

Femtosecond Laser-Assisted Corneal Small Incision Allogenic Intrastromal Lenticule Implantation in Monkeys: A Pilot Study

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PURPOSE. Lenticule implantation can be used to correct vision problems. However, it is significantly restrained by the sources of autologous lenticules. The aim of the present study was to investigate the feasibility and effects of femtosecond laser-assisted corneal small incision allogenic intrastromal lenticule implantation (AILI) in monkeys.

METHODS. Six healthy adult monkeys were included in this study. Femtosecond lenticule extraction (−4.0 diopter [D] correction, 5.0-mm optical zone) was performed in one eye of two monkeys and both eyes of one monkey. Each extracted refractive lenticule was allogeneically transplanted into a femtosecond laser-created corneal stromal pocket in one eye of the other two monkeys and one monkey's both eyes. Pre- and postoperative (1 or 3 days, 1 month, and 6 months) slit lamp microscopy, corneal topography, anterior segment optical coherence tomography, and in vivo confocal microscopy were performed.

RESULTS. Corneal edema occurred in the early postoperative days with a large number of hyperreflective particles around the borders. Corneal tissue edema gradually decreased. Nerve fiber regeneration could be detected in the lenticule layer at 6 months. Overall, 3.27 ± 1.2 D corneal power was increased at 6 months, accounting for 82% of the intended correction. At the same time point, corneal stroma was 69 ± 11 μ m thicker than preoperative ones and was roughly equal to the maximum thickness of implanted lenticules. No significant complications were observed.

CONCLUSIONS. The AILI technique seems to be feasible and safe for increasing corneal stromal thickness and changing corneal refractive power, which may provide a useful method for treatment of keratoectasia, presbyopia, and hyperopia.

Keywords: allogenic intrastromal lenticule implantation, femtosecond laser, cornea, refractive lenticule

Corneal refractive surgery is routinely used to correct myopia, astigmatism, and hyperopia. However, excimer lasers will reduce the corneal thickness. Therefore, recent technologies aim to correct myopia or hyperopia without the loss of corneal tissue. With the recent use of femtosecond laser (FSL), laser-assisted in situ keratomileusis (LASIK) has become more safe and precise in the creation of a corneal flap.¹ Very recently, the introduction of refractive lenticule extraction (ReLEx) has marked a new approach using the precision and accuracy of FSL, making the entire refractive procedure of flap creation and stromal tissue ablation feasible to be performed with a single laser.² Based on the incision used to remove stromal lenticule, ReLEx can be further used for femtosecond lenticule extraction (FLEx) and small incision (usually 2–3 mm) lenticule extraction (SMILE). In the FLEx procedure, the lenticule is generally extracted through a fully lifted flap, similarly to a LASIK procedure, or a partially lifted flap (pseudo-SMILE). The corneal flap obtained by the FLEx procedure with partially lifted flap is actually a corneal cap instead of a real flap.

Obviously, the lenticule obtained with ReLEx could be used to correct refractive defects.

The fact that the intrastromal lenticule is created and extracted all in one piece with high accuracy allows the possibility of using it for transplantation. Previous studies in rabbit models have shown that autologous lenticule implantation is feasible and that the implanted lenticule survives in the host cornea.^{3–5} Pradhan et al.⁶ have reported the case of a hyperopia patient who received lenticule implantation from a donor patient with high myopia, using the SMILE procedure, and the results indicate that intrastromal lenticule implantation could be a promising and effective procedure to correct hyperopia without significant adverse effects. These studies indicate a possible application of lenticule implantation in the clinical setting. However, it is significantly restrained by the sources of autologous lenticules. Therefore, to obtain lenticules from donations would be an alternative solution to solve this issue. Ganesh et al.⁷ have reported nine cases involving implantation of cryopreserved corneal lenticules extracted from myopia-correction patients to treat hyperopia. However,

the freeze/thaw process of the lenticule probably damages the lenticule collagen structure and viability.⁸⁻¹⁰

Therefore, in the present study, fresh intrastromal lenticules extracted from donor monkeys were immediately transplanted into recipient monkeys. The aim of the present pilot study was to assess the feasibility and safety of allogenic intrastromal lenticule implantation (AILI).

MATERIALS AND METHODS

Animals

Six 4- to 5-year-old healthy adult rhesus monkeys (4.5-5.5 kg) obtained from XiShan Zhongke Laboratory Animals Co. Ltd. (Jiangsu, China) were used in this study. The use of animals adhered to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

Methods

Monkeys were randomly divided into three groups of two monkeys. In the first two groups, one was selected to be the intrastromal lenticule donor and the other was the recipient. For the third group, intrastromal lenticules were derived from one monkey's two eyes and implanted to the other monkey's two eyes.

Monkeys were anesthetized with an intramuscular injection of ketamine hydrochloride (15-20 mg/kg) and acepromazine maleate (0.15-0.2 mg/kg) before each operation and measurements. For donor monkeys, the Pentacam system (Oculus Optigerate GmbH, Wetzlar, Germany) was used to measure corneal thickness pre and post FLEx to make sure that the residual cornea bed thickness was no less than 250 μm . The FLEx procedure was performed by using a VisuMax FSL system (Carl Zeiss Meditec, Jena, Germany), in which the FSL performed a hinged LASIK-type flap incision that was lifted up, and the underlying refractive stromal lenticule was peeled away through the incision (Fig. 1A).

The intended diameter of the corneal flap was 6.0 mm, 100 μm in thickness with a side-cut angle of 90°. An optical zone of 5.0 mm with a side-cut angle of 90° was used to correct -4.0 diopters (D), which resulted in a 60- μm maximum central lenticule thickness with a minimum edge thickness of 15 μm . Extracted lenticules were kept with a balanced salt solution (BSS)-dipped sponge that was swabbed regularly to prevent dehydration. A corneal flap was then performed on one eye of the recipient monkeys. Firstly, a femtosecond laser was used to create a 6.0-mm-diameter and 100- μm corneal flap with a side-cut angle of 90°. The edge of the upper quadrant was then separated in the normal fashion and the incision was limited as short as possible to produce a lamellar pocket (corneal cap). Secondly, the lenticule extracted from the donor monkey was immediately inserted into the intrastromal pocket with an iris repository and smooth microforceps (Figs. 1B, 1C). The lenticule was considered as being centered by the operator when the center of the lenticule was aligned with the center of the pupil in the recipient monkeys. Tobramycin and dexamethasone eye drops (two drops each) were applied after surgery and three times per day for 2 weeks. The average central corneal thickness for donor monkeys (four eyes) was 462 ± 25 μm (range, 441-496 μm) preoperatively and was decreased to 390 ± 29 μm (range, 371-434 μm) 1 day postoperatively. The calculated residual bed thickness was more than 250 μm after subtracting from the 100- μm flap thickness.

Slit lamp microscopy was used to observe corneas and incisions pre and post operation. Cornea and transplanted

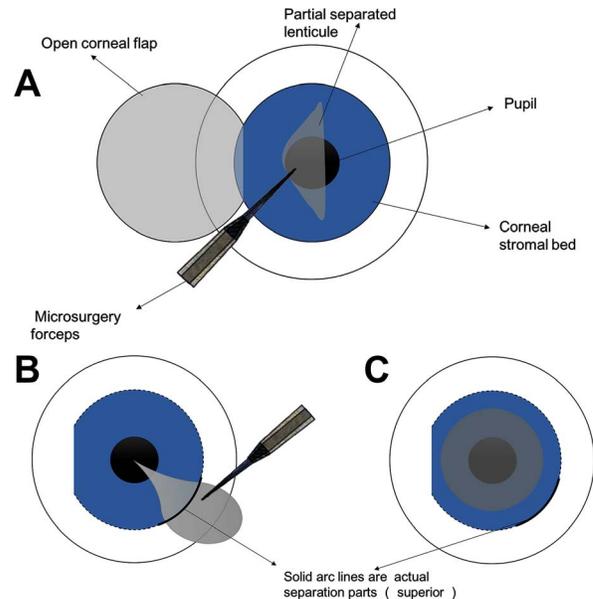


FIGURE 1. Diagram to show the separate and insert procedures. (A) Refractive lenticule was extracted from the donor monkey through FLEx procedure. (B) Corneal flap was performed on the intended recipient monkey by means of FLEx; only the edge of the upper quadrant was then separated in the normal fashion and incision. Extracted refractive lenticule was then inserted between the flap and stromal bed, instantly (C) spread out as well as its position adjusted until its center was overlapped with the center of the pupil.

lenticule thicknesses were measured with a RTVue OCT (software version 6.2; Optovue, Inc., Fremont, CA, USA) before lenticule transplantation, and 1 and 6 months after transplantation. Corneal front and posterior topography were provided by using the Pentacam system preoperatively, and 1 and 6 months postoperatively. We also performed in vivo confocal microscopy (HRT 3; Heidelberg Engineering GmbH, Heidelberg, Germany) at 3 days, 1 month, and 6 months after transplantation.

Statistical Analysis

Data are expressed as mean \pm standard deviation. Statistical analysis was performed by using SPSS Statistics 22.0 (IBM Corp., Armonk, NY, USA). The differences between any two time points were analyzed by using paired samples *t*-tests. A two-tailed *P* value < 0.05 indicated statistical significance.

RESULTS

Slit Lamp Microscopy

Corneal epithelial defects and mild to moderate corneal edema were noticed on day 1 after lenticule transplantation, with tissue edema around the implanted lenticules (Fig. 2A). Corneal epithelium was restored to full integrity an average of 6 (range, 3 to 10) days after operation. Corneal edema gradually improved from postimplantation days 5 to 21. Corneas recovered their transparency in all eyes by postoperative month 6. Although the cornea appeared normal on postoperative month 6, the edge of the corneal cap was visible and the density of the anterior stroma under the cap was increased under slit lamp microscopy (Fig. 2A, right image).

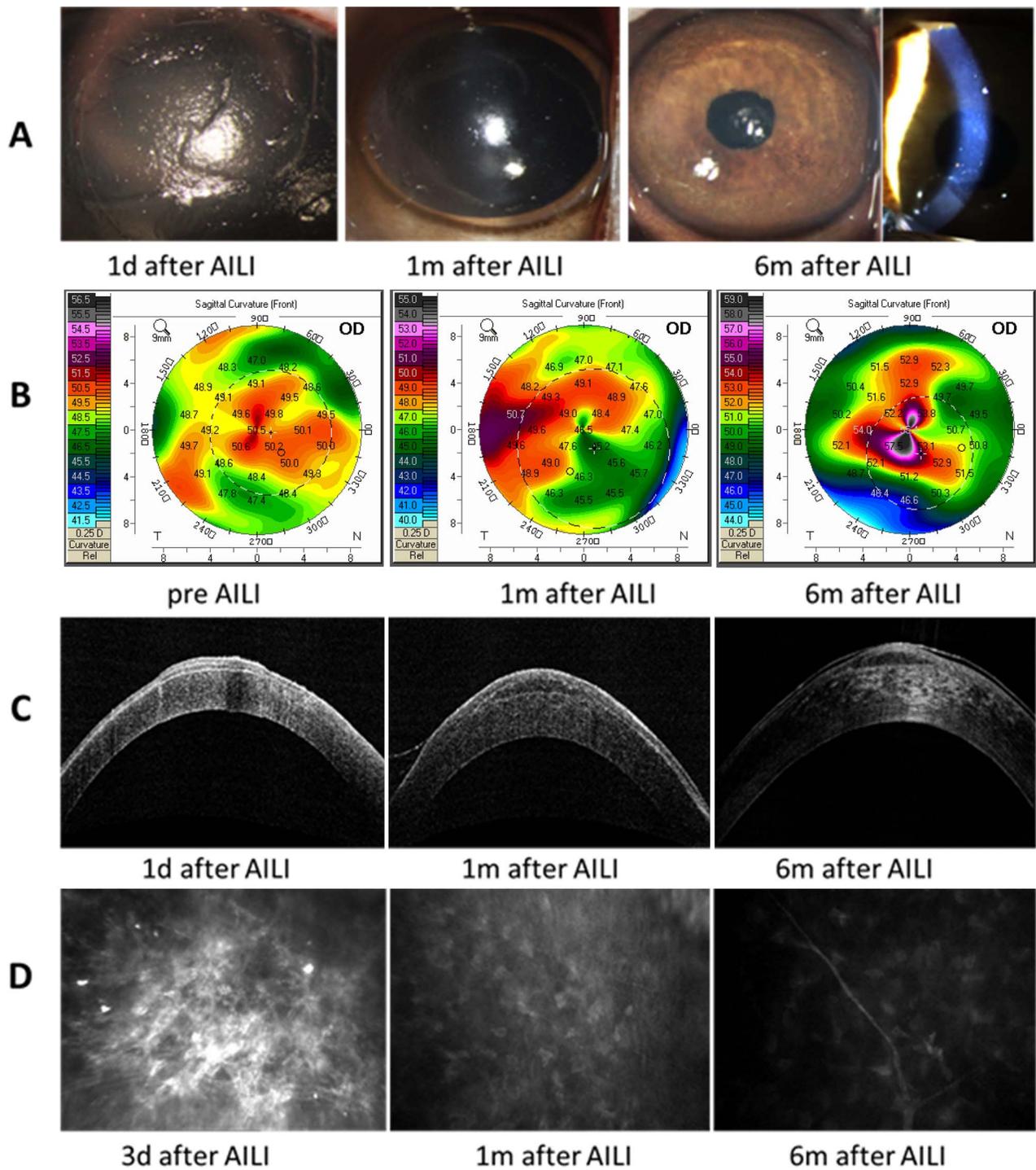


FIGURE 2. Slit lamp microscopy, corneal topography, anterior segment optical coherence tomography, and in vivo confocal microscopy of eyes that underwent AILI. (A) Slit lamp photographs showing the cornea before, and on months 1 and 6, after AILI. Corneal edema decreased gradually after AILI and returned back to clearness. Note that in the image at month 1 after AILI, the pupil was dilated by a mydriatic agent. The *far right* slit illumination image showed slightly increased density of the anterior stroma at 6 months. (B) Corneal topography before, and 1 and 6 months after, AILI. The anterior corneal surface became significantly steeper after operation. (C) Anterior segment optical coherence tomography images on day 1, and months 1 and 6 after AILI. Borders of implanted refractive lenticule were clearly detected after AILI. (D) In vivo confocal images on day 3, and months 1 and 6 after AILI. Corneal stroma nerve regeneration was observed at 6 months postoperatively. Field size was $400 \times 400 \mu\text{m}$.

Corneal Topography and Keratometry

Pentacam corneal topographic maps (Fig. 2B) showed obvious steepened corneal surface in all cases after lenticule transplantation. Mean radius of the anterior curvature was 6.75 ± 0.13

mm before operation and 6.57 ± 0.08 mm at 1 month and 6.4 ± 0.13 mm at 6 months after operation ($P = 0.057$, pre versus 1 month; $P = 0.033$, pre versus 6 months); there was no difference on the anterior curvature between 1 and 6 months ($P > 0.05$). Mean radius of the posterior curvature for the

operated eyes were 5.49 ± 0.25 mm before operation and 5.3 ± 0.24 mm at 1 month and 5.66 ± 0.52 mm at 6 months postoperatively ($P > 0.05$). By combining both the anterior and posterior changes of the radius curvature, the lenticule transplantation led to a 3.27 ± 1.2 D total increase in corneal refractive power at 6 months ($P < 0.01$).

Anterior Segment Optical Coherence Tomography

The contour profile of the transplanted lenticule was easily detected, which was surrounded by swelling stroma 1 day after operation (Fig. 2C, left). At 1 month, no edema was found in the lenticule-implanted cornea, while the corneal epithelium still appeared less smooth than the preoperative state (Fig. 2C, middle). At 6 months, all the corneas were clear with a little higher density in stroma around the lenticule (Fig. 2C, right). The anterior and posterior surfaces of the lenticule were still discernible. Corneal pachymetry increased by 69 ± 11 μ m, from 475 ± 11 μ m before the procedure to 544 ± 17 μ m at 6 months postoperatively.

In Vivo Confocal Microscopy

The stroma near the border of the lenticule was filled with many hyperreflective particles of variable size and was acellular on day 3 after transplantation (Fig. 2D, left). At 1 month, the reflection near the lenticule border was less prominent and active keratocytes were visible (Fig. 2D, middle). At 6 months, inactive keratocytes with clear borders were visible and nerve fibers could be detected in the lenticule layer (Fig. 2D, right). Except for a few small particles accumulating around the anterior and posterior surfaces of the lenticule, the cornea showed a relative normal and quiet state.

Complications

One eye of the donor monkeys lost suction before flap creation during the FLEx procedure, and FLEx procedure was repeated on the same eye successfully after suction was restored. FLEx was terminated on one eye of the donor monkey owing to loss of suction after flap creation, and FLEx was finished in the other eye instead. No serious complications were recorded during lenticule transplantation on the recipient monkeys. During follow-up, no complications, such as eye infection, diffused lamellar keratitis, significant corneal haze, or transplanted lenticule rejection, were noted in all monkeys.

DISCUSSION

Results of the present study may suggest that AILI may be used to increase the corneal thickness and alter the corneal power as intended without significant complications. As more and more surgeons are inclined to use the ReLEx procedures (including FLEx and SMILE) to correct refractive errors, owing to its excellent efficacy, safety, and predictability,^{11–14} an intact refractive lenticule (RL) as the immediate by-product of ReLEx could be used during operation. Refined RL storage technique^{5,9} and RL supplied by the ReLEx procedure could make possible the use of AILI in a clinical setting.

Despite the occurrence of corneal and lenticule edema combined with epithelial defects within the first few days after AILI, these signs progressively disappeared by 1 month, and the corneas were clear by 6 months, comparable to intact eyes.⁹ This recovery course was also confirmed by in vivo confocal microscopy. Similar to autologous lenticule reimplantation following FLEx, corneal inflammatory cells and edema

could be seen at the anterior and posterior surfaces of the implanted lenticule on in vivo confocal microscopy early after surgery.^{4,5,15} These characteristics resolved over 1 month. More importantly, corneal reinnervation was noted in lenticules on postoperative month 6, which was in accordance with corneal nerve damage recovery procedure reported following refractive surgery.^{13,16}

The discrepancy of corneal thickness before and after AILI was measured as 69 ± 11 μ m at 6 months, which was roughly equal to the predicted lenticule thickness of 60 μ m. With regard to corneal morphology, anterior corneal surface was significantly steepened after AILI and the total corneal refractive power increased by 3.27 ± 1.2 D when the posterior corneal curvature was taken into account. These findings are in agreement with previous studies that have shown that corneal thickness and corneal curvature could be restored to preoperative state by autologous lenticule reimplantation following ReLEx procedure.^{4,5} In the present study, the ultimate alteration of corneal refractive power by surgery accounted for 82% of the intended correction, which was 0.73 D less than the predicted 4.0-D correction and possibly caused by corneal cap creation, wound healing reaction after surgery, and measurement errors. Similar findings have been reported in a few studies with 0.6 D less in keratometry after lenticule reimplantation, compared with preoperative values, which accounted for 90% of the intended correction (-6.0 D).^{4,5} Pradhan et al.⁶ have reported a case of +5.25 D hyperopia correction by implantation of a -10.5 D sphere lenticule (127- μ m maximum central lenticule thickness) obtained by SMILE from a myopic donor. The rate of 50% of the intended correction is thought to be induced by posterior surface changes and epithelium remodeling.⁶ In the present study, we did not observe any statistically significant changes of posterior corneal curvature at any time points after AILI, and this may be the result from much less power of RL that we used (-4.0 D, 60- μ m maximum central lenticule thickness). As described by Pradhan et al.,⁶ the lenticule reimplantation procedure is designed to restore corneal stromal volume after ReLEx, and the corneal thickness is almost the same as the preoperative one after the surgery. Different from that, there was a significant increase of the stromal thickness after allogeneic lenticule transplantation in the present study. These findings implied that postoperative corneal thickness may contribute to the prediction of postoperative refractive status. In all probability, a maximum threshold of postoperative corneal thickness could exist for keeping high efficiency of refractive correction in lenticule transplantation, beyond which its efficacy and predictability will decrease dramatically.

The suction cone lost its vacuum during surgery owing to a tendency of the monkey eye to move involuntarily even under anesthesia. For the same reason, the actual ReLEx procedure was difficult to center accurately and consistently on the pupil in monkeys. No adverse effects were observed after AILI. Slit lamp examination and in vivo confocal microscopy revealed virtually no corneal haze formation. During follow-up, we paid attention to the rate of corneal allograft rejection. However, we did not observe any sign of allogeneic transplanted lenticule rejection in all operated eyes under regular eye drop administration. In the present study, AILI was a kind of selective lamellar keratoplasty rather than a full-thickness transplant procedure; the results suggest a high rate of graft survival and rejection reversibility that were higher than in previous studies.^{17–19} It is possible that the transplanted stromal lenticule was protected by the cap above and the stromal bed below. The lenticule could then be isolated from tears and aqueous humor that contain high immune factor concentrations, which may reduce the risk of immune factor-induced graft rejection.²⁰ Thirdly, the stromal lenticule was

transplanted to an avascular recipient bed, and high-risk factors of corneal graft rejection, such as the presence of corneal neovascularization, active ocular inflammation, and ocular surface disease, were not present, which could improve graft survival.²¹⁻²³ Finally, RL implantation was performed immediately after lenticule extraction, which maintained the freshness of the implantation material and reduced the rejection risk. Taken together, these factors could have led to the high success rate observed in the present study. However, further study is still necessary to examine adequately these issues.

The functions of AILI include an increase of the corneal thickness and alteration of the corneal power, which may be useful in a number of potential clinical situations such as postrefractive corneal ectasia and presbyopia or hyperopia treatment. However, using the available technologies, this approach might be more useful than practical. Corneal ectasia has been estimated to occur in 0.004% to 0.6% of patients post LASIK.²⁴ The clinical onset of keratoectasia can occur months or even years after surgery.²⁵⁻²⁷ Minimally invasive surgical options, including intracorneal ring segment implantation, collagen cross-linking, and techniques combining collagen cross-linking with intracorneal ring, have been documented to stop the progression of ectasia.^{25,28-30} However, these surgical procedures actually cannot compensate the lost lamellae in corneal ectasia, which gives rise to thinning and weakening of the corneal stroma.³¹ The AILI method may restore patients' corneal stroma and achieve a normal corneal thickness that provides patients with a unique opportunity for performing further refractive surgery to correct residual astigmatism or refractive problems.¹⁵ Another application of the AILI could be the treatment of presbyopia and hyperopia. In the treatment of presbyopia, RL could act as a kind of biologic intrastromal inlay in the nondominant eye and reserve low myopia, thereby resulting in presbyopia monovision and reducing biocompatibility-related complications with nonbiological inlay.^{32,33} On the basis of a similar principle, obtained RL could be modified and refined to customized size and refractive power to correct hyperopia. Given the unpredictability of intended correction may increase over certain postoperative recipient corneal thickness, hyperopia correction in this scenario is supposed to be restricted to mild to moderate degrees, particularly in preoperative normal corneal thickness. Using the current ReLEx technology, we can only obtain RL with positive power for hyperopic patients. Refractive lenticule with negative power will be available in the future, and high myopic patients, particularly those with thin cornea, will benefit from AILI.

The AILI method described in the present study was similar to the surgery reported by Pradhan et al.,⁶ but their study is limited to an individual case without confocal microscopy. Correction of a hyperopic patient is also presented by Ganesh et al.⁷ with the implantation of a cryopreserved lenticule donated from myopic patients. However, most previous investigators have observed the reimplantation of cryopreserved lenticules^{4,5} or the feasibility of LASIK following lenticule reimplantation,¹⁵ which are methods that are radically different from the method described in the present study. Lenticule implantation on rabbit cornea is well described in a previous work,^{3,4,15} but the present study was the first to evaluate AILI in monkeys, based on a previous experiment from our group on autologous lenticule transplantation in rabbits.³

The results of the present study could be a basis to perform clinical trials in humans in order to investigate further the safety and efficacy of the procedure in human beings. The present study may suggest that the use of lenticules from a donor could be used to treat hyperopic or presbyopic patients with thin corneas or keratoconus, since this approach has the

potential to increase corneal stromal thickness, without having to rely on autologous transplantation. This approach could also help to respond to the shortage issues of lenticules.

The present study is not without limitations. We did not evaluate AILI's effects on cornea endothelial function and biomechanical properties, since corneas thickened postoperatively. In the present and previous studies,⁶ corneal edema only occurred within the first few days after transplantation, and corneal clarity gradually recovered without Descemet's folds. This recovery procedure indicated that there were no obviously adverse effects on endothelial function. Since corneal thickness and volume are positively associated with corneal viscosity and elasticity, we deduced that the corneal stiffness would increase postoperatively.³⁴ Secondly, this was a preliminary study and we did not test keratocyte repopulation of the lenticules with immunohistochemistry. The sample size was small, but larger numbers of animals are difficult to obtain because the use of nonhuman primates is strictly regulated. Correction of astigmatism will require the correct orientation of the lenticule, which was not performed in the present study and will be the focus of future investigations. Finally, more studies should address the potential immune responses under AILI and the establishment of algorithms between RL power and intended refractive corrections. Meticulous clinical trials will then be designed to test the clinical utility of this approach.

In conclusion, the present study may suggest that AILI could safely, reliably, and effectively increase corneal thickness and refractive power with a possible low rate of adverse effects, which may provide new avenues in the treatment of corneal ectasia, presbyopia, and hyperopia. More investigations should focus on the potential immune responses under AILI and on the establishment of algorithms between RL power and intended refractive corrections before meticulous clinical trials may be designed.

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