BMC Ophthalmology

RESEARCH

Open Access

Long-term follow-up of SMILE-derived corneal stromal lenticules preserved in nutrient capsules for treating corneal diseases

Zhe Zhang[1,2,3†], Yanze Yu[1,2,3†], Bingqing Sun[1,2,3], Meng Li[1,2,3], Peijun Yao[1,2,3], Xiaoying Wang[1,2,3], Xingtao Zhou[1,2,3*] and Jing Zhao[1,2,3*]

Abstract

Purpose To investigate the long-term safety and efficacy of using keratorefractive lenticule extraction (KLEx)-derived corneal stromal lenticules preserved in nutrient capsules for the treatment of corneal diseases.

Setting Eye and ENT Hospital of Fudan University.

Design Observational study.

Methods Ten eyes of 10 patients with corneal diseases (6 males, 4 females, age 38.10 ± 9.11 years,) were treated with phototherapeutic keratectomy combined with epikeratophakia or intrastromal keratoplasty using KLEx-derived lenticules. Corneal stromal lenticules which used for transplantation was extracted from healthy myopia patients and stored in nutrient capsules at 4 °C for 28 days. Patients underwent slit-lamp microscopy, uncorrected and corrected distance visual acuity (CDVA), subjective refraction, corneal topography, and anterior segment optical coherence tomography evaluations preoperative and were followed-up at 1-week, 1-month, 3-months, and 1-year postoperatively.

Results No complications occurred intra- or postoperatively. CDVA increased by at least two lines in seven eyes and by one line in one eye, while remaining unchanged in two eyes, by the final follow-up. There was no significant difference found in the comparison of each postoperative corneal thickness to preoperative measure. The lenticules maintained a good degree of transparency 1-year post-operatively and adhered closely to the adjacent tissue. Intraocular pressure remained stable from preoperatively to all points postoperatively. There was no significant difference in corneal endothelial cell density values between pre and all post-operative time points.

†Zhe Zhang and Yanze Yu contributed equally to this study and were considered as co-first authors.

*Correspondence:
Xingtao Zhou
doctzhouxingtao@163.com
Jing Zhao
zhaojing_med@163.com

Full list of author information is available at the end of the article

Conclusion KLEx-derived corneal stromal lenticules preserved in nutrient capsules for up to 28 days can be safely and effectively utilized as allografts for the treatment of corneal diseases, demonstrating favorable long-term outcomes.

Keywords Keratorefractive lenticule extraction, Corneal stromal lenticules, Nutrient capsule, Phototherapeutic keratectomy combined with epikeratophakia, Lenticule intrastromal keratoplasty (LIKE) surgery

Introduction

Since Barraquer performed the first stromal keratophakia in the 1960s, reuse of corneal lenticules have been a research focus [1]. The development of keratorefractive lenticule extraction (KLEx) has provided abundant corneal stromal lenticules for transplantation. Compared with previous transplant materials, corneal lenticules have the advantages of better biocompatibility, transparent, precise cutting, and easy access. Lenticules are currently mainly reused for the treatment of various eye diseases, including keratoconus, corneal dystrophy, corneal ectasia, presbyopia, and hyperopia, with feasibility and safety confirmed in many in vitro and in vivo studies [2–7].

The activity and structural integrity of corneal stromal lenticules is the key of lenticule transplantation, which has great influence on postoperative visual effect and complications. Most of the current clinical studies use fresh corneal stromal lenticules for transplantation, which limits the clinical application and promotion of lenticule transplantation.

Preservation of lenticules is the major challenges in the reuse of corneal stromal lenticules. In general, the entire clinical workflow of tissue organ donation should include qualified acquisition, preservation, and transport in order to ensure quality and success of transplantation [8]. Once the stromal lenticules are removed from the cornea, damage due to hypoxia and dehydration can disrupt the original morphology and microstructure of the corneal stroma [9]. Irregular collagen fiber structure has been reported to affect corneal transparency [10]. Therefore, maintaining the integrity and sustaining the activity of the lenticules is essential to preserve the corneal stromal lenticules. Cryopreservation technology is the most widely used tissue and organ preservation method in clinical practice, which is economical and adaptable, with low requirements for equipment [11, 12]. Most of the existing corneal preservation solution is to preserve the whole cornea, but there are few preservation methods for corneal stromal lenticules and maintaining the activity of corneal stromal cells, which need to be further studied.

In our previous study, we constructed a novel nutritional capsule that mimics the human tear film for the preservation of corneal tissue with multiple roles of physical barrier, nutrient supply and surface lubrication. It was shown that the nutrient capsule prolonged the storage time and maintained the structural integrity, transparency, and cellular activity of human corneal lenticules, inhibited the growth of microorganisms, and reduced the corneal immunogenicity [13].

In the present study, we investigated the safety and efficacy of using KLEx-derived corneal stromal lenticules that had been preserved at 4 °C in nutrient capsules for 28 days as allografts to treat corneal diseases such as granular corneal dystrophy, corneal ectasia, and band keratopathy. Our aim was to provide a safe and feasible strategy for the preservation and reuse of the extracted corneal tissues.

Patients and methods

Study population

Ten patients (mean age: 38.10 ± 9.11 years) with corneal diseases who visited at the Eye and ENT Hospital of Fudan University from June to October 2021, including six males (six eyes) and four females (four eyes), were included in this study. The inclusion criteria: patients aged ≥ 18 years, with a remaining stromal thickness after phototherapeutic keratectomy (PTK) treatment ≥ 250 μm, and with indications for lenticule transplantation which include corneal opacity or degeneration, keratoconus, corneal ectasia. The exclusion criteria: patients with severe dry eyes, retinal detachment, cataract, or other eye diseases; with active systemic inflammation, keratitis, or conjunctivitis; with autoimmune system diseases, such as systemic lupus erythematosus; with scarring diathesis, with no light perception, or who were pregnant or breastfeeding. Preoperative examinations included uncorrected distance visual acuity (UDVA), subjective refraction and corrected distance visual acuity (CDVA), corneal topography, and anterior segment optical coherence tomography (AS-OCT), as well as slit lamp microscopy. The preoperative information is shown in Table 1. The corneal lenticule donors underwent a regular KLEx procedure with blood sampled preoperatively to exclude infections.

Ethics

The study was approved by the Ethics Committee of the Eye & ENT Hospital of Fudan University (2021026) and was registered on the clinical trial website (www.http://www.chictr.org.cn/) (ChiCTR2100050623). All work was conducted in accordance with the Declaration of Helsinki. Donors and recipients were fully informed and written informed consent was obtained from all patients.

Table 1 Baseline ocular biometrics of the patients

No.	Age(y)	Gender	UDVA	Eye	Diagnosis	Refraction (D)	CDVA	Surgery
1	31	Male	4/20	OS	CD	-1.00-0.75*45	12/20	PTK-EP
2	41	Female	2/20	OD	CD	-4.75*165	6/20	PTK-EP
3	46	Female	2/20	OS	CD	-2.25	2/20	PTK-EP
4	40	Male	2/20	OS	CD	-2.00-1.00*30	6/20	PTK-EP
5	32	Male	4/20	OS	CD	+3.25-5.00*175	12/20	PTK-EP
6	48	Female	2/20	OD	CD	-4.00-1.50*30	8/20	PTK-EP
7	48	Female	2/20	OS	CD	-0.75-2.00*155	4/20	PTK-EP
8	32	Male	2/20	OS	Hyperopia after RK	+3.50–2.50*85	8/20	PTK-EP
9	20	Male	8/20	OS	Keratectasia	-2.25-4.00*100	12/20	LIKE
10	43	Male	LP	OD	Band keratopathy, aphakic	undetectable	LP	PTK-EP

UDVA: Uncorrected distance visual acuity; CDVA: Corrected distance visual acuity; D: Diopters; LP: light perception; RK: Radial Keratotomy; LP: light perception; CD: corneal dystrophy; PTK-EP: Epikeratophakia combined with transepithelial phototherapeutic keratectomy; LIKE: lenticule intrastromal keratoplasty; OD: right eye; OS: left eye

Table 2 Transplanted donor lenticules and postoperative parameters of patients

No	UDVA	Lenticular refraction (D)	Lenticule central thickness (mm)	Refraction (D)	CDVA
1	10/20	-6.25-0.50*90	110	-1.50	18/20
2	12/20	-5.75-0.50*70	120	-0.75-2.00*170	16/20
3	4/20	-7.75-0.25*60	110	-6.00	12/20
4	4/20	-7.50	120	-3.25-1.75*60	10/20
5	4/20	-5.50	120	-1.50-2.00*160	16/20
6	6/20	-6.75-0.50*110	110	-4.00-0.50*30	12/20
7	10/20	-5.75	120	-2.75-2.00*45	14/20
8	4/20	-5.35-0.50*10	120	1.00*90	8/20
9	8/20	-8.00-0.50*5	110	-1.75*115	12/20
10	1/20	-8.00-0.50*150	120	+8.00	1/20

UDVA: Uncorrected distance visual acuity; CDVA: Corrected distance visual acuity; D: Diopters

Preparation of nutrient capsules and preservation of lenticules

The lenticules used for transplantation were extracted from healthy patients with myopia. The extracted corneal lenticules were placed directly in medium DMEM/F-12 (Gibico, USA) containing 2.5% chondroitin sulfate (CS), 1% penicillin-streptomycin, 0.1% sodium hyaluronate, 1% sodium alginate, and 0.5% alginate. The corneal lenticules were then transplanted into a 100 mM $CaCl_2$ solution to form a hydrogel nutrient capsule [13]. Corneal medium containing chondroitin sulfate was added and corneal lenticules stored at 4 °C for 28 days. Prior to human corneal lenticules transplantation, the nutrient capsules were immersed in 100 mM sodium citrate solution to dissolve the capsule shell layer. The preserved corneal lenticules were released from the nutrient capsules. The capsules were soaked with 0.5% levofloxacin and 0.5% fluconazole for 5 min, respectively. Subsequently, the corneal lenticules was rinsed with saline in preparation for the next step.

Surgical method

All procedures were performed by the same surgeon at the Eye & ENT Hospital of Fudan University (Shanghai, China). Nine eyes with corneal opacity underwent phototherapeutic keratectomy combined with epikeratophakia (PTK-EP) surgery and 1 eye with keratectasia underwent the lenticule intrastromal keratoplasty (LIKE) surgery (Table 1). Corneal stromal lenticules of appropriate refraction and thickness was selected for transplantation based on the recipient's preoperative refraction (Table 2).

PTK-EP procedure. The corneal epithelium was first removed by PTK with a MEL 90 excimer laser (Carl Zeiss Meditec, Jena, Germany) with a 250-kHz repetition frequency, a pulse energy of 1.1 mJ, an optical zone set to 6.5–7 mm, and a laser ablation depth of 90–130 μm, based on the degree of corneal opacity. Then, the prepared lenticule was transplanted onto the center of the recipient' cornea. Under the microscope, the surgeon ensured that the center of the lenticule was aligned with the beam. The edge of the lenticule was carefully spread with a lenticule spatula and cotton swabs. Once the lenticule was flat, a soft contact lens was placed onto the cornea. It was removed 1–2 weeks after surgery upon complete remodeling of the corneal epithelium.

LIKE. The original corneal flap was lifted with a spatula to expose the stroma. Then, the prepared lenticule was transplanted onto the corneal stromal. Under the microscope, the surgeon ensured that the center of the

lenticule was aligned with the beam. The edge of the lenticule was carefully spread with a lenticule spatula and cotton swabs. Once the lenticule was laid flat, the corneal flap was returned to its original position. A bandage soft contact lens was placed on the cornea and was removed 1 day after surgery.

Postoperative medication

Postoperative medications were prescribed as follows: 0.5% Levofloxacin eye drops were prescribed 4 times per day for 7 days; The 0.1% fluorometholone was prescribed 8 times per day, reduced to 7 times per day after 3 days, then to 6 times per day after another 3 days, with the frequency decreasing every 3 days until discontinuation. Sodium hyaluronate eye drops, 4 times per day for 3 months. Dosing adjustments were made during follow-up visits based on the patient's specific conditions.

Postoperative examinations and follow-ups

Follow-up was conducted 1 week, 1 month, 3 months, and 1 year after surgery. UDVA, subjective refraction, and CDVA were assessed. Slit lamp microscopy was performed to assess lenticule transparency and to detect the presence or absence of displacement, curling, autolysis, epithelial implantation, etc. The corneal thickness was measured pre- and postoperatively using a corneal topography (Pentacam HR, Oculus Optikgeräte GmbH, Wetzlar, Germany) to calculate corneal refractive parameters and the changes in thickness. AS-OCT was used to evaluate the changes in lenticule location and healing response after lenticule transplantation at various postoperative time-points. Intraocular pressure (IOP) and corneal endothelial cell density (ECDs) were also measured at each time point of follow-up before and after surgery.

Statistical analysis

SPSS software (Version 23; SPSS Inc., Armonk, NY, USA) was used for data analysis. All data are presented as mean ± standard deviation. The Kolmogorov–Smirnov test was employed to assess the normality of data distribution. Paired t-tests were utilized to compare intraocular pressure (IOP), endothelial cell density (ECD), and corneal thickness between different postoperative time points and their respective preoperative value. Repeated measures analysis of variance (ANOVA) was applied to analyze IOP, ECD, and corneal thickness across the multiple postoperative time points. A Pvalue < 0.05 was considered statistically significant.

Results

Safety and efficacy

Ten patients underwent postoperative follow-ups for 1 year. No complications occurred during the postoperative follow-up period, including filamentary keratitis, diffuse lamellar keratitis, microstriae, epithelial sloughing, epithelial ingrowth, and recurrent corneal erosions. Additionally, complications related to lenticule transplantation, as well as lenticule-related complications like displacement, curling, and autolysis. The transplanted lenticules remained transparent. The postoperative visual acuity and refractive test results are listed in Table 2.

At the final follow-up, the postoperative UDVA had increased by at least two lines in four of the 10 eyes and by one line in another four eyes, as compared with the preoperative UDVA. The postoperative UDVA remained the same as the preoperative UDVA in two eyes (Fig. 1A).

At the final follow-up, the postoperative CDVA had increased by at least two lines in seven of the 10 eyes and by one line in one eye as compared with preoperative CDVA, and remained comparable to the preoperative CDVA in two eyes (Fig. 1B).

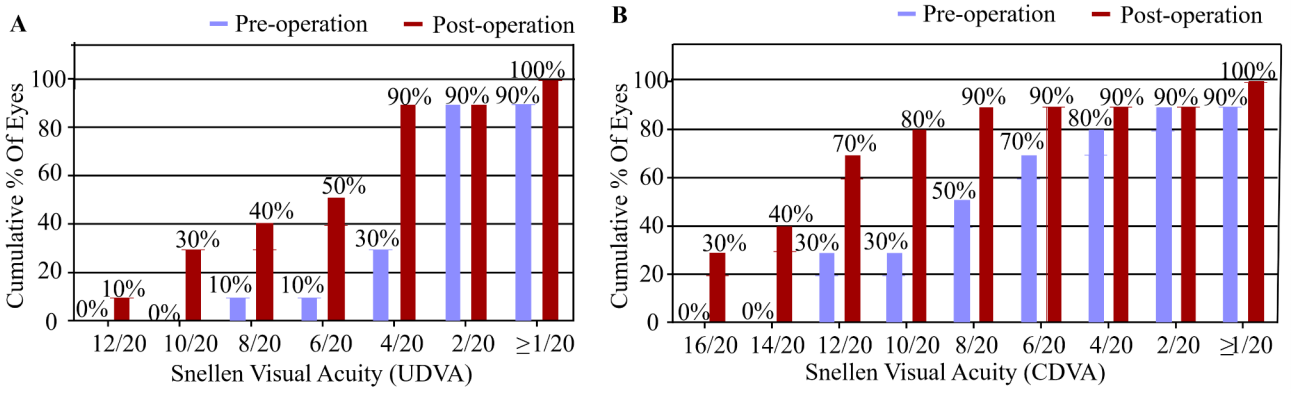

Fig. 1 Changes in subjects' visual acuity during follow-up. UDVA (**A**) and CDVA (**B**) changes during one year of follow-up

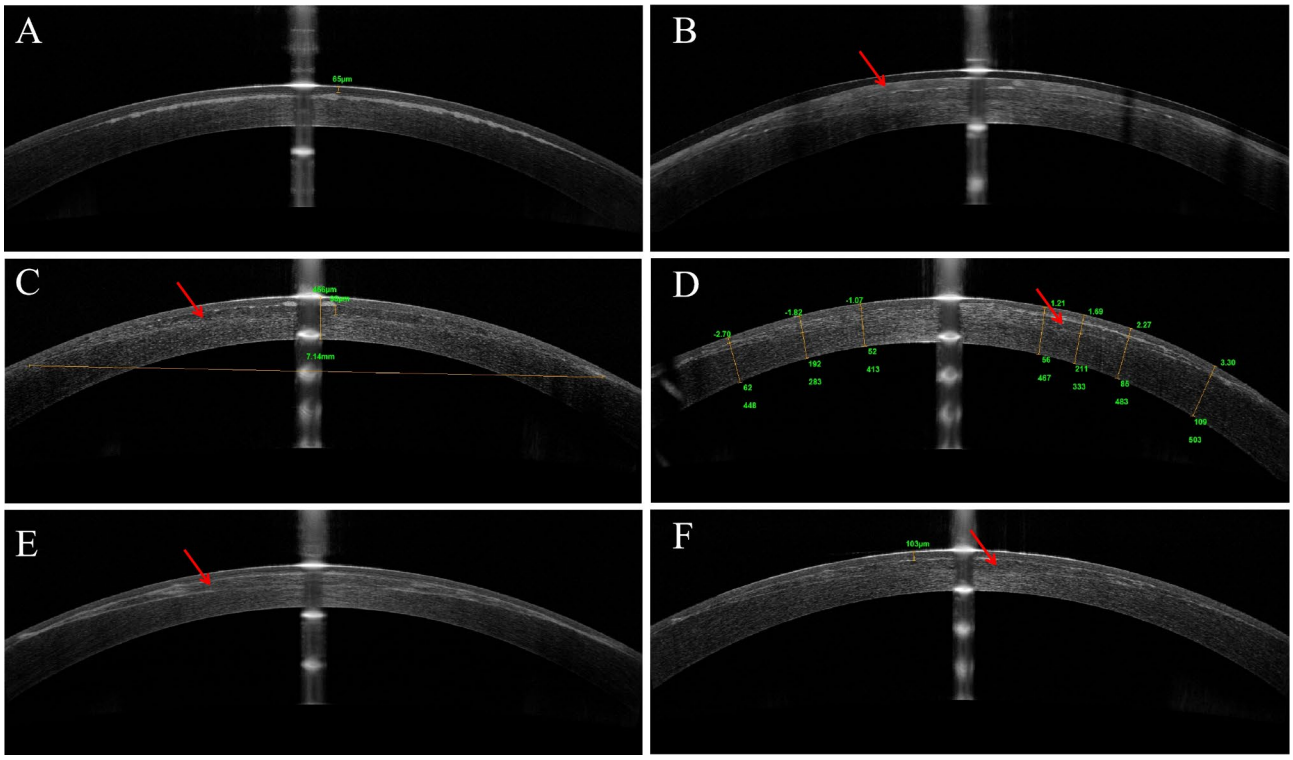

Fig. 2 Healing response after corneal lenticules transplantation. AS-OCT observation at pre-operation (**A**), 1 day (**B**), 1 week (**C**), 1 month (**D**), 3 months (**E**), and 1 year (**F**) after surgery

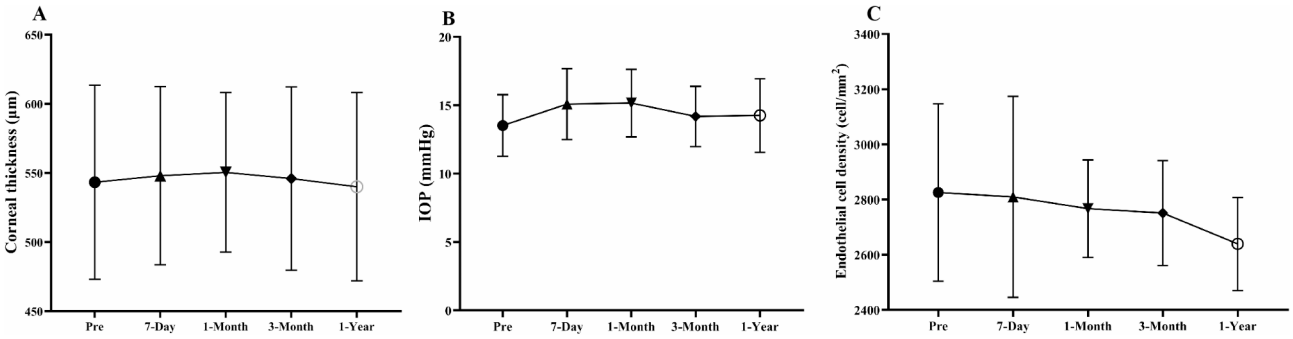

Fig. 3 Changes in subjects' corneal thickness (**A**), intraocular pressure (**B**) amd corneal endothelial cell density (**C**) during follow-up

AS-OCT observation

AS-OCT images showed that, by one day after corneal lenticule transplantation, the lenticules were smoothly positioned on the stromal. By one week post-surgery, an intact high-reflection band appeared on the anterior surface of the lenticules, signaling the completion of corneal epithelial remodeling. One month after surgery, the lenticules remained intact and clear, with no displacement, curling, or wrinkling. By one year post-surgery, the reflection of the lenticles is comparable to the surrounding stroma and epithelium, with no displacement and the edges becoming gradually indistinct, indicating tight adhesion to the adjacent tissue and blurred demarcation lines with the original corneal tissue (Fig. 2).

Changes in corneal thickness

A comparison of the corneal thicknesses of the 10 eyes before and 1 year after surgery is shown in Fig. 3A. Corneal thicknesses before surgery, as well as 1 week, 1 month, 3 months, and 1 year after surgery, were 559.9 ± 65.78 μm, 548.0 ± 64.46 μm, 550.4 ± 57.70 μm, 546.0 ± 66.32 μm, and 559.2 ± 54.69 μm, respectively. There was no significant difference found in the comparison of each postoperative corneal thickness to their corresponding preoperative measures ($P_{7d} = 0.99$; $P_{1m} = 0.99$; $P_{3m} = 0.99$; $P_{1y} = 0.57$) and there were also no significant differences among the corneal thicknesses of all postoperative time points ($P = 0.99$; $F = 0.03$).

Intraocular pressure

The mean intraocular pressure (IOP) before surgery, as well as at 1 week, 1 month, 3 months, and 1 year after surgery were 14.51 ± 2.31 mmHg, 15.09 ± 2.59 mmHg, 15.16 ± 2.47 mmHg, 14.18 ± 2.20 mmHg, and 14.26 ± 2.70 mmHg, respectively. The IOP at any postoperative time-point was not significantly different from its corresponding preoperative measurements ($P_{7d} = 0.70$; $P_{1m} = 0.66$; $P_{3m} = 0.99$; $P_{1y} = 0.98$) and no significant differences were found among the IOPs at the different postoperative time-points ($P = 0.54$; $F = 0.79$) (Fig. 3B).

Corneal endothelial cell density

The corneal endothelial cell density (ECDs) before surgery, as well as 1 week, 1 month, 3 months, and 1 year after surgery were 2671 ± 218.6 cell/mm^2, 2810 ± 364.0 cell/mm^2, 2767 ± 177.1 cell/mm^2, 2751 ± 190.0 cell/mm^2, and 2639 ± 168.5 cell/mm^2. There was no significant difference between the postoperative corneal ECD at each time-point and its corresponding preoperative ECD ($P_{7d} = 0.99$; $P_{1m} = 0.99$; $P_{3m} = 0.99$; $P_{1y} = 0.57$), and there were also no significant differences among the ECDs assessed at all postoperative time-points ($P = 0.57$; $F = 0.68$) (Fig. 3C).

Discussion

In this investigation of the safety and efficacy of using KLEx-derived corneal stromal lenticules preserved in nutrient capsules for treating corneal diseases, most of the patients have currently obtained better UDVA and CDVA after receiving the corneal lenticule transplantation. These results verified the safety and efficacy, as well as the good prospects, of using lenticules preserved in nutrient capsules as allografts.

Corneal stromal lenticules have good biocompatibility and less risk of graft rejection. The feasibility, safety, and efficacy of corneal lenticule transplantation have been verified in some animal and clinical studies, opening new avenues for the treatment of hyperopia, presbyopia, keratoconus, and corneal perforation [14–16]. Compared to synthetic corneal grafts, corneal stromal lenticules have reduced risks of corneal opacity and rejection, while also being more accessible [17–19]. As they are inherently refractive, they can be used to correct refractive errors. The lenticule can remain transparent for a long time after transplantation and appear to retard disease progression, helping to postpone or avoid further corneal transplantation, reducing surgical trauma, and easing the burden on patients and society.

The safety and efficacy of using corneal stromal lenticules, as well as the importance of constructing a corneal lenticule bank to optimize the reuse of corneal stromal lenticules, have been widely recognized. In most previous studies, corneal stromal lenticular transplants were freshly collected [14, 20–23]. Establishment of a corneal stromal lenticule bank would allow for lenticule donor screening to avoid the spread of infectious diseases, such as acquired immunodeficiency syndrome (AIDS), hepatitis B, and hepatitis C. Additionally, lenticule quality in terms of structural integrity, optical transmission, diameter, and thickness can be evaluated to achieve a better and more rational selection of lenticules for reuse. Therefore, establishing a reasonable and optimized lenticule preservation method, to maintain maximum activity, structure, and transmission, is particularly important for the construction of a corneal lenticule bank. With the increased availability of femtosecond laser-assisted surgery and the construction of a standardized corneal stromal lenticule bank, patients will be able to receive a more compatible corneal stromal lenticule for transplantation, which may improve the predictability of post-transplantation refractive power, assisting more patients with refractive error and keratoconus to gain better vision.

Based on this, developing reliable and novel lenticule preservation methods is crucial. One commonly used method for preserving donor corneal tissue clinically is Optisol, which can store corneal endothelial cells at 4 °C for up to two weeks, but it has limitations for long-term preservation [24]. To address this, decellularization of corneal stromal lenticules has been explored as a potential long-term preservation method [25]. Decellularization involves using agents like SDS or Triton X-100 to remove cellular components from the tissue, creating an acellular scaffold [25]. This process effectively reduces immunogenicity and lowers the risk of immune rejection after transplantation [26, 27]. Several studies have investigated the application of decellularized corneal tissue in clinical or animal studies. For instance, Shang et al. found that decellularization of porcine lenticules with 0.1% SDS significantly reduced immunogenicity. However, decellularization disrupts the collagen fiber structure, leading to decreased transmittance, weakened mechanical properties, and potential corneal haze after transplantation [28]. Although decellularization offers advantages such as reduced immunogenicity and prolonged preservation time, it also induces significant structural changes, including collagen disorganization and compromised optical clarity [29]. In our preliminary in vitro study [13], we investigated the effects of nutrient capsule preservation on corneal stromal cells and lenticules, focusing on their physiological activity, collagen fiber structure, and transparency. Specifically, the sulfate chondroitin in the preservation solution has a special affinity for corneal lenticules, helping to maintain their original tissue structure and transparency. Additionally, the nutritional components in the solution support the metabolic needs of the lenticules, promoting cell viability and structural integrity. The non-gelling sodium alginate and trehalose

in the solution further reduce the loss of proteoglycans in the corneal stroma, contributing to the maintenance of corneal transparency. The porous nature of the nutrient capsules also facilitates the exchange of nutrients and metabolic waste between the lenticules and the surrounding environment, which is vital for maintaining tissue health over extended periods. Moreover, the spherical structure of the capsule helps preserve the anatomical curvature of the lenticules, making it possible to differentiate the front and rear surfaces of the corneal stromal lenticules after storage. The results showed that nutrient capsules exhibited satisfactory biocompatibility with human corneal stromal cells, and the cell viability, assessed by TUNEL assay and Live/Dead staining, remained high at different time points (7 to 28 days). The corneal lenticule tissue preserved in the nutrient capsules maintained its intact structure and high transmittance, with the lenticule surface being smooth and slightly irregular after 28 days of preservation. Compared to decellularization, our nutrient capsule preservation method may better maintain tissue integrity, preventing haze and structural degradation, thus offering improved long-term preservation for clinical use. In the present study, we further translated the preliminary findings into clinical applications, and verified the safety and efficacy of reusing nutrient capsule-preserved corneal stromal lenticules for treating corneal diseases.

Previous studies have examined the long-term safety and efficacy of corneal stromal lenticule transplantation, as well as the morphological and histopathological changes in these lenticules following transplantation [15, 30]. Zhao et al., found that corneal stromal lenticule allografts fused to the recipient corneal stroma by 6-months post-transplantation with a density similar to that of the adjacent tissue. However, the boundary between the corneal stromal lenticule and the adjacent tissue could still be observed under a light and transmission electron microscope [31]. Li et al. retrospectively evaluated micromorphological changes in the corneas in five patients who underwent autologous corneal stromal lenticule transplantation, and the branching of nerve fibers into the corneal stromal lenticules could be observed by 12-months post-surgery [32]. In this study, we transplanted preserved corneal stromal lenticules to treat corneal dystrophy, corneal ectasia, and band keratopathy. Most of the opacities disappeared from the corneal central area after being treated with PTK and lenticule transplantation. Epithelial remodeling was achieved by 1-week postoperatively, with significantly improved corneal transparenc, thus, improving the patient's visual acuity. No complications, including corneal subepithelial fibrosis, corneal cloudiness, graft rejection, or corneal neovascularization, were observed throughout the 1-year follow-up period in all patients, illustrating the safety of using nutrient capsule-preserved corneal stromal lenticules for transplantation. Throughout the follow-up period, the lenticules remained clear. Consistent with the study conducted by Sun et al., there was no significant difference in the central corneal thickness at 1-day post-transplantation of autologous corneal stromal lenticules as compared to that measured at the last follow-up [15]. Removal of the opacity zone by PTK during the surgery procedure results in a thinning of the corneal thickness, but since the lenticules graft maintains the original corneal thickness, there is no significant difference in corneal thickness before and after the surgery.

In our study, both UDVA and CDVA were improved in most patients, as compared to their preoperative measurements, suggesting the efficacy of this treatment. All treated patients had refractive powers of -1.50 to -6.00 D, except for one patient with band keratopathy and aphakia who had a postoperative refractive power of + 8.00 D at the final follow-up. The postoperative refractive power was evaluated based on an overall consideration of the preoperatively determined correction needed for the lenticule donor, the preoperative refractive status of the recipient eye, and the depth of PTK ablation. It is difficult to assess the preoperative refractive power of the recipient eyes accurately, because diseases, such as corneal dystrophy and ectasia can lead to corneal cloudiness or surface irregularities. Additionally, since corneal epithelium healing and remodeling and corneal thickness changes can affect the postoperative refractive power, it is currently difficult to predict post-transplantation refractive power accurately before surgery. Strategies for predicting refractive power after corneal stromal lenticule transplantation require further exploration [33, 34]. Further studies should investigate the long-term stability of the nutrient capsule-preserved corneal stromal lenticule allografts used in this study.

Our study had some limitations. To date, the corneal stromal lenticules used have only been preserved in nutrient capsules for 28 days. The reuse of corneal stromal lenticules after long-term cryopreservation in these capsules needs to be investigated. Additionally, our study did not include patients who received fresh lenticule transplantation as controls. Due to the diversity of diseases, as well as the differences in disease severity and healing responses in patients requiring corneal lenticule transplantation, it is difficult to conduct a fully matched and controlled study of the outcomes of transplantation using preserved and fresh lenticules. Moreover, the long-term stability of the lenticule grafts used in this study needs to be further investigated.

Conclusion

KLEx-derived corneal stromal lenticules preserved in nutrient capsules at 4 °C for 28 days demonstrate excellent long-term safety and efficacy as allografts for treating corneal diseases, providing a viable and safe strategy for the long-term preservation and reuse of corneal tissue.

Abbreviations

KLEx	Keratorefractive lenticule extraction
CDVA	Uncorrected and corrected distance visual acuity
LIKE	Lenticule intrastromal keratoplasty
UDVA	Uncorrected distance visual acuity
AS-OCT	Anterior segment optical coherence tomography
PTK-EP	Phototherapeutic keratectomy combined with epikeratophakia
ECD	Corneal endothelial cell density
AIDS	Acquired immunodeficiency syndrome
IOP	Intraocular pressure

Acknowledgements

Not applicable.

Author contributions

ZZ，YZY: study design, manuscript draft and statistical analysis. ZZ，BQS，FX, PJY, ML and XYW，XTZ，ZZ: study execution and manuscript revision. ZZ，XTZ，JZ: conception of study. JZ: manuscript approval for publication.

Funding

This work was supported by National Natural Science Foundation of China (Grant No.82471116, 82401300, 82271119, 82371096); National Funded Postdoctoral Program of China (GZB20230158); China Postdoctoral Science Foundation (2024M750550); Shanghai Rising-Star Program (23QA1401000); Healthy Young Talents Project of Shanghai Municipal Health Commission (2022YQ015); Sailing Program of Shanghai Science and Technology Commission (No. 22YF1405900). The funding agencies had no role in study design, data collection and analysis, interpretation of data, or writing the manuscript.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Eye & ENT Hospital of Fudan University (2021026) and was registered on the clinical trial website (www.http://www.chictr.org.cn/) (ChiCTR2100050623). All work was conducted in accordance with the Declaration of Helsinki. Donors and recipients were fully informed and written informed consent was obtained from all patients.

Consent for publication

Not applicable.

Competing interests

The authors declare that there is no competing interest.

Author details

[1]Department of Ophthalmology and Vision Science, Eye and ENT Hospital, Fudan University, 19 Baoqing Road, Xuhui District, Shanghai, China
[2]Key Laboratory of Myopia and Related Eye Diseases, NHC, Shanghai, China; Key Laboratory of Myopia and Related Eye Diseases, Chinese Academy of Medical Sciences, Shanghai, China
[3]Shanghai Research Center of Ophthalmology and Optometry, Shanghai, China

Received: 4 November 2024 / Accepted: 30 January 2025
Published online: 11 February 2025

References

1. Riau AK, Liu YC, Yam GHF, Mehta JS. Stromal keratophakia: corneal inlay implantation. Prog Retin Eye Res. 2020;75:100780.
2. Wu F, Jin X, Xu Y, Yang Y. Treatment of corneal perforation with lenticules from small incision lenticule extraction surgery: a preliminary study of 6 patients. Cornea. 2015;34(6):658–63.
3. Damgaard IB, Ivarsen A, Hjortdal J. Biological Lenticule Implantation for correction of Hyperopia: an Ex vivo study in human corneas. J Refractive Surg (Thorofare NJ: 1995). 2018;34(4):245–52.
4. Liu YC, Teo EPW, Ang HP, Seah XY, Lwin NC, Yam GHF, Mehta JS. Biological corneal inlay for presbyopia derived from small incision lenticule extraction (SMILE). Sci Rep. 2018;8(1):1831.
5. Li M, Wei R, Yang W, Shang J, Fu D, Xia F, Choi J, Zhou X. Femtosecond Laser-assisted allogenic lenticule implantation for corneal Ectasia after LASIK: a 3-Year in vivo Confocal Microscopic Investigation. J Refractive Surg (Thorofare NJ: 1995). 2020;36(11):714–22.
6. Mastropasqua L, Nubile M, Salgari N, Mastropasqua R. Femtosecond Laser-assisted stromal Lenticule Addition Keratoplasty for the treatment of Advanced Keratoconus: a preliminary study. J Refractive Surg (Thorofare NJ: 1995). 2018;34(1):36–44.
7. Zhao J, Miao H, Han T, Shen Y, Zhao Y, Sun L, Zhou X. A pilot study of SMILE for Hyperopia: corneal morphology and surface characteristics of Concave Lenticules in Human Donor eyes. J Refractive Surg (Thorofare NJ: 1995). 2016;32(10):713–6.
8. Steichen C, Giraud S, Bon D, Barrou B, Badet L, Salame E, Kerforne T, Allain G, Roumy J, Jayle C, et al. Barriers and advances in kidney preservation. Biomed Res Int. 2018;2018:9206257.
9. Xia F, Zhao J, Fu D, Qin B, Chen Z, Zhao Y, Shen Y, Hou J, Zhou X. Comparison of the effects of Temperature and Dehydration Mode on glycerin-based approaches to SMILE-Derived Lenticule Preservation. Cornea. 2022;41(4):470–7.
10. Liang G, Wang L, Pan Z, Zhang F. Comparison of the different preservative methods for refractive Lenticules following SMILE. Curr Eye Res. 2019;44(8):832–9.
11. Maathuis MH, Leuvenink HG, Ploeg RJ. Perspectives in organ preservation. Transplantation. 2007;83(10):1289–98.
12. Yang J, Pan C, Sui X, Cai N, Zhang J, Zhu Y, Zhang L. The hypothermic preservation of mammalian cells with assembling extracellular-matrix-mimetic microparticles. J Mater Chem B. 2017;5(8):1535–41.
13. Zhao J, Zhang Z, Xia F, Li G, Saiding Q, Xiang L, Xu H, Yao P, Wang X, Cai Z, et al. Nutrient capsules maintain tear film homeostasis for human corneal lenticule transplantation. Chem Eng J. 2022;450:138078.
14. Zhao J, Zhao F, Huang J, Xu H, Chen Y, Zhou X. Two-year outcome of a patient treated with Phototherapeutic Keratectomy and Autologous SMILE Lenticule transplantation for flap-related complications following LASIK. J Refract Surg. 2018;34(4):281–5.
15. Sun L, Yao P, Li M, Shen Y, Zhao J, Zhou X. The Safety and predictability of implanting Autologous Lenticule obtained by SMILE for Hyperopia. J Refractive Surg (Thorofare NJ: 1995). 2015;31(6):374–9.
16. Li T, Zhou X, Chen Z, Zhou X. Corneal thickness Profile Changes after Femtosecond LASIK for Hyperopia. Eye Contact Lens. 2017;43(5):297–301.
17. Avadhanam VS, Liu CSJBJO. A brief review of Boston type-1 and osteo-odonto keratoprostheses. 2015;99(7):878–87.
18. Zhang Z, Niu G, San Choi J, Giegengack M, Atala A, Soker SJBM. Bioengineered multilayered human corneas from discarded human corneal tissue. 2015;10(3):035012.
19. Hu X, Wei R, Liu C, Wang Y, Yang D, Sun L, Xia F, Liu S, Li M, Zhou X. Recent advances in small incision lenticule extraction (SMILE)-derived refractive lenticule preservation and clinical reuse. Eng Regeneration. 2023;4(2):103–21.
20. Sakowska J, Glasner P, Zieliński M, Trzonkowski P, Glasner L. Corneal Allografts: Factors for and against Acceptance. Journal of immunology research. 2021;2021:5372090.
21. Shang Y, Li Y, Wang Z, Sun X, Zhang F. Risk evaluation of human corneal stromal Lenticules from SMILE for reuse. J Refract Surg. 2021;37(1):32–40.
22. Zhao Y, Zhao F, Han T, Zhao J, Zhou X. A pilot study: lenticule quality of hyperopic small incision lenticule extraction (SMILE) in rabbits. BMC Ophthalmol. 2020;20(1):158.
23. Zhao J, Sun L, Shen Y, Tian M, Yao P, Zhou X. Using Donor Lenticules obtained through SMILE for an epikeratophakia technique combined with Phototherapeutic Keratectomy. J Refract Surg. 2016;32(12):840–5.

24. Javadi MA, Rezaeian Akbarzadeh A, Chamani T, Rezaei Kanavi M. Sinasol versus Optisol-GS for cold preservation of human cornea: a prospective ex vivo and clinical study. Cell Tissue Banking. 2021;22(4):563–74.
25. Hashimoto Y, Funamoto S, Sasaki S, Honda T, Hattori S, Nam K, Kimura T, Mochizuki M, Fujisato T, Kobayashi HJB. Preparation and characterization of decellularized cornea using high-hydrostatic pressurization for corneal tissue engineering. 2010;31(14):3941–8.
26. Li Q, Wang H, Dai Z, Cao Y, Jin CJC. Preparation and biomechanical properties of an acellular porcine corneal stroma. 2017;36(11):1343–51.
27. Yoeruek E, Bayyoud T, Maurus C, Hofmann J, Spitzer MS, Bartz-Schmidt KU. Szurman PJAo: Reconstruction of corneal stroma with decellularized porcine xenografts in a rabbit model. 2012;90(3):e206–10.
28. Shang Y, Li Y, Wang Z, Sun X, Zhang FJJRS. Risk evaluation of human corneal stromal lenticules from SMILE for reuse. 2021;37(1):32–40.
29. Yu N, Chen S, Yang X, Hou X, Wan L, Huang Y, Qiu J, Li Y, Zheng H, Wei HJAB. Comparison of fresh and preserved decellularized human corneal lenticules in femtosecond laser-assisted intrastromal lamellar keratoplasty. 2022;150:154–67.
30. Williams GP, Wu B, Liu YC, Teo E, Nyein CL, Peh G, Tan DT, Mehta JS. Hyperopic refractive correction by LASIK, SMILE or lenticule reimplantation in a non-human primate model. PLoS ONE. 2018;13(3):e0194209.
31. Zhao J, Shen Y, Tian M, Sun L, Zhao Y, Zhang X, Zhou X. Corneal Lenticule Allotransplantation after Femtosecond Laser Small Incision Lenticule extraction in rabbits. Cornea. 2017;36(2):222–8.
32. Li M, Li M, Sun L, Han T, Ding L, Xiang J, Zhou X. In vivo confocal microscopic investigation of the cornea after autologous implantation of lenticules obtained through small incision lenticule extraction for treatment of hyperopia. Clin Exp Optom. 2018;101(1):38–45.
33. Paparo LG, Rapuano CJ, Raber IM, Grewal S, Cohen EJ, Laibson PR. Phototherapeutic keratectomy for Schnyder's crystalline corneal dystrophy. Cornea. 2000;19(3):343–7.
34. Sorour HM, Yee SB, Peterson NJ, Li FT, Macsai MS, Zhao XC, Yee RW. Recurrence of chromosome 10 Thiel-Behnke corneal dystrophy (CDB2) after excimer laser phototherapeutic keratectomy or penetrating keratoplasty. Cornea. 2005;24(1):45–50.

Publisher's note