An Experimental Study of Femto-Laser in Assisting Xenograft Acellular Cornea Matrix Lens Transplantation

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Source of support: This work was supported by Project of Shandong Natural Science Foundation of China (No. ZR2015HL053); Shandong medical and health science and technology development plan (No. 2015WS0383); Project of Shandong Natural Science Foundation of China (No. ZR2012HM019) and Project on the National Natural Science Foundation of China (No. 3057199)

Background: The aim of this study was to evaluate the feasibility of using a femto-laser in assisting xenograft cornea matrix lens transplantation in correcting ametropia, along with evaluating the effectiveness and predictability of this procedure.

Material/Methods: A corneal matrix pouch was prepared on the right eyes on 8 healthy New Zealand rabbits by a femto-laser that was also employed to perform small incision lenticule extraction (SMILE) on 8 bovine cornea matrix lenses (+6D). A lens was treated acellular and implanted into a right rabbit cornea matrix pouch. Surface inflammation was observed at 1, 2, 4, 8, 12, and 24 weeks after surgery. Anterior ocular segment optical coherence tomography (OCT), corneal topography, retinoscopy, and cornea endothelial cell enumeration were performed.

Results: All the surgeries were successfully performed without any complications. The hyperopia condition of the rabbit eyes transformed into myopia status at an early stage and gradually developed hyperopia. Diopter at 24 weeks after surgery was 1/3 of that before surgery. Central corneal thickness stabilized at 4 weeks after surgery. Anterior segment OCT showed a clear lens edge at early post-operative stage, and blurred edge at 24 weeks later, indicating gradual fusion with the rabbit corneal matrix.

Conclusions: Femto-laser assisted xenograft corneal matrix lens transplantation is safe and effective in correcting ametropia, with satisfactory predictability, thus providing novel choice for correcting ametropia.

MeSH Keywords: Cornea • Feasibility Studies • Transplantation, Heterologous

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/909294
VisuMax femto-laser is a near-infrared pulse laser with unique feature, including short pulse duration, high peak power, and minor heating effective region [1–5]. Small incision lenticule extraction (SMILE) is generally performed by 2 laser scans at different depths by femto-laser within the corneal matrix to generate one corneal matrix lens with specific thickness and size, which can be extracted via 2–4 mm side incision, thus correcting ocular ametropia status and treating myopia or astigmatism [6–10].

During SMILE, one piece of corneal matrix lens tissue with certain diopter degree is removed at one time, and consequent autograft/xenograft transplantation largely improves corneal ametropia surgery, thus providing new insights for treating hyperopia or conical cornea [11–14]. This study prepared xenograft (bovine) corneal matrix lenses with certain diopter degree, followed by acellular treatment and transplantation into rabbit corneal matrix pouches to observe the effect of xenograft corneal matrix lens transplant on diopter status, corneal thickness, and corneal endothelial cells after surgery, in order to evaluate the feasibility, effectiveness, and predictability of xenograft corneal matrix lens transplantation in correcting ametropia.

Material and Methods

Preparation of acellular bovine corneal matrix lens

A total of 8 fresh bovine eyeballs were treated with VisuMax femto-laser SMILE. Surgical parameters were: corneal flap diameter=7.5 mm; corneal flap thickness=120 μm; side incision locates at 270° location; arch length=5 mm; dioptere=-6.0 DS; lens diameter=7.00 mm; maximal central thickness of corneal matrix lens=126 μm. Extracted corneal lenses were treated with 0.5% sodium dodecyl sulfate (SDS) for 24 hours to generate acellular bovine corneal matrix lenses, which were sterilized by ethylene oxide.

Experimental animals

A total of 8 healthy adult New Zealand rabbits (males and females, body weight 2.5–3 kg) were purchased from Lukang Experimental Animal Center (Certificate No. SCXK-201500001) and were kept for 2 weeks. Then 24 hours before surgery, slit lamp microscopy was performed to examine any abnormality in ocular tissues. One eye from each rabbit was selected as the surgical eye.

Acellular reagent

SDS was purchased from Sigma (US).

Surgical approach and post-op medication

For anesthesia, we used 10% hydrate chloroform (3 mL/kg, intraperitoneal injection) and 0.4% Benoxil (ocular surface dropping). FS-LASIK surgery under femto-laser was performed with following parameters: corneal flap diameter=7.9 mm; corneal flap thickness=100 μm; side incision angle=70°; pedicle locations are 240° right and 305° left; pedicle angle=315°; pedicle width=21.72 mm. The pouch was prepared by iris reposition for laminar separation. Acellular bovine corneal matrix lens was implanted into the pouch and was placed in the central site. Tobramycin-dexamethasone paste was applied inside the conjunctival pouch after surgery. The surgical protocol is shown in Figure 1. One week after surgery, tobramycin-dexamethasone eye drops were applied 3 times daily, along with tobramycin-dexamethasone paste once every night.

Post-operation examinations

Ocular surface inflammation

The eye was examined for ocular surface inflammation. A slit-lamp was used to observe any congestion, edema, corneal swelling or turbidity, angiogenesis, anterior chamber inflammation, transparency or translocation of implant materials, and any detachment of implants.

Anterior segment OCT

Anterior segment optical coherence tomography (OCT) was employed to observe any reductus or translocation of implanted cornea matrix lens. Build-in measurement software quantified central thickness of cornea in triplicates.

Retinoscopy

A retinoscopy examination was performed. Anterior segment analyzing system (Oculus pentacam, Germany) was applied to plot retinoscopy at specific time points before and after surgery in triplicated measurements. Averaged k-value was obtained from multiple measurements.

Retinoscopy optometry

A retinoscopy optometry examination was performed. Mydriasis was performed using tropicamide eye drops before and 2, 4, 8, 12, and 24 weeks after surgery. Rabbit eyes were examined under optometry by the same clinicians.

Enumeration of corneal endothelial cells

Corneal endothelial cell examination was performed. A cell counter for retinal endothelial cells (TOPCON, Japan) was used.
to measure density of corneal endothelial cells, and percentage of hexagonal cells, for statistical analysis.

**Plasma CD4 and CD8 assay**

A plasma CD4 and CD8 assay was performed 2 weeks after surgery, using 2 mL venous blood samples collected from rabbit ear vessels. Anti-coagulated blood samples were centrifuged to separate plasma. Dual-antibody sandwich approach was employed to quantify protein expression level. In brief, micro-well plate with purified antibody pre-coating was prepared and serum proteins added, followed by binding with HRP-labelled antibody to form antibody-antigen-enzyme labelled antibody complex. After complete washing, TMB substrate was added. Under HRP enzymatic catalyze, TMB was transformed to produce blue precipitations, and later, under acid treatment a final yellow color. The intensity of color is positively correlated with sample protein concentration. Optical density (OD) value at 450 nm was measured under a microplate reader. Serum protein concentration was calculated based on standard curves.

**Corneal morphometry**

Rabbits were scarified 24 weeks after surgery using air thrombosis. The corneas were extracted and fixed in 10% formalin, embedded in paraffin, and stained in hematoxylin and eosin (H&E) staining method. Pathology and morphometry were observed under a light field microscope. Representative fields were captured.

**Results**

**General conditions of post-operative ocular tissues**

All rabbit eye transplants showed no dissolving or necrosis during the observation window, without any reductus or translocation of lenses. At the first day after surgery, surgical eye retinas showed edema, becoming transparent at 2 weeks after surgery. Until 24 weeks post-operation, no corneal inflammation, turbidity, or angiogenesis was observed (Figure 2).

**Anterior segment OCT**

Two weeks after surgery, corneas presented with edema and obvious anterior/posterior lens edge. Four weeks after surgery, corneal edema disappeared, and showed blurred lens edge at 24 weeks post-operation (Figure 3).

**Thickness of central cornea**

Central corneal thickness was increased by 186.87 μm at 2 weeks post-operation compared to before surgery, with a statistically significant difference (P<0.001). The thickness of the central cornea stabilized at 4 weeks after surgery. One-way analysis of variance (ANOVA) indicated that all data fitted equal variance hypothesis (F=2.501, P=0.08 >0.05). No statistical significance was found at 4, 8, 12, and 24 weeks after surgery (Table 1).

**Retinoscopy results**

All rabbit eyes showed hyperopia before surgery and reached expected diopter at 2 weeks after surgery. Diopter degree...
then drifted towards hyperopia and stabilized at 8 weeks after surgery. At 24 weeks after surgery, the diopter was only 1/3 of that before surgery. One-way ANOVA showed equal variance among all dataset (F=2.501, P=0.08 >0.05). Therefore all 8 groups showed no statistically significant differences at 8, 12, and 24 weeks after surgery (Table 2).
Corneal endothelial cell density

As shown in Table 3, one-way ANOVA showed all data fitted equal variance hypothesis (F=1.559, P=0.193 >0.05), indicating no statistically significant differences among all data points before or after surgery.
Corneal topography

Due to larger pupil size and worse gazing performance of rabbit eyes, corneal topography showed relatively larger variations, making the statistical analysis more difficult (Figure 4).

Plasma CD4 and CD8 assay results

Relative expression levels of plasms CD4 and CD8 were compared at various time points between experimental and control groups using independent samples t-test. No statistical significant difference was found among the 2 groups (Figure 5).

Histopathology examination

At 24 weeks after surgery, the intact corneal structure was observed, with fusion between the acellular corneal matrix lens and peripheral matrix, and no significant cleft or infiltration of inflammatory cells. Few matrix cells invade into the corneal matrix lens (Figure 6).

Discussion

Currently, the major surgical approach to correct ametropia is a “resection” plan, including the resection of the cornea to change its curvature. This method, however, makes the cornea thinner, leading to potential risk of cornea dilation or...
conical cornea after surgery [15,16]. This study thus evaluated the possibility of femto-laser assisted xenograft cornea matrix lens transplantation, which would revolutionize astigmatism surgery. Previous studies have focused on autograft or allograft transplantation. Sun et al. performed femto-laser assisted lens extraction surgery to implant lenses from myopia eyes of patients through transplantation into the pouches of hyperopia eyes [17], and reported short-term safety and effectiveness, with satisfactory predictability.

Zhang et al. performed autograft lens transplantation by the SMILE approach and showed satisfactory bio-compatibility [18]. However, there are limited sources for human corneal lenses for transplants and only a few cases meet the criteria for autograft transplants. Therefore, xenograft transplantation may have a more promising future. Bovine corneas are in sufficient supply and can be used to prepare corneal matrix lenses with specific diopter, and can be used to correct severe myopia, hyperopia, or presbyopia after acellular treatment. Zhang et al. utilized acellular bovine corneal matrix by histo-engineering (Ainier Corneal Engineering Corp, China) for laminar corneal transplantation, and prepared bovine lenses with acellular treatment and consequent transplantation into rabbit eyes. Results showed safety and effectiveness with good biocompatibility with acellular corneal lens transplants [20,21].

This study further observed the effect on diopter, corneal thickness, and endothelial layer after transplantation of bovine lenses. Within days of surgery, cornea tissues showed edema, with transparency occurring at 2 weeks after surgery, which was consistent with results from rabbit autograft lens transplants or monkey xenograft lens transplants. Implanted lenses showed satisfactory bio-compatibility, probably due to the lack of blood vessels or lymph tubes in the corneas that help reduce immune system rejection after cornea transplantation. Moreover, the donor corneal matrix lens used in our experiment underwent acellular treatment to remove cell and antigen components. Rejection of transplanted cornea is mainly due to late onset hypersensitive response induced mainly by T lymphocytes [22]. Host CD4 and CD8 positive cells have been directly correlated with rejection of transplantation [23]. In our study, plasma CD4 and CD8 levels were measured at various time points in the experimental group and the control group, and we found no statistically significant differences between the 2 groups, and thus no rejection after lens implantation.

Anterior ocular segment OCT and corneal thickness showed remodeling of the cornea after lens transplantation. Corneal endothelial cell density assay showed no major effect on corneal endothelial layer of the xenograft matrix lens transplantation. Histopathology results showed fusion between corneal matrix lens and peripheral corneal matrix of recipient, without inflammatory cell infiltration. The traumatic-healing response of corneal matrix lens extraction and pouch preparation was minor due to small incision and no suture lines.

Our retinoscopy, however, did not obtain expected results. All rabbit eyes showed hyperopia status before surgery and reached expected diopter at 2 weeks after surgery. The diopter then drifted towards hyperopia and stabilized at 8 weeks after surgery. At 24 weeks after surgery, the diopter degree only reached 1/3 of that before surgery. Diopter is determined not only by thickness of implanted lens, but also includes changes in corneal curvature at both front and back side, as well as corneal epithelial remodeling. Other possible reasons included worse gazing of rabbit eyes and consequent off-axis of lens. A previous study also showed difficulty in determining central point during lens extraction, mainly due to the rotation away from the center of rabbit eyes after anesthesia [24]. Such circumstances, however, may not occur in patients with satisfactory gazing performance. Pradhan et al. extracted corneal matrix lenses from severe myopia patient eyes and then implanted the lenses into the corneal matrix pouches of hyperopia patients [25]. The corneas maintained transparent after surgery, but only reached 1/2 of the expected correction of diopter degree. Therefore, surgical plan of lens implantation to correct hyperopia should include the effect of corneal

Figure 6. Corneal tissue structures at 24 weeks after surgery. (A–C) show different angles (hemoxyn and eosin staining, 400×).
reshaping on the dioptric degree, rather than the simple summation of dioptric degree. In considering both physical and biological effects, larger-sample experimental datasets are required to elucidate a related formula, indicating infeasibility of promoting this transplantation approach into clinical practice.

Conclusions

Femto-laser assisted xenograft acellular corneal matrix lens transplantation has satisfactory safety and predictability. The SMILE approach can be used to prepare bovine corneal matrix lenses with specific dioptric degree and shape. After acellular treatment, femto-laser can be applied to prepare the recipient corneal matrix pouch. Such xenograft corneal matrix lens transplants can be used to correct myopia and hyperopia, revolutionizing the ametropia surgery from “subtraction” into “addition”, and also provide novel insights for treating corneal dilation and conical cornea.

Conflict of interest

None.

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