Effects Of SMILE-Derived Decellularized Lenticule As An Adhesion Barrier In A Rabbit Model of Glaucoma Filtration Surgery

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Research Article

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Abstract

Background: To investigate the effects of small incision lenticule extraction (SMILE)-derived decellularized lenticule on intraocular pressure (IOP) and conjunctival scarring in a rabbit model of glaucoma filtration surgery.

Methods: Trabeculectomy was performed on both eyes of New Zealand rabbits. Decellularized lenticule was placed in the subconjunctival space in one eye of the rabbits (decellularized lenticule group), and no adjunctive treatment was performed in the fellow eye (control group). The filtering bleb features and IOP were evaluated 0, 3, 7, 14, 21, and 28 days after surgery, and histopathologic examination was performed 28 days after surgery.

Results: Decellularized lenticule significantly increased bleb survival and decreased IOP postoperatively in the rabbit model with no adverse side effects. Histopathologic results showed a larger subconjunctival space and less subconjunctival fibrosis in the decellularized lenticule group.

Conclusions: Decellularized lenticule can prevent postoperative conjunctiva-sclera adhesion and fibrosis, and it may represent a novel antifibrotic agent for trabeculectomy.

Background

Glaucoma is one of the leading causes of irreversible blindness worldwide.

Lowering of intraocular pressure (IOP) remains the only proven treatment to slow the progression of glaucoma[1]. Trabeculectomy, in which a drainage bypass is created to allow excess aqueous humour to drain into a conjunctival filtering bleb, is one of the most effective glaucoma filtration surgeries in reducing IOP[2]. However, filtration bleb dysfunction often occurs due to excessive scar tissue formation at the surgical site[3]. To reduce excessive scar formation, antimetabolic agents such as mitomycin C (MMC) and 5-fluorouracil (5-FU) are often used during trabeculectomy surgery and have been shown to improve the surgical outcome[4, 5]. However, these antimetabolic agents may lead to serious postoperative complications, such as persistent postoperative hypotony, corneal toxicity, filtering bleb leakage, blebitis, and endophthalmitis[4, 5]. Thus, a more physiological approach to suppressing subconjunctival fibrosis is needed.

Several investigations have been conducted of the prevention of bleb adhesion and fibrosis using physical barriers that are placed in the subconjunctival space or underneath the scleral flap. These include hyaluronate hydrogel, biodegradable polymers, or and nonbiodegradable polymers[5–7]. Recently, small incision lenticule extraction (SMILE) has proved to be a safe, efficient, and predictable corneal refractive surgery[8, 9]. With increasing patients undergoing SMILE, the extracted lenticules could be used for various purposes in the ophthalmic field, such as hyperopia correction, keratoconus treatment, and management of corneal perforation[10–12]. Decellularized lenticules are thin and transparent and also

exhibit good biocompatibility *in vivo*[13]. Therefore, we suggest that decellularized lenticule will act as a physical adhesion barrier during trabeculectomy surgery.

The aim of this study was to evaluate the efficacy of SMILE-derived decellularized lenticule in reducing adhesions between the conjunctiva and sclera, and keeping filtering bleb active after trabeculectomy in rabbit eyes.

Materials And Methods

The use of SMILE-derived lenticules was approved by the Ethics Committee of the Second Affiliated Hospital, Zhejiang University School of Medicine, and the procedures used conformed to the tenets of the Declaration of Helsinki. Male New Zealand white rabbits (weighing 2-2.5 kg, aging 3-4 months) were supplied by the Academy of Medical Sciences of Zhejiang province. All animal experiments were approved by the Animal Ethics Committee of the Second Affiliated Hospital, School of Medicine, Zhejiang University and were in accordance with the Association for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in ophthalmic and vision research. Study was carried out in compliance with the ARRIVE guidelines.

Decellularization of SMILE-derived lenticule

SMILE-derived lenticule were collected during refractive surgery using the VisuMax femtosecond laser system (Carl Zeiss Meditec AG, Jena, Germany) as our previous study[13]. Lenticules with a diameter of 6.6mm and a central thickness of 50 μm were selected for the following procedure. The fresh lenticules were decellularized using sodium chloride (NaCl) and nucleases as our previous study (Fig. 1A)[13].

Surgical Procedure

As different rabbits had significantly different baseline IOP and wound-healing reactions, surgery was performed on both eyes of the rabbits. After creating the scleral flap, the eye was randomly assigned to the decellularized lenticule group or control group. Twelve eyes of 6 rabbits were used in this study. The rabbits were anesthetized with an auricular vein injection of sodium pentobarbital (30 mg/kg), and topical anaesthesia using 0.4% oxybuprocaine hydrochloride eye drops was administered before surgery. Trabeculectomy was then performed with previously reported methods by an experienced glaucoma specialist (J.F.Y) with few modifications[14]. Briefly, a fornix-based flap of conjunctiva was carefully dissected and a 3 × 3 mm partial thickness scleral flap was separated. After a 1 × 2 mm trabecular tissue was removed, peripheral iridectomy was performed. The scleral flap was not sutured, but the conjunctiva was closed with a 10-0 nylon suture. In the decellularized lenticule group, the decellularized lenticule was loosely secured by suturing on the sclera with 10-0 nylon (Fig. 1B). Only trabeculectomy was conducted on the control group, and no decellularized lenticule was placed.

Clinical evaluation

After topical anaesthesia, IOP was measured by Tono-pen (Reichert, Depew, NY, USA) at baseline and 3, 7, 14, 21, and 28 days after surgery. An average of three measurements taken from each eye was recorded. Bleb appearance was examined via a slit lamp and was graded as previously described at 3, 7, 14, 21, and 28 days after surgery.

Histological analysis and immunohistochemistry

Rabbits were euthanized 28 days after surgery by an overdose intravenous injection of sodium pentobarbital. Eyeballs were enucleated and fixed in 4% paraformaldehyde solution overnight. Then the eyeballs were dissected at the equator and embedded in paraffin. Four-micrometre-thick serial sections were cut through the centre of the operation site, and stained with hematoxylin and eosin (H&E) for general histologic examination. Masson trichrome staining was performed to evaluate scar tissue formation. To examine the myofibroblast adjacent to the surgical site, we immunohistochemically measured the expressions of α-smooth muscle actin (α-SMA).

Statistical analysis

Each measurement was expressed as the mean ± standard deviation (SD). The Mann-Whitney *U* test and an unpaired *t* test were used to compare Bleb score and IOP between the 2 groups. A *P* value less than 0.05 was considered statistically significant. All analyses were performed using Statistical Package for the Social Sciences software (version 22.0, International Business Machines Corp.)

Results

Slit-lamp examination and bleb appearance

Slit-lamp examination revealed no severe postoperative inflammation in the anterior chamber, and no bleb leakage, blebitis, or endophthalmitis was observed during the postoperative period in both groups. Bleb morphology was scored based on its appearance and size as previously described at 3, 7, 14, 21, and 28 days after surgery[15]. Filtering blebs were maintained over the scleral flap in the decellularized lenticule group for at least 28 days in the decellularized lenticule group, whereas the filtering bleb collapsed within 14 days after surgery in the control group. Bleb scores were significantly higher in the decellularized lenticule group than those in the control group on day 3 and from day 14 to day 28 ($P<0.05$, Fig. 2).

Postoperative IOP changes

There was no statistically significant difference of the initial IOP between the decellularized lenticule group and the control group (Fig. 3). The IOP was reduced 3 days after surgery in both groups, and it did not differ significantly between two groups within 7 days after surgery (Fig. 3). However, IOP began to increase again in the control group 7 days after surgery, and the IOP of the decellularized lenticule group was significantly lower than that of the control group from day 14 to day 28 ($P<0.05$, Fig. 3).

Histopathologic features

Histopathologic examination was performed 28 days after surgery to evaluate the effects of decellularized lenticule on bleb scarring. H&E staining revealed that filtering space between the conjunctiva and lenticule remained prominent in the decellularized lenticule group while no filtering space was observed in the control group; however, massive scarring was observed in the control group (Fig. 4A and 4B). No evidence of obvious inflammatory change or tissue damage was observed in either group (Fig. 5B). To assess the degree of subconjunctival fibrotic response, we performed immunohistochemical staining for α-SMA (a marker of myofibroblasts). Many cells with intensive α-SMA expression were observed in the subconjunctival area in the control group, which indicated severe fibrosis (Fig. 5A). However, the bleb fibrosis was significantly attenuated in the decellularized lenticule group (Fig. 5B). Consistent with α-SMA expression, Masson's trichrome staining demonstrated significant collagen deposition in the subconjunctival region of the control group (Fig. 6A). In contrast, there was less collagen deposition in the decellularized lenticule group (Fig. 6B).

Discussion

The present study demonstrates for the first time that the use of decellularized lenticule for trabeculectomy in rabbits keeps the filtering bleb active and maintains IOP reduction by inhibiting the formation of subconjunctival fibrosis.

Antimetabolites such as MMC and 5-FU are commonly used during trabeculectomy to inhibit subconjunctival fibrosis[4, 5]. However, the usage of these antimetabolites has been associated with higher risk for wound healing disorders and severe infections due to their non-selectivity[4, 5]. In recent years, adhesion barriers between the conjunctiva and sclera have been investigated as alternative methods for preventing bleb adhesion and fibrosis, including PDMAA polymer, expanded polytetrafluoroethylene (Gore-Tex) membrane, seprafilm, biodegradable collagen, and honeycomb-patterned film[5–7, 14]. Although some of these adhesion barriers have proven effective in reducing bleb adhesion and fibrosis in animal models, clinical trials showed inconsistent results concerning the surgical outcome[5].

SMILE has become clinically available as an alternative to laser in situ keratomileusis since 2011[16, 17]. The extracted lenticule is the immediate by-product of this procedure and is typically discarded after surgery. The increasing popularity of this surgery has made it easier to obtain SMILE-derived lenticules. The decellularized lenticule is a thin stromal layer with low immunogenicity and good biocompatibility, which makes it an excellent candidate for corneal stromal regeneration[18, 19]. We have previously shown that decellularized lenticule could safely and effectively repair damage to the anterior cornea in rabbits[13]. Recently, Gu *et al* also reported that subretinally transplanted decellularized lenticule exhibited excellent biocompatibility without obvious adverse reactions and fibrosis[20]. Therefore, decellularized lenticule might be a useful biomaterial in various types of ophthalmic surgery.

The present study reveals that decellularized lenticule promotes IOP reduction and prolongs bleb survival in trabeculectomy in rabbits with no complications. A strategy to reduce scar formation following glaucoma filtration surgery is to reduce the adhesion of the tenon fibroblasts to the underlying sclera at the surgical site[21]. Our *in vivo* studies suggest that decellularized lenticule has a space-keeping effect that prevents adhesion between the tenon fibroblasts and sclera (Fig. 1C). To fix the decellularized lenticule precisely in the desired area, it was loosely sutured onto the sclera. However, we speculate that there is a passage between the sclera and the decellularized lenticule to divert aqueous humour from the anterior chamber to the subconjunctival space, given that the IOP reduction and bleb formation observed in the postoperative period (Fig. 1C).

Myofibroblast accumulation and excessive collagen deposition in the subconjunctiva are major causes of bleb failure[22]. Histopathologic examination showed that myofibroblasts infiltrated the subconjunctival area with compact collagen deposition in the control group; however, there were fewer myofibroblasts and less collagen deposition in the subconjunctiva in the decellularized lenticule. These findings indicate that decellularized lenticule may effectively inhibit excessive scar formation in glaucoma filtering surgery and improve the surgical outcome.

Conclusions

Although further studies with larger sample sizes and longer follow-ups are warranted to clarify the safety and efficacy of decellularized lenticule in glaucoma filtering surgery, our study provides a novel separating agent to prevent subconjunctival fibrosis after trabeculectomy and increase the success rate of glaucoma filtering surgery.

Abbreviations

5-FU: 5-fluorouracil; α-SMA: α-smooth muscle actin; ARVO: Association for Research in Vision and Ophthalmology; H&E: hematoxylin and eosin; IOP: intraocular pressure; MMC: mitomycin C; NaCl: sodium chloride; SD: standard deviation; SMILE: small incision lenticule extraction.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of the Second Affiliated Hospital, Zhejiang University School of Medicine, and the research followed the tenets of the Declaration of Helsinki. Written consent was obtained from all patients and controls. All animal experiments were approved by the Animal Ethics Committee of the Second Affiliated Hospital, School of Medicine, Zhejiang University and were in accordance with the Association for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in ophthalmic and vision research.

Consent for publication

Not Applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Participated in study design: HF Yin, XY Chen and YB Yang. Conduct of the study: HF Yin, XG Hong, F Wu, T Wan, YW Sang, QL Fu, W Wu, and JF Yin. Performed data analysis: XY Chen, ZW Qin, and DN Lyu. Wrote or contributed to the writing of the manuscript: HF Yin, XY Chen, J Ma and YB Yang. All authors have read and approved the final manuscript.

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Not Applicable.

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Figures

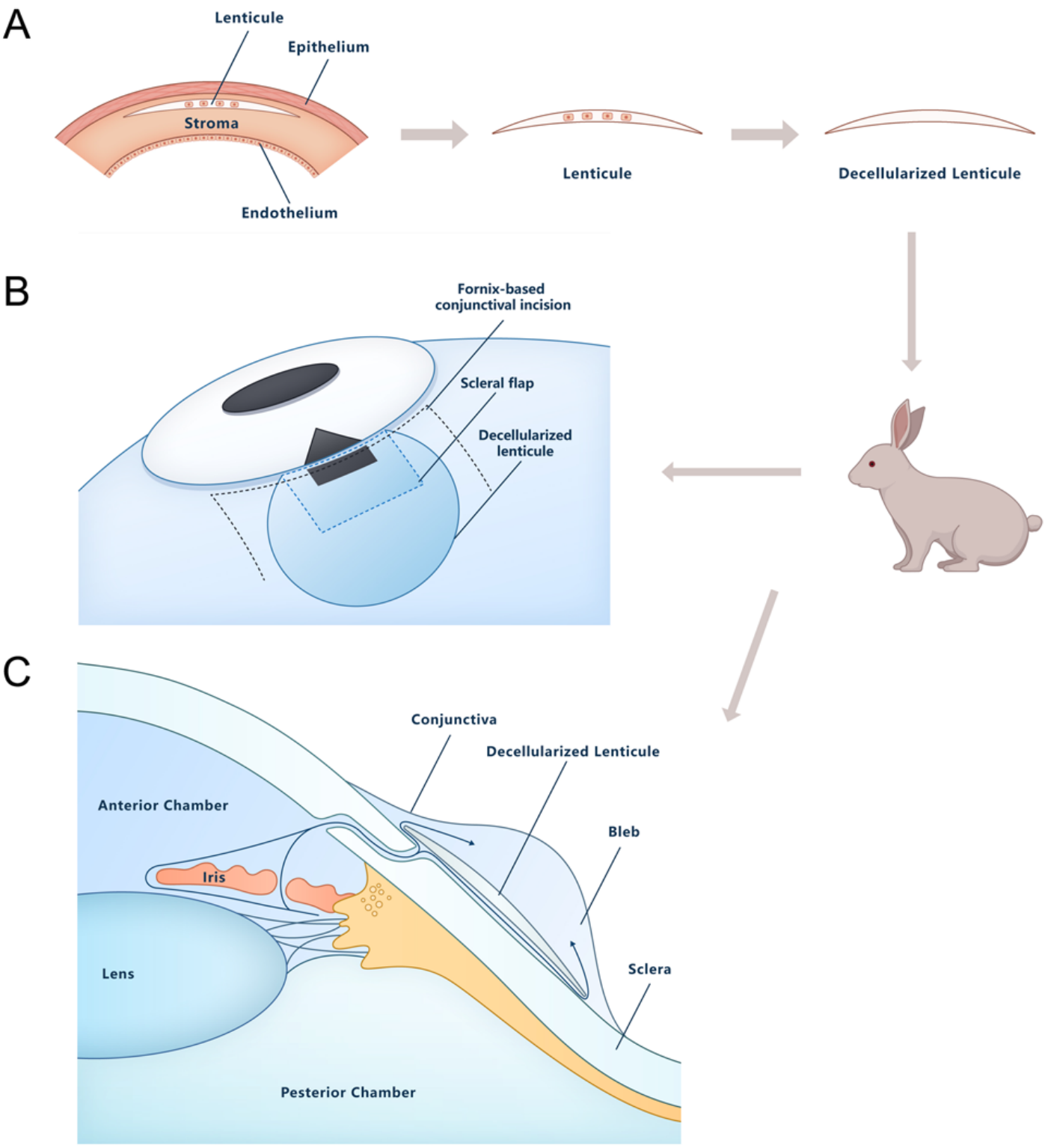

Figure 1

The schematic illustration of decellularized lenticule for the prevention of subconjunctival fibrosis after trabeculectomy. (A) Decellularization of SMILE-derived lenticule. (B) Schematic diagram to illustrate the surgical procedures used to place the decellularized lenticule. (C) Schematic depiction of the concept to prevent subconjunctival fibrosis after trabeculectomy.

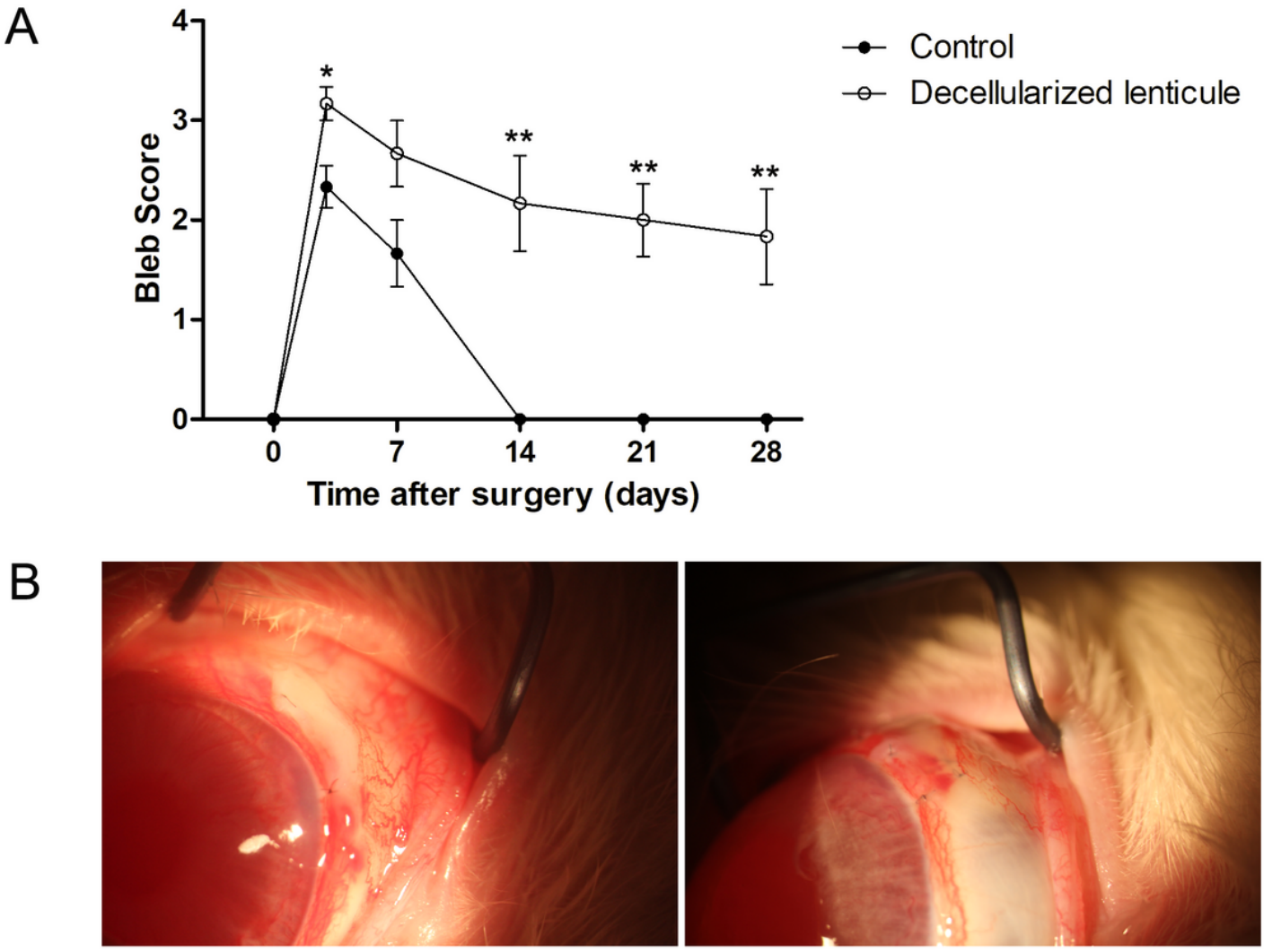

Figure 2

Bleb scoring via slit-lamp examination. (A) Bleb score changes in the control group and the decellularized lenticule group. *P < 0.05, **P < 0.01 versus the control group. (B) Representative photographs of blebs in the control group and the decellularized lenticule group 7 days after surgery.

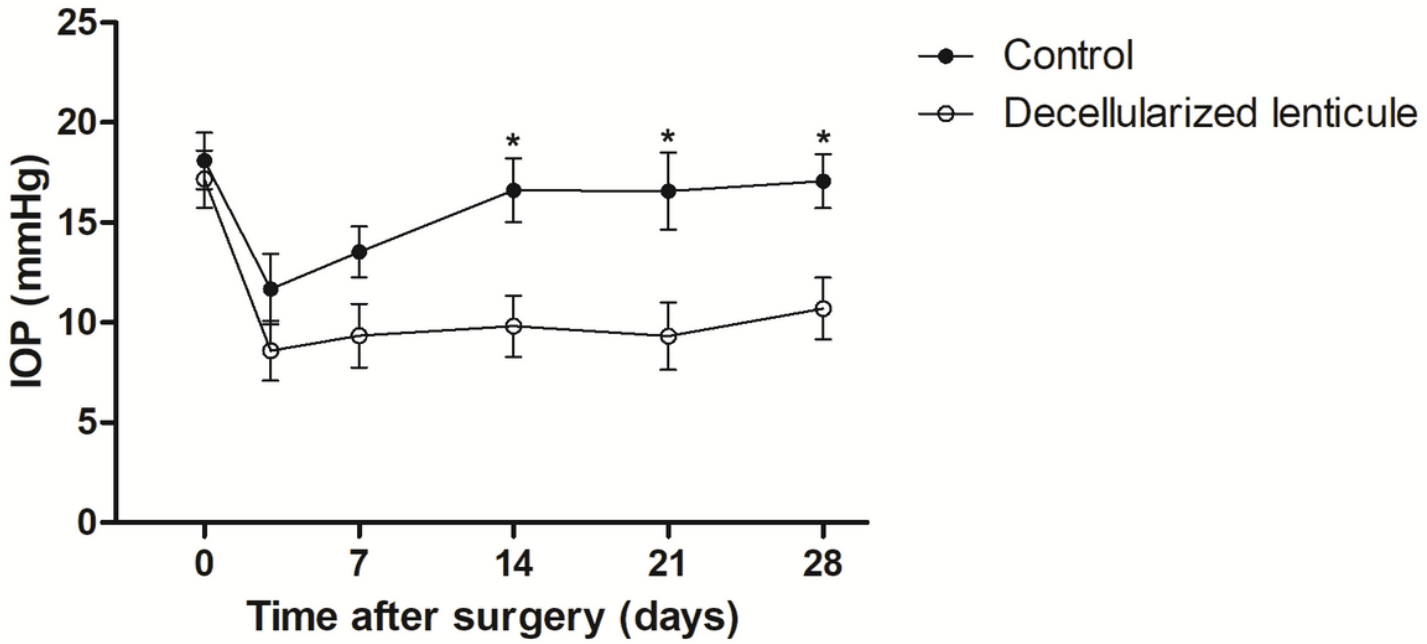

Figure 3

IOP changes in the control group and the decellularized lenticule group. *P < 0.05 versus the control group.

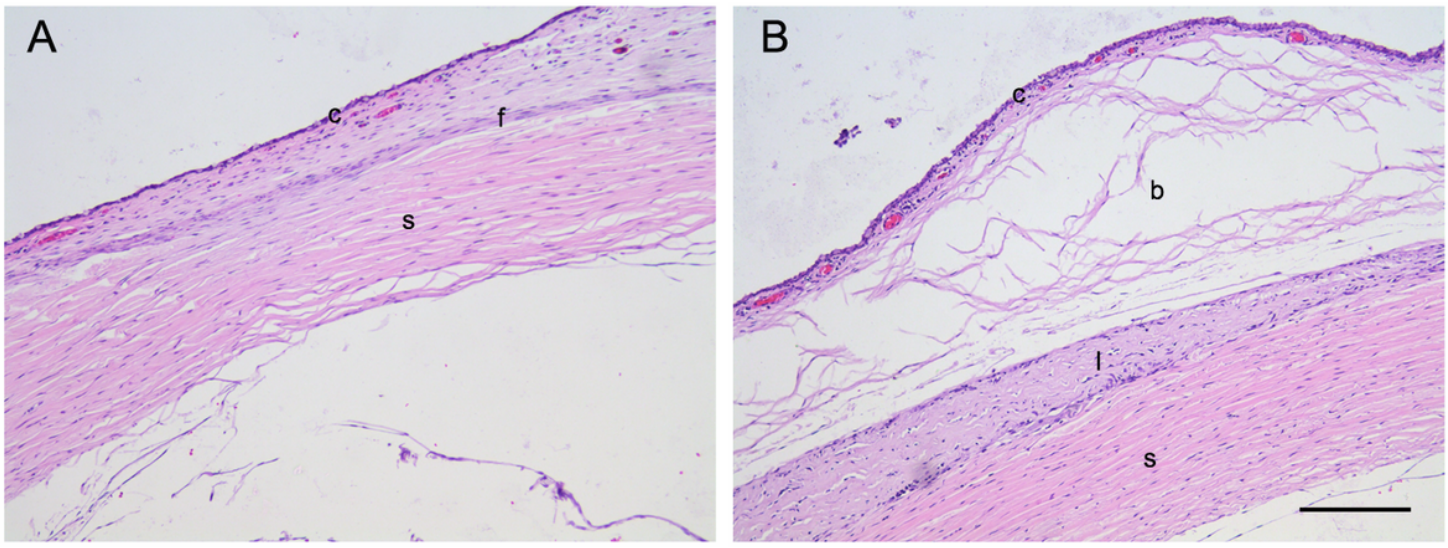

Figure 4

Histologic characteristics of the filtration site stained with H&E 28 days after surgery. (A)The control group. (B) The decellularized lenticule group. Prominent filtering space was observed in the decellularized lenticule group; however, filtering space disappeared and scar tissues significantly deposited in the

subconjunctival area in the control group. c, conjunctiva; b, subconjunctival space; f, scar tissues; l: decellularized lenticule; s, sclera. Scale bar: 100 μm.

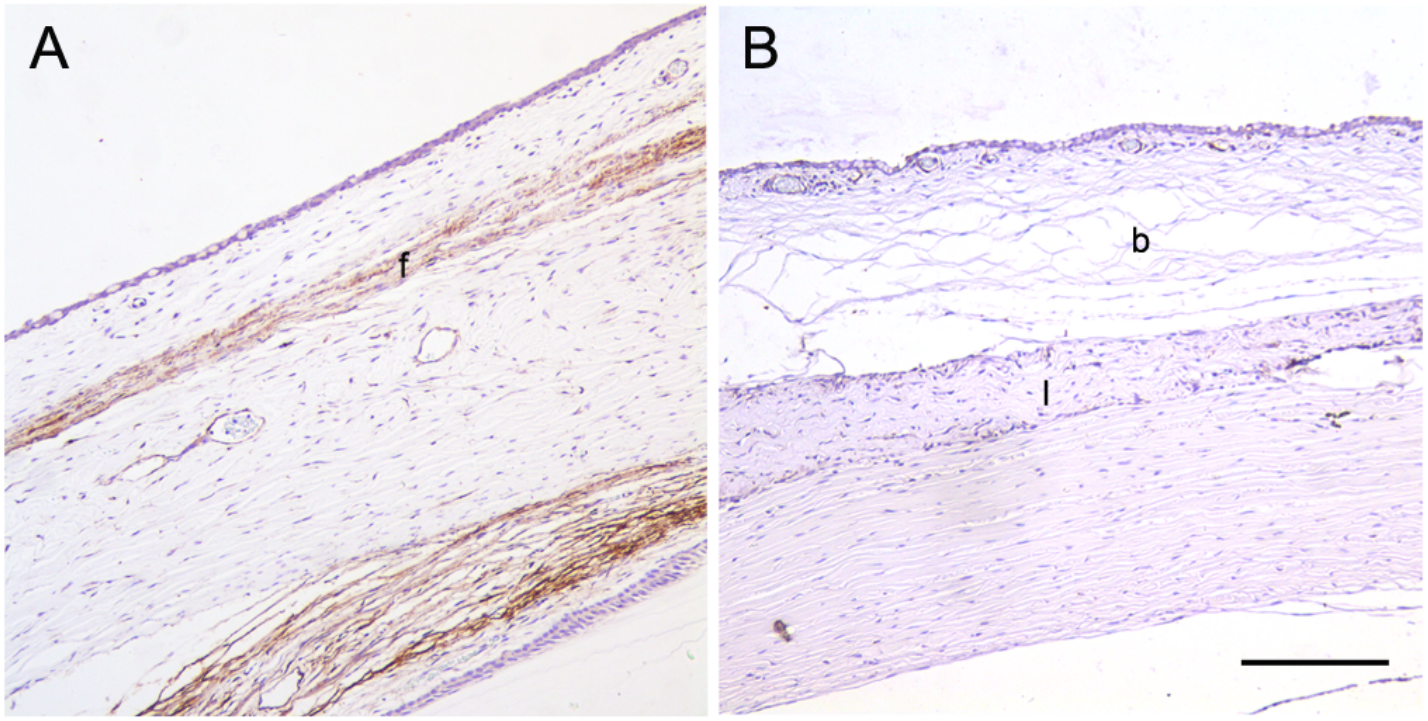

Figure 5

Immunohistochemical staining for α-SMA in the filtration site 28 days after surgery. (A)The control group. (B) The decellularized lenticule group. The subconjunctival area showed increased expression of α-SMA in the control group. In contrast, only a few positive-staining cells were observed in the decellularized lenticule group. b, subconjunctival space; f, α-SMA positive -staining cells; l: decellularized lenticule; s, sclera. Scale bar: 100 μm.

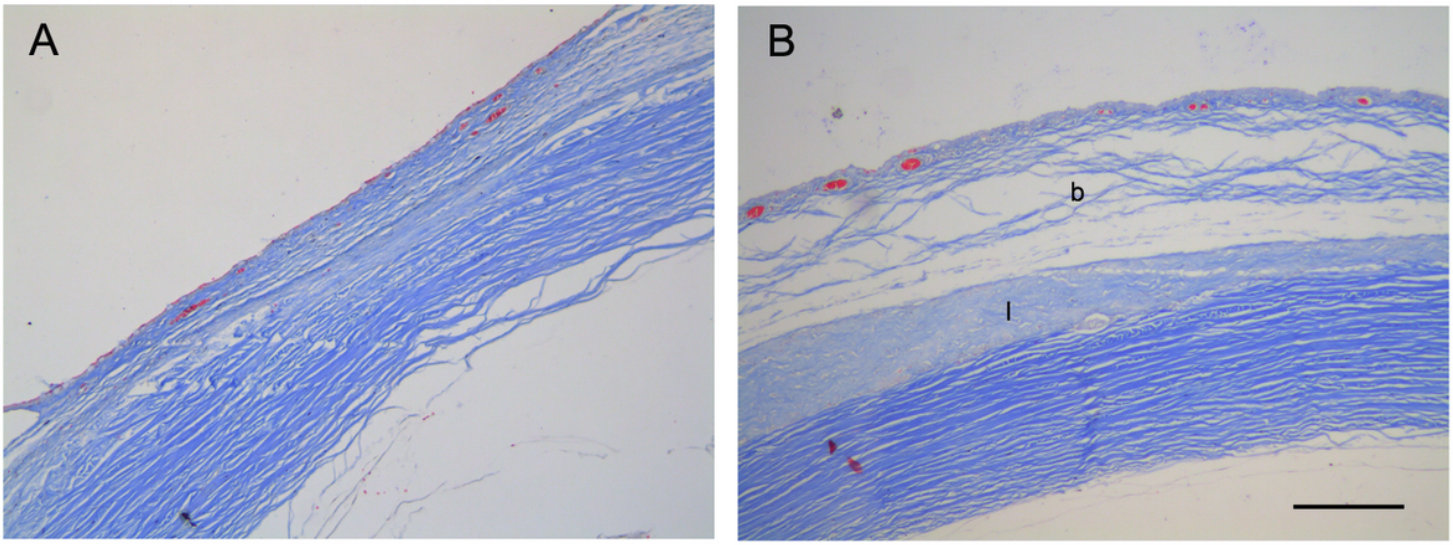

Figure 6

Histologic characteristics of the filtration site stained with Masson's trichrome 28 days after surgery. (A)The control group. (B) The decellularized lenticule group. The subconjunctival area showed reduced collagen deposition in the decellularized lenticule group. b, subconjunctival space; l: decellularized lenticule. Scale bar: 100 μm.