• Clinical Research •

A novel decellularized conjunctival stroma biomaterial for conjunctival reconstruction following pterygium surgery

Shang Li, Jing-Yi Wang, Shi-Jing Deng, Xiao-Dan Hu, Fei Luo, Ying Jie

Beijing Institute of Ophthalmology, Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University; Beijing Ophthalmology & Visual Sciences Key Laboratory, Beijing 100730, China

Co-first Authors: Shang Li and Jing-Yi Wang

Correspondence to: Ying Jie. Beijing Institute of Ophthalmology, Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University; Beijing Ophthalmology & Visual Sciences Key Laboratory, Beijing 100730, China. jie_yingcn@aliyun.com

Received: 2025-02-19 Accepted: 2025-07-16

Abstract

● **AIM:** To evaluate the efficacy and safety of decellularized conjunctival stroma (DCS) as a novel biomaterial by comparing its grafting outcomes with amniotic membrane (AM) when used for conjunctival reconstruction after primary pterygium excision.

● **METHODS:** This randomized, parallel-controlled study with allocation concealment enrolled 40 patients with primary pterygium. Participants were randomly assigned to two groups using the sealed envelope method: the DCS group (n=20) and the AM group (n=18), receiving DCS and AM grafts respectively. Slit-lamp photography of the operative eyes was performed preoperatively and at 1, 3, 5, 7, 10, 30, 90, and 180d postoperatively. Best-corrected visual acuity (BCVA) and symptom scores were recorded simultaneously. *In vivo* confocal microscopy was conducted at 3 and 6mo postoperatively.

● **RESULTS:** All participants exhibited improved postoperative symptoms. The mean age was 60±9y (male/female ratio: 6/14) in the DCS group and 56±12y (male/female ratio: 7/11) in the AM group. The average epithelial healing time was 9.89±3.54d in the DCS group and 8.17±1.34d in the AM group (P=0.084). One recurrence case was observed in each group. Postoperative graft hemorrhage was significantly more severe in the DCS group than in the AM group only at 30d postoperatively (P=0.011). *In vivo* confocal microscopy revealed conjunctival epithelial cell growth in both groups at 90d postoperatively, while clear corneo-conjunctival cell boundaries were observed until 180d postoperatively.

● **CONCLUSION:** DCS used in primary pterygium surgery has a safety profile comparable to AM. It promotes rapid postoperative conjunctival healing, achieves a relatively low pterygium recurrence rate, and yields outcomes similar to AM. DCS provides a novel biomaterial option for conjunctival reconstruction after pterygium excision and the treatment of other conjunctival injuries.

● **KEYWORDS:** pterygium; decellularized conjunctival stroma; amniotic membrane; conjunctival reconstruction; recurrence; graft hemorrhage

DOI:10.18240/ijo.2026.01.06

Citation: Li S, Wang JY, Deng SJ, Hu XD, Luo F, Jie Y. A novel decellularized conjunctival stroma biomaterial for conjunctival reconstruction following pterygium surgery. *Int J Ophthalmol* 2026;19(1):48-55

INTRODUCTION

Pterygium is a triangular-shaped tissue located in the nasal and temporal conjunctiva overgrown towards the cornea, which in essence is overgrown conjunctival fibrovascular[1-2]. As a common ocular surface disease, pterygium has an average prevalence of up to 12% worldwide[3-4]. With the progression of pterygium, it not only affects vision, but also irritates the ocular surface and even causes other complications that can affect the patient's quality of life. The pathogenesis of pterygium is still unclear, but previous studies have shown that it is influenced by several factors, with a strong correlation with ultraviolet light exposure[5-7]. Surgery is the only effective way to treat pterygium, however, simple excision is often accompanied by a high recurrence rate[8]. To achieve better surgical results, approaches such as amniotic membrane (AM) transplantation, supplemented with mitomycin C, as well as autologous conjunctival transplantation are gradually emerging[9-10].

AM is rich in growth factors and cytokines and other bioactive components that promote epithelialization, mitigate inflammation and fibrosis and relieve pain, and is therefore widely used in ophthalmology and surgery to reduce scar formation[11-13]. However, AM differs structurally from the conjunctiva and typically dissolves within 2 to 3wk of covering the conjunctival defect, serving only as a temporary substitute

for conjunctival repair[14-15]. In addition, AM needs to be preserved under strict conditions, with freezing being the most used technique. However, freezing reduces the activity of the AM cells and decreases the level of growth factors, and thus should not be prolonged. Freeze-drying preservation involves decellularizing the AM and then freezes it for preservation[16-17]. Although the preservation time of the AM is prolonged to a certain extent in this way, the composition of the AM is altered, and its properties are somewhat compromised[18]. Therefore, identifying reliable and safe alternatives for conjunctival repair remains an urgent challenge.

Decellularized tissue stroma is a biologically derived material for tissue repair, which obtains a certain biological activity by decellularizing allogeneic tissues and removing associated antigens. In addition to its stable, elastic and non-immunogenic properties, the extra-conjunctival matrix also plays a crucial role in the differentiation of conjunctival epithelial cells and the avoidance of scarring[19-20]. With the development of materials science, synthetic materials based on 3D-printed membrane, branched polyethylene and polylactic acid-coglycolic acid (PLGA) have been successfully applied in animal models of ocular surface reconstruction and repair[21-23]. However, there are still some differences in composition and properties between synthetic materials and natural conjunctiva. In contrast, acellular conjunctival matrix obtained by virus inactivation, decellularization, and antigen removal has an extracapsular matrix that is highly similar to the natural conjunctiva[24]. Although numerous *in vitro* studies have explored the use of acellular materials for conjunctival injury treatment[25-26], the restoration of conjunctival function, scar formation, and angiogenesis following *in vivo* transplantation remain to be further investigated.

This study compared the efficacy and safety of decellularized conjunctival stroma (DCS) and AM for the management of conjunctival defects following pterygium excision. The aim was to investigate whether DCS, as a novel biomaterial for conjunctival reconstruction, provides a safe, effective and stable option for conjunctival repair after pterygium excision.

PARTICIPANTS AND METHODS

Ethical Approval Ethical approval for this study was obtained from the Ethics Committee of Beijing Tongren Hospital (No. TREC2021-116). Informed consent was obtained from all participants.

Study Design This randomized, parallel-controlled trial was conducted at Beijing Tongren Hospital from March 2021 to June 2023. A total of 40 participants undergoing pterygium surgery were initially enrolled, but ultimately only 38 participants successfully enrolled and completed the 180-day follow-up. Inclusion criteria: 1) age between 18y and 70y; 2) diagnosis of primary pterygium; 3) conjunctival defect not larger than 1/3 of the bulbar conjunctiva. Exclusion criteria included: 1) severe vascular disorders; 2) severe dry eye disease (Schirmer I test ≤2 mm/5min); 3) psychiatric abnormalities; 4) systemic connective tissue diseases; 5) severe allergies. All participants were randomly assigned to two groups using sealed envelopes based on a pre-generated randomization sequence.

Surgical Materials

Decellularized conjunctival stroma DCS (Byo Diesel Biotechnology Co., LTD, Sichuan, China) graft, derived from porcine conjunctiva, underwent decellularization, antigen removal, and cobalt-60 irradiation sterilization to obtain a bio-compatible extracellular matrix primarily composed of collagen. This matrix includes both the fibrous layer of the natural conjunctiva and the underlying subconjunctival tissue. Before transplantation, the graft was soaked in sterile saline for 2min. A piece of appropriate size was then trimmed and applied to the conjunctival defect, with the smooth (upper cortical) surface facing upward, and secured with sutures.

Amniotic membrane AM (Ruiji Bioengineering Technology Co., LTD, Jiangxi, China) is derived from the placental tissue of healthy women who have undergone cesarean section. It is freeze-dried, sealed, and preserved for clinical use. AM contains various types of collagen fibers, laminin, and multiple biologically active factors, and possesses histological, immunological, and physiological properties beneficial for tissue repair. During application, the convex surface of the AM (corresponding to the basement membrane side) was placed in direct contact with the conjunctival defect. Sterile saline was instilled onto the concave surface of the AM, and suturing was performed after 5min.

Surgical Procedure All surgical procedures were performed by a single experienced surgeon (Jie Y). Participants in the test group received DCS grafts, while those in the control group received AM grafts. The surgical steps were standardized across both groups. After subconjunctival injection of 2% lidocaine for anesthesia, the pterygium head was gently dissected from the corneal limbus using a sharp blade. The neck and body of the pterygium were excised with scissors to expose the underlying bare sclera. Hemostasis was achieved with cautery when necessary. A sponge soaked in 0.3 mg/mL mitomycin C was then applied to the exposed scleral area for 2min, followed by thorough irrigation with 0.9% saline. The area of exposed sclera was measured using a caliper, and a graft of corresponding size was trimmed. The graft was sutured to the scleral bed surrounding the conjunctival defect using 10-0 nylon sutures (Johnson & Johnson, Somerville, New Jersey, USA). Finally, a bandage contact lens was applied to the operative eye to protect the cornea from suture-related irritation.

Postoperation Postoperatively, tobramycin and dexamethasone eye ointment was applied, and the eyes were covered with dressing for 24h. Tobramycin and dexamethasone eye drops (1 to 2 drops, 3 times daily) and 0.1% (or 0.3%) sodium hyaluronate eye drops (1 drop, 3 times daily) were used from the first day after surgery. The bandage contact lens and the nylon sutures were removed 2wk after surgery. At that time, the medication was switched to 0.1% (or 0.02%) fluorometholone eye drops (1 to 2 drops, 3 times daily) and 0.1% (or 0.3%) sodium hyaluronate eye drops (1 drop, 3 times daily). The dosage of eye drops was gradually tapered based on the degree of wound hyperemia and discontinued once no signs of ocular surface inflammation were observed. In principle, corticosteroid eye drops were not used for more than three months.

Outcome Measures

Preoperative examinations Preoperative examinations included best-corrected visual acuity (BCVA), symptom score and the size, the redness and the fleshiness of pterygium. The symptom score was evaluated according to the level of ocular discomfort and key indicators of ocular surface disease severity, including photophobia, tearing, foreign body sensation, and pain. Depending on the severity of these symptoms, the score ranged from 0 (no) to 4 (severe). The size of the pterygium was assessed based on the extent of its encroachment onto the cornea relative to the corneal diameter: Grade 1 (G1), involving up to one-third of the corneal diameter; Grade 2 (G2), extending beyond one-third of the corneal diameter but not reaching the pupil; and Grade 3 (G3), extending into the pupillary area[27]. Pterygium redness was graded based on the extent of conjunctival hyperemia: GI, no redness or faint pinkish hue; GII, scattered areas with moderate redness; GIII, significant diffuse redness[28]. The fleshiness of pterygium was evaluated by the relative clarity of the pterygium: Tl, atrophic; T2, moderate; T3, fleshy[27].

Postoperative examinations Postoperative assessments were conducted at 1, 3, 5, 7, 10, 30, 90, and 180d following surgery, and included BCVA, conjunctival defect size, healing time, symptom score, recurrence, as well as evaluation of graft-associated hemorrhage and inflammation. The degree of recurrence was evaluated by the episcleral vessels and the fibrous tissues: 1, normal appearance; 2, some fine episcleral vessels in the excised area extending up to but not beyond the limbus and without any fibrous tissues; 3, additional fibrous tissues in the excised area that did not invade the cornea; 4, true recurrence with fibrovascular tissue invading the cornea[29]. The haemorrhage of the graft was evaluated by the area: 0, none; 1, ≤25% of the size of the graft; 2, ≤50% of the size of the graft; 3, ≤75% of the size of the graft; 4, involving the entire graft[30]. The inflammation of the graft was evaluated by the number and degree of congestion of corkscrew vessel: 0, no dilated corkscrew vessel in the graft; 1, 1 bright red, dilated corkscrew vessel crossing the graft-bed margin; 2, 2 bright red, dilated corkscrew vessels crossing the graft-bed margin; 3, 3 bright red, dilated corkscrew vessels crossing the graft-bed margin; 4, ≥3 bright red, dilated corkscrew vessels crossing the graft-bed margin[31].

In-vivo confocal microscopy (IVCM, Heidelberg Retina Tomograph, Heidelberg Engineering, GmBH, Dossenheim, Germany) was also performed 90 and 180d postoperatively for the operated eye. Conjunctival epithelial regeneration and corneal conjunctivalization were evaluated by clinical examination of the surgical area.

Statistical Analysis Statistical analysis was performed using SPSS software version 25.0 (IBM Corp., Chicago, IL, USA). Normality of data distribution was assessed with the Shapiro-Wilk test. Chi-squared test was used to compare gender distribution between groups. Student's *t*-test was applied for comparisons of age, healing time, and symptom scores. Mann-Whitney *U* test was employed to compare pterygium size, redness, fleshiness, recurrence degree, as well as graft haemorrhage and inflammation between the two groups. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Demographic Information of Participants and Preoperative and Postoperative Parameters of Pterygium There are 20 participants in the DCS group and 18 participants in the AM group. The mean age in the DCS group was 60y, with a male/female ratio of 6/14. The mean age in the AM group was 56y, with a male/female ratio of 7/11. There were no significant differences in age, gender, symptom scores, pterygium size, pterygium redness and pterygium fleshiness between the two groups. All operations were successfully completed and there were no statistically significant differences in the size of conjunctival defect, healing time, degree of recurrence, symptom scores and BCVA changes between the two groups (all $P>0.05$; Table 1).

Assessment of Haemorrhage and Inflammation of the Grafts Graft haemorrhage was more severe in the DCS group than in the AM group only on postoperative day 30 ($P=0.011$), with no significant differences observed at other time points. Throughout the 180-day postoperative follow-up, no statistically significant differences were found between the two groups in terms of graft inflammation (Figures 1 and 2).

Comparison of conjunctival epithelial regeneration and transformation between the 2 groups at 90 and 180d postoperatively In both groups, conjunctival epithelial

Table 1 Demographic information of participants and preoperative and postoperative parameters of pterygium *n* (%)

Parameters	DCS group (*n*=20 eyes)	AM group (*n*=18 eyes)	*P*
Gender (male/female)	6/14	7/11	0.569
Age±SD (y)	60±9	56±12	0.416
Pterygium size			0.206
G1	8 (40)	10 (55.6)	
G2	6 (30)	6 (33.3)	
G3	6 (30)	2 (11.1)	
Redness			0.666
I	7 (35)	5 (27.8)	
II	11 (55)	11 (61.1)	
III	2 (10)	2 (11.1)	
Fleshiness			0.237
T1	9 (45)	5 (27.8)	
T2	7 (35)	7 (38.9)	
T3	4 (20)	6 (33.3)	
Preoperative symptom score, mean±SD	0.92±1.11	0.94±1.22	0.952
Size of conjunctival defect (mm^2), mean±SD	7.02±2.53	7.56±3.14	0.815
Healing time, mean±SD (d)	9.89±3.54	8.17±1.34	0.084
Postoperative symptom score, mean±SD	0.07±0.26	0.31±1.07	0.417
Recurrence degree			0.352
G1	16 (80)	16 (88.9)	
G2	3 (15)	1 (5.55)	
G3	1 (5)	1 (5.55)	
G4	0 (0)	0 (0)	
BCVA			0.826
Increased	10 (50)	7 (38.9)	
Unchanged	6 (30)	5 (27.8)	
Decreased	4 (20)	6 (33.3)	

DCS: Decellularized conjunctival stroma; AM: Amniotic membrane; SD: Standard deviation; BCVA: Best-corrected visual acuity; *P*: Compared between the DCS group and the AM group.

cells were found in the surgical area at 90d postoperatively, with irregular polygons, and the boundary between corneal epithelium and conjunctival epithelium was unclear. At 180d postoperatively, the structure of conjunctival epithelium was more regular and clearer, and there was a clear boundary between the conjunctival epithelium and the corneal epithelium. However, there was no significant difference in IVCM findings between the two groups at 90 and 180d postoperatively (Figure 3).

DISCUSSION

In this study, there were no significant differences between the two groups in terms of age, gender, symptom scores, or preoperative pterygium characteristics, including size, redness, and fleshiness, indicating comparable baseline conditions. The proportion of participants with improved postoperative BCVA was 50% in the DCS group and 38.9% in the AM group, representing the largest subgroup in both cohorts. The improvement in BCVA was mainly attributed to the reduction in corneal astigmatism following pterygium excision, which led to a noticeable enhancement in visual acuity for many participants. Healing time was comparable between the two groups, with complete epithelialization achieved within 10d postoperatively, indicating favorable cosmetic outcomes. These results are closely related to the structural characteristics of both AM and DCS: the intact basement membrane of the AM and the extracellular matrix composition of the DCS, which closely resembles that of human conjunctiva, both facilitate epithelial regeneration and wound healing. At 180d postoperatively, symptom scores in both the DCS and AM groups were significantly reduced compared to preoperative levels, this suggests that the irritation caused by pterygium was effectively alleviated by the surgery, and that the materials used for conjunctival repair did not induce additional ocular surface discomfort. Overall, the use of DCS was effective in the management of primary pterygium, yielding outcomes comparable to those of AM in terms of symptom relief, improvement in BCVA, and satisfactory postoperative cosmetic appearance[32-33].

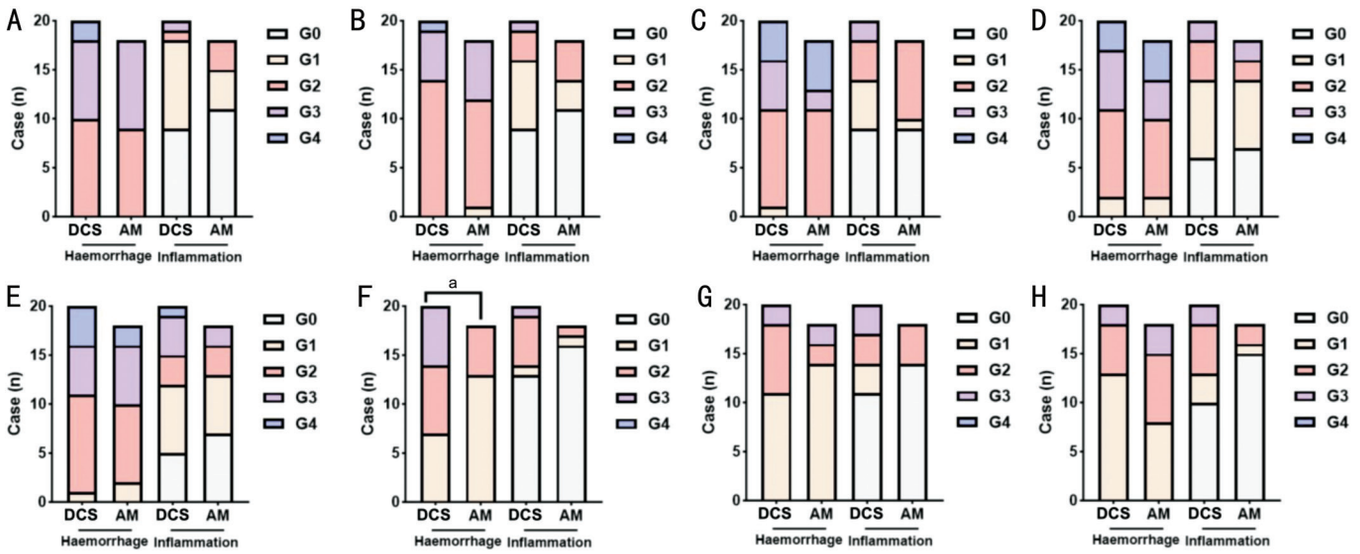

Figure 1 The changes in graft haemorrhage and inflammation in the DCS and the AM groups during the 180-day follow-up The changes in graft haemorrhage and inflammation in the DCS group and the AM group 1d postoperatively (A), 3d postoperatively (B), 5d postoperatively (C), 7d postoperatively (D), 10d postoperatively (E), 30d postoperatively (F), 90d postoperatively (G), and 180d postoperatively (H). The graft haemorrhage was significantly different between the two groups only at 30d postoperatively ($^{a}P<0.05$), and there was no statistically significant difference between the two groups for at other time points. The inflammation was not significantly different between the two groups at 180d postoperatively. DCS: Decellularized conjunctival stroma; AM: Amniotic membrane; G0: Grade 0; G1: Grade 1; G2: Grade 2; G3: Grade 3; G4: Grade 4.

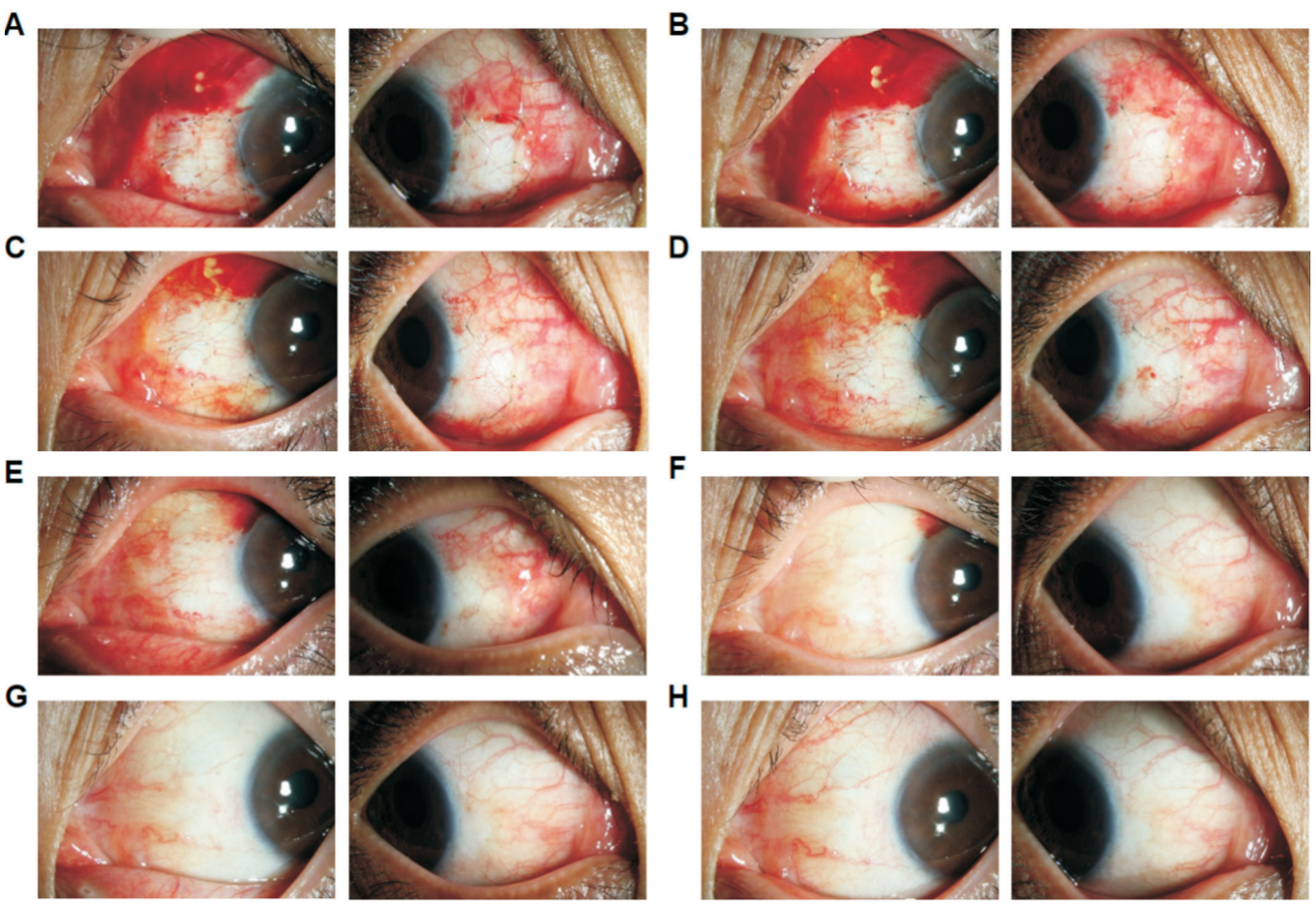

Figure 2 The external ocular photos in the DCS group (left) and the AM group (right) during the 180-day follow-up The external ocular photos in the DCS group and the AM group 1d postoperatively (A), 3d postoperatively (B), 5d postoperatively (C), 7d postoperatively (D), 10d postoperatively (E), 30d postoperatively (F), 90d postoperatively (G), and 180d postoperatively (H). The degree of graft haemorrhage and the inflammation status of the graft in both the DCS group and the AM group gradually alleviated on the 1, 3, 5, 7, and 10d postoperatively, and tended to stabilize after 30d postoperatively. DCS: Decellularized conjunctival stroma; AM: Amniotic membrane.

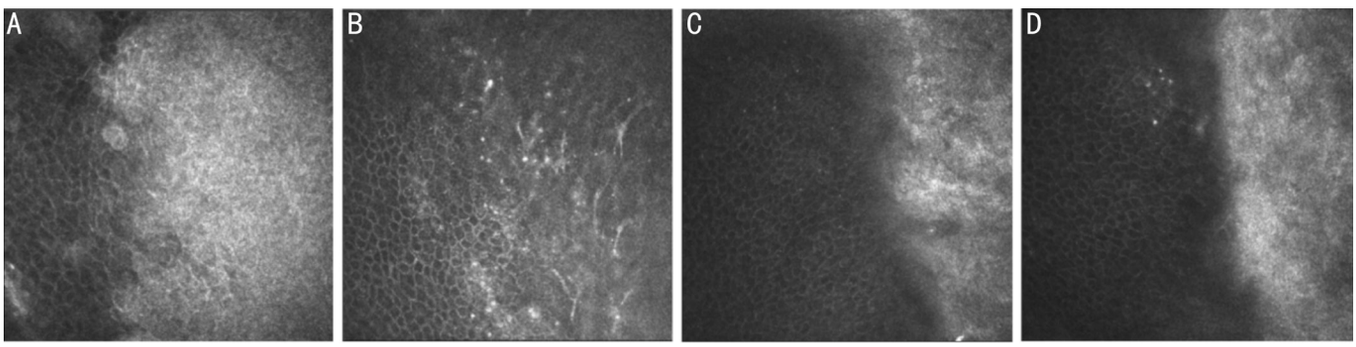

Figure 3 The IVCM findings of the DCS group and the AM group at 90 and 180d postoperatively A: At 90d postoperatively, conjunctival epithelial cells (left) in the DCS group exhibited slightly blurred boundaries and enlarged morphology, with an indistinct transition between conjunctival and corneal epithelium (right); B: At 90d postoperatively, the AM group showed conjunctival epithelium (left) with mildly blurred boundaries and irregular morphology, and the boundary with the corneal epithelium (right) remained unclear; C: At 180d postoperatively, the conjunctival epithelium (left) in the DCS group displayed well-defined boundaries and regular cell morphology, with a clearly distinguishable border between conjunctival and corneal epithelium (right); D: At 180d postoperatively, conjunctival epithelial cells (left) in the AM group also exhibited well-defined boundaries and regular morphology, and the boundary between conjunctival and corneal epithelial cells (right) was clearly visible. IVCM: *In-vivo* confocal microscopy; DCS: Decellularized conjunctival stroma; AM: Amniotic membrane.

Since pterygium consists of fibrovascular tissue proliferating at various ocular surface locations, inflammation plays a key role in the pathogenesis of both fibrosis and neovascularization[34-35]. Therefore, controlling the postoperative inflammatory response is crucial in preventing pterygium recurrence. In this study, the inflammatory response in grafts from both groups gradually subsided over time, with no statistically significant differences observed between the DCS and AM groups at any follow-up point. This suggests that both DCS and AM, as materials for conjunctival repair, do not elicit severe inflammatory reactions. A statistically significant difference in graft haemorrhage between the two groups was observed only at postoperative day 30, with the AM group exhibiting significantly less haemorrhage than the DCS group. Interestingly, graft haemorrhage in the AM group increased at 90 and 180d compared to day 30, whereas haemorrhage in the DCS group decreased progressively over time. Graft haemorrhage may reflect a physiological response to reperfusion injury and represents the process by which the donor tissue integrates with the host conjunctival vasculature[30]. This may be attributed to the fact that AM expresses a range of anti-inflammatory factors such as interleukin-10 (IL-10) and interleukin-1 receptor antagonist (IL-1RA) and suppresses the expression of pro-angiogenic factors, thereby effectively inhibiting early angiogenesis. In contrast, DCS is composed of conjunctival extracellular stroma collagen and retains vascular structure. As a result, graft haemorrhage was less pronounced in the AM group during the early postoperative period. However, vascularization is essential for the long-term survival of the graft. The superior biocompatibility of DCS likely contributes to more stable graft conditions in the later stages[25,33,36]. Ultimately, no significant differences in overall prognosis were observed between the two groups.

At the 180-day postoperative follow-up, the recurrence rate was 5.0% in the DCS group and 5.6% in the AM group, with no statistically significant difference between the two. These findings indicate that DCS is comparable to AM in preventing postoperative pterygium recurrence. AM contains components such as anti-inflammatory agents, anti-angiogenic factors and transforming growth factor-β (TGF-β) inhibitors, which effectively suppress inflammation and angiogenesis, thereby significantly reducing pterygium recurrence. Consequently, AM has become a widely used material for conjunctival reconstruction in pterygium surgery[37-38]. DCS exhibits excellent extensibility and elasticity, along with a unique extracellular matrix component that effectively promotes cell migration, proliferation and differentiation. Consequently, DCS demonstrates superior biocompatibility, stability, and resistance to degradation compared to AM, making it a more reliable and effective option for controlling pterygium recurrence[25,33]. Based on data from previous studies, the recurrence rate of pterygium after AM transplantation is about 14%[39-40], while the recurrence rate can be reduced to about 8% following AM transplantation combined with intraoperative mitomycin application[41-42]. The recurrence rate in the AM group observed in this study was lower than that reported in previous studies using AM grafts, which may be attributed to differences in surgeon experience across studies and the relatively small sample size in the present study.

The corneal epithelium, Bowman's layer, and part of the stroma in this region are damaged following pterygium excision[43-44]. IVCM allows visualization of the microscopic cellular structures of the cornea, corneoconjunctival limbus, and various layers of the conjunctiva[45-46]. Therefore, IVCM

can be used to monitor the recovery of the cornea and damaged conjunctiva following repair with DCS or AM. In this study, conjunctival epithelial cell growth within the surgical area was observed by IVCM at 90d postoperatively in both groups, revealing irregular cell shapes and indistinct boundaries between conjunctival and corneal epithelial cells. However, by 180d postoperatively, clear corneoconjunctival boundaries and more regularly shaped conjunctival epithelial cells were evident in both groups under IVCM. The IVCM findings in this study were similar to those in the study by Pedrotti *et al*[47]. The IVCM findings suggest that both DCS and AM effectively support epithelial healing during conjunctival repair without promoting scar formation. These findings are consistent with the clinical signs observed in the operated eyes throughout the follow-up period.

There are some limitations of this study. First, the small sample size may limit the stability and statistical power of the results. For instance, the observed recurrence rates might not accurately reflect the true recurrence patterns in the broader population, and certain potential factors significantly influencing recurrence may remain unidentified. Moreover, the limited sample size reduces the power of statistical tests, increasing the risk of failing to detect differences that are actually significant. Second, this study included only participants with primary pterygium and excluded those with recurrent pterygium or other types of conjunctival injuries. Therefore, the effectiveness of DCS in repairing other types of conjunctival injuries remains to be determined. In future studies, we plan to expand the sample size and include participants with various similar conjunctival injuries to further investigate the efficacy of DCS across different types of conjunctival damage.

In conclusion, DCS demonstrates comparable efficacy to AM in primary pterygium surgery, promoting rapid postoperative conjunctival repair with a relatively low recurrence. Therefore, DCS is a safe and effective option for conjunctival restoration and can serve as a viable alternative to AM in clinical practice.

ACKNOWLEDGEMENTS

Foundations: Supported by grants from the National Natural Science Foundation of China (No.82171018; No.82371022); Beijing Hospitals Authority's Ascent Plan (No.DFL20240202); The Youth Beijing Scholars Program (No.022); High Level Public Health Technical Talents Construction Project from Beijing (Jie Y); Beijing Municipal Public Welfare Development and Reform Pilot Project for Medical Research Institutes (No.2023YFC2410401).

Conflicts of Interest: Li S, None; **Wang JY,** None; **Deng SJ,** None; **Hu XD,** None; **Luo F,** None; **Jie Y,** None.

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