chamber. **b** The same cornea after pocket creation and lenticule implantation. The pocket cut is larger than the lenticule; the lenticule is inserted into the pocket without folding it; the posterior stroma is flattened following insertion of the lenticule. We used a caliper to show the thickness of the anterior and posterior stroma and the stromal lenticule. The lenticule appears thicker than expected possibly due to the edema induced during its insertion

parameters showed a trend to a more prolate profile, however.

Prominent flattening of the posterior surface by means of SMILA was confirmed by a reduction in both the steep and flat posterior meridians, without significantly influencing the posterior astigmatism, which was actually slightly increased. Posterior flattening was also demonstrated by the statistically significant reduction in the mean posterior elevation of the higher point from the BFTE after SMILA. This result also confirmed regularization of the posterior profile, which was more similar to the BFTE, i.e., to the normal posterior curvature. Differently, posterior asphericity was not significantly affected by lenticule insertion ($P = 0.17$). The spherical aberration calculated at 6 mm was increased after lenticule implantation (not statistically significant).

Discussion

Previous studies in animal models showed that corneal stroma lenticule implantation is technically feasible and that the implanted lenticule was integrated and survived in the host stroma [3–5]. In their ex vivo study, Mastropasqua et al. [2] demonstrated that insertion inside the optical zone of a hyperopic-shaped stromal lenticule 5 to 6 mm in diameter, thinner at the

Table 4 Mean tomographic values following excimer ablation and SMILA

	Excimer	SMILA	Difference	*P* value
CCT, µm	610.65 ± 114.50	887.41 ± 125.84	276.76 ± 136.26	< 0.0001
TCT, µm	478.41 ± 108.70	725.71 ± 91.60	246.29 ± 104.88	< 0.0001
SimK1 anterior, D	42.73 ± 3.79	43.99 ± 4.42	1.25 ± 3.77	0.19
SimK2 anterior, D	45.33 ± 2.57	47.41 ± 6.43	2.08 ± 5.71	0.15
Sim K anterior astigmatism, D	2.79 ± 2.33	4.56 ± 4.90	1.77 ± 5.30	0.19
Max posterior elevation from BFTE, µm	135.95 ± 42.6	78.34 ± 43.90	− 57.6 ± 36.7	< 0.0001
K1 posterior, D	7.50 ± 2.07	5.42 ± 2.14	− 2.1 ± 2.5	0.004
K2 posterior, D	8.26 ± 1.98	6.50 ± 2.75	− 1.8 ± 3.2	0.041
Posterior astigmatism, D	0.75 ± 0.69	1.08 ± 1.04	0.3 ± 1.2	0.29
Spherical aberration Z, µm [@ 6 mm]	0.06 ± 0.72	0.39 ± 0.80	0.4 ± 1.1	0.12
Anterior asphericity Q [@ 4.5 mm]	− 0.43 ± 0.30	− 0.49 ± 0.42	− 0.05 ± 0.4	0.63
Anterior asphericity Q [@ 8 mm]	− 0.30 ± 0.49	− 0.42 ± 0.41	− 0.1 ± 0.6	0.43
Posterior asphericity Q [@ 8 mm]	0.39 ± 1.14	1.40 ± 2.31	1.01 ± 2.9	0.17

CCT denotes central corneal thickness, *TCT* thinnest corneal thickness, *BFTE* best-fitted toric ellipsoid

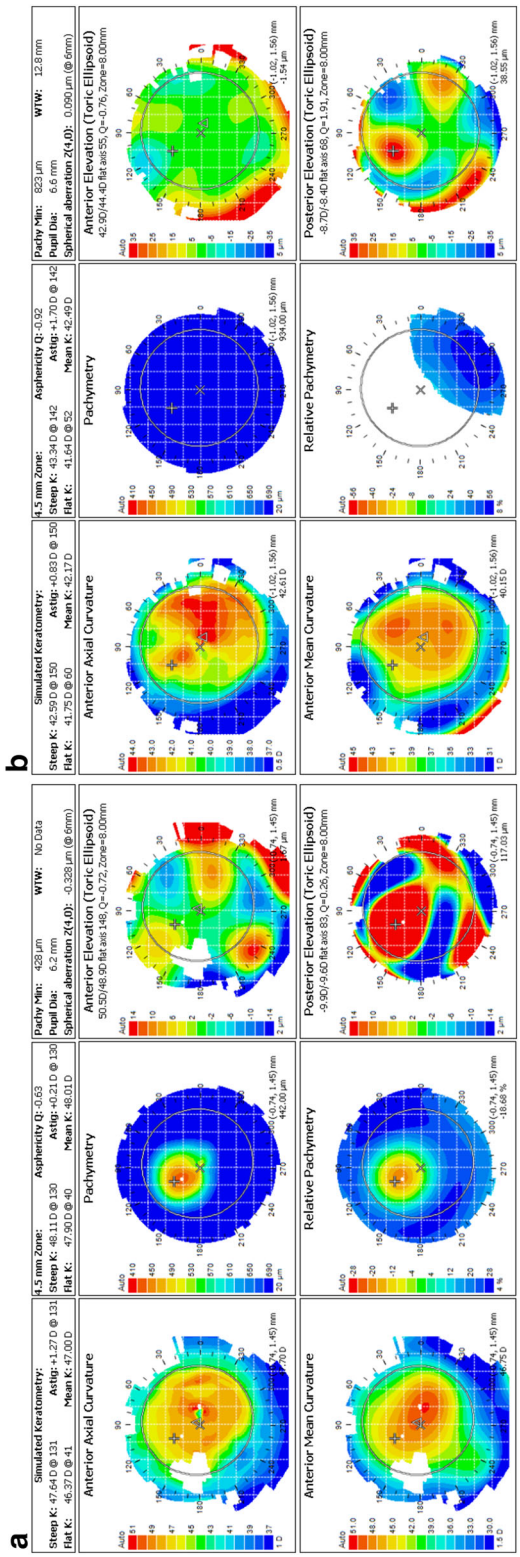

Fig. 2 Representative example of the Holladay reports from the Visante Omni after excimer ablation (**a**) and small-incision lenticule addition (SMILA) (**b**). **a** Steepening in the anterior curvature and posterior elevation from the best-fitted toric ellipsoid corresponding to the thinnest corneal thickness at the pachimetry map producing a keratoconus-like pattern following excimer ablation. **b** Thickening of the cornea, particularly of the thinnest corneal thickness, flattening of the anterior and posterior curvature, and a more regular pattern in the posterior best-fitted toric ellipsoid following SMILA

center that at the periphery, flattens the central corneal profile and increases corneal pachimetry. In a more recent study, they confirmed these results in vivo: They assumed that the mechanism of action of their technique is analogous to intracorneal ring insertion and proposed this surgery as an alternative treatment for KC [6].

However, because ectasia in KC starts from the posterior stroma and eventually comprises the anterior stroma, the posterior surface warrants particular attention when the aim is to restore a physiological corneal setting. For this reason, we set up SMILA to restore thickness in the area of the greatest thinning, while flattening the posterior surface at the same time.

We observed an increase in anterior curvature after SMILA, albeit not statistically significant, due to the addition of stroma to the central cornea. Induced steepening of corneal curvature is undoubtedly key to the final useful refractive power of the eye in KC. For the best visual acuity, however, we believe that, compared to the posterior surface, flattening of the anterior surface is a secondary goal since it can be definitely modified later by any of several approaches that regularize the surface or change its curvature (i.e., wearing contact lenses or by excimer laser ablation).

Creating a model of ectatic cornea ex vivo on which we could test our hypothesis was of pivotal importance. The tomographic results confirmed that localized excimer laser ablation of the posterior surface followed by high-pressure inflation of the AAC with BSS up to 100 mm Hg was effective in creating the expected model.

We were thus able to show that insertion of biconvex lenticules restores corneal thickness in the ectatic area and induces good restoration of the posterior surface to a more physiological profile (as shown by the reduction in the elevation from the BFTE), without inducing a significant increase in astigmatism in the anterior or the posterior surfaces. The implanted additional stromal tissue may provide biomechanical support and central corneal thickening to the RC, while the FSL-sculpted pocket in the recipient stroma is believed to minimally modify its biomechanical strength [7].

This study has several limitations, the major one being that the corneal epithelium was not removed before assessment, and manipulation of the RC could have altered the epithelial surface, probably masking possible modifications in the anterior keratometric data and slightly reducing reliability. Furthermore, we acknowledge that the RC was analyzed only during the immediate post-treatment period and that a component of stromal edema was observed as a statistically significant difference in the intended and actually induced modification of corneal thickness. This could derive from posterior surface ablation, which eliminated endothelium in the ablated area, and from the high pressure to which the RC were subjected.

Moreover, one could argue that the affected stroma left in place might lead to progression of disease: While this is possible, cross-linking can be proposed and the stroma added with SMILA will render it even safer for the endothelium. In the present era of customized medicine, we think that patients should receive tailored treatment based on specific keratoconus features. The SMILA technique could be a means to achieve this objective in the treatment of KC. Patients candidate for SMILA should have been diagnosed with advanced KC but still have a clear corneal stroma for both the efficacy of FSL and for the achievement of qualitatively good visual performance after the procedure. Although the lenticule thickness in our study was standardized (200 μm), when performing the procedure in clinical practice, lenticule thickness should be customized to the individual patient depending on the degree of thinning the patient presents. Results recently reported by Aliò et al. [8] at 6 months after intrastromal lenticule implantation in 9 patients with KC are extremely promising. However, these authors transplanted lenticules that were thinner (120 μm) and larger (9 mm diameter) than ours, and they did not report the effects on the posterior surface.

The impact of stromal haze that could develop in vivo after lenticule implantation is a major concern. However, stromal haze is rarely an issue in partial thickness lamellar keratoplasty, a widely performed procedure [9]. Finally, regularization of the anterior surface, although not significant in our study, could improve contact lens fitting, thus reducing or deferring the need for traditional corneal transplantation. If SMILA proves feasible in patients with KC, as suggested by early results, it could become an effective alternative for the management of patients with KC.

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