Original Article

Efficiency and safety of human acellular dermal matrix for repair of leaking filtering blebs in rabbit eye

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Abstract: This study was aimed to investigate the efficiency and safety of human acellular dermal matrix (ADM) for the repair of leaking glaucoma filtering blebs in the rabbit eye model. A total of 48 New Zealand rabbits with leaking filtering blebs were randomized into ADM group, amniotic membrane group, and conjunctiva overlap group. The intraocular pressure (IOP) was measured preoperatively, 1 day, 1 week, 1 month, 3 months and 6 months post-operation. Slit lamp, anterior segment optical coherent tomography (AS-OCT) images, and histopathological changes were recorded 1 week, 1 month, 3 months, and 6 months post-operation. The IOP of the ADM group was lower than the control groups after the operation, but statistical significance was observed at 1 month post-operation (ANOVA F=13.006, P<0.001, LSD t-test P=0.001, <0.001). Slit lamp and AS-OCT images revealed transparent ADM patches, the filtering blebs up to 3 months post-operation, and the conjunctival neovascularization occurred within 3 months post-operation. However, the filtering bleb could only be maintained up to 1 month, and the conjunctival neovascularization occurred in 1 month post-operation with continual corneal neovascularization in the control groups. Histopathologically, the ADM patch was found up to 6 months post-operation. ADM might be effective and a safe biomaterial for repairing the leaking filtering blebs.

Keywords: Acellular dermal matrix, filtering blebs leaking, repair, amniotic membrane

Introduction

After a patient underwent trabeculectomy, aqueous humor would flow beneath the scleral flap to subconjunctiva by intraocular pressure (IOP) and form a specific “pool”. Hence, there is a “blister”-like appearance on the conjunctival surface of the patient’s eye, which is commonly known as “filtering blebs” [1]. The ideal filtering blebs should be dispersive and extensively backward without neovascularization [2]. Filtering bleb leakage is a common bleb-related complication after filtering surgery with the incidence from 7-25% [1, 3-5]. It may induce hypotony [6], hypotonic maculopathy [7], bleb infection, and bleb-related endophthalmitis [2, 8]. Thus, filtering bleb leakage is a disease that needs emergency treatment.

Currently, the treatment of filtering bleb leakage includes conservative and surgical treatments. The conservative treatment includes package, antibiotic ointment, autologous serum, and biological glue [9, 10]; however, they are only effective in the early stage leakage or minor leakage and may induce scar healing. The surgical treatment indicates repairing with other tissues. The surgical methods include autologous conjunctiva advancement or conjunctival patch transplantation [11, 12], allograft scleral patch or corneal patch [13], amniotic membrane (AM) transplantation [14], buccal mucous membrane [15], human pericardium graft [16], and biodegradable collagen-glycosaminoglycan matrix [17]. Nevertheless, all the materials mentioned above have some limitations. Incision dehiscence and tissue regression may occur after conjunctival tissue advancement or transplantation. The conjunctival patch may induce secondary incision, and the size of such patch is also limited. The allograft scleral patch is associated with the risk of infection. The AM can liquease and degrade easily that may induce recurrent leakage [18]. The studies on the buccal mucous membrane, a human pericardium graft, and other artificial biomaterials are limited, and
Efficiency and safety of HADM for repair of leaking filtering blebs

Thus, the efficiency and safety necessitate further investigation [15-17].

Therefore, we aspired to find a new biomaterial, which is flexible, easy to obtain, and without immunological activity, to repair the filtering bleb leakage.

The acellular dermal matrix (ADM) originates from the human dermis and is currently used as a commercial biomaterial. The cellular and antigen components of dermis were removed by mechanical and chemical methods while the intact extracellular matrix was retained [19]. ADM has an intact appearance and insoluble matrix components, which could not only maintain the physiological structure but also serve as the stent of cell regeneration. The ADM graft is not rejected as all of the cellular components that may induce the immunological reaction are excluded. ADM was first applied for the repair of burn injury in 1992, resulting in a satisfactory clinical effect [20]. After that, ADM was applied in an increasing number of surgeries for tissue repair and reconstruction, such as recurrent umbilical hernia, inguinal hernia, and closure of a contaminated gynecologic wound with satisfactory results [20-22].

ADM was applied in ophthalmology since 1999 [23] and is now widely used in eyelid defect, conjunctival sac plasty, and the replacement of conjunctiva and sclera [24, 25]. ADM could act as a stent of tissue regeneration. Furthermore, it could accelerate repair, decrease the incidence of infection, and reduce adhesion. In addition, it also advantageous in tensile resistance and extension, which are crucial for tissue repair. After the ADM patch was implanted, the wound would heal with surrounding cells; the ADM patch would degrade and be replaced by peripheral health tissue. Previously, we demonstrated that ADM could be used in corneal reconstruction [26], and hence, whether such a biomaterial could be used in the treatment of filtering bleb leakage, located at limbus of cornea necessitated further studies.

The current study established a filtering bleb leakage model in rabbit eyes. We repaired the leakage region with ADM, AM, or conjunctiva overlapping without the tissue patch. The IOP, bleb morphology and histopathology of the three groups were compared with respect to the efficiency and safety in repairing the leaking filtering blebs of ADM. To the best of our knowledge, this is the first study attempting to repair the filtering bleb leakage by ADM.

Materials and methods

Materials and animals

A total of 48 New Zealand white rabbits (Center for Experimental Animals of Peking University Health Science Center, Beijing, China), weighing 2.0-2.5 kg, were used for establishing the animal filtering bleb leakage model. All animals were treated in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animal in Ophthalmic and Vision Research. All animal experiments were approved by the Medical Ethics Committee of Peking University Third Hospital.

Surgical methods

Forty-eight eyes from 48 rabbits were randomly divided into three groups: the ADM group, the AM group, and the conjunctiva overlap group. Under general anesthesia, the conjunctiva was opened at the superior quadrant, a 4×3 mm with 1/2 scleral thickness scleral flap was removed, and a 3×1 mm trabecular meshwork was cut with aqueous humor flowing out. Then, iridectomy was performed. For the ADM group, an ADM patch was sutured with the scleral surface at the limbus. The front portion of the ADM patch was inserted into the corneal stromal layers while the posterior part laid flat on the scleral surface such that the trabecular meshwork excision region could be fully covered. Finally, the conjunctival incision was sutured. The procedure for the AM group was similar to that of the ADM group except that the ADM patch is replaced by the AM patch. In the case of the conjunctiva overlap group, after the conjunctiva covered the trabecular meshwork excision, it was sutured post-iridectomy. Eye drops comprising of tobramycin and dexamethasone (Debox, Alcon, USA) were used twice daily until 2 weeks after the operation.

Clinical and histological analysis

The follow-up clinical examinations included IOP at 1 day, 1 week, 1 month, 3 months, and 6 months post-operation. Slit-lamp and anterior segment optical coherent tomography (AS-OCT) were conducted at 1 week, 1 month, 3 months, and 6 months post-operation to examine the...
Efficiency and safety of HADM for repair of leaking filtering blebs

The eyeballs of the rabbits harvested at different follow-up time points (1 week, 1 month, 3 months, and 6 months, post-operative, n=4, respectively) were fixed in 10% formaldehyde and embedded in paraffin, followed by slicing into 4 mm thick sections. Half of the specimens were stained with hematoxylin and eosin (H&E) and observed under a light microscope while the remaining were stained with picrosirius red and observed under a polarizing microscope (dark and light fields).

Statistical analysis

Statistical analysis was performed by SPSS 17.0. The three groups and the intragroup differences were compared by one-way analysis of variance (ANOVA), and the difference between the two groups was confirmed by LSD t-test. The statistical significance was set at P<0.05.

Results

Filtering function

IOP was used to evaluate the filtering function in our study. The preoperative and postoperative IOP was listed in Table 1.

At most of the follow-up time-points, the IOP of ADM was lower than the control groups; however, the statistically significant difference was achieved at 1 month post-operation (F=13.006, P=0.000). The LSD t-test showed a significant difference between the ADM and AM groups and ADM and conjunctiva overlap groups. (P=0.001 and P<0.001, respectively).

The intragroup comparison of IOP in all the three groups demonstrated a statistically significant difference by the ANOVA analysis among different follow-up time-points. (F=8.895, P<0.001 for the ADM group, F=10.897, P<0.001 for the AM group, and F=11.617, P=0.000 for the conjunctiva overlap group).

In the ADM group, the IOP fluctuated from 11.0-11.4 mmHg at 1 week to 3 months post-operation, which differed significantly from that of the pre-operation. The IOP increased to 11.8 mmHg at 6-months post-operation, which did not differ significantly from that of pre-operation. In the AM group, the IOP of 1-week post operation (7.4 mmHg) was significantly different from that of pre-operation. The IOP fluctuated from 13.0-13.1 mmHg at 1-6 months post-operation, which was not significantly different from that of pre-operation. On the other hand, the IOP of 1-week post-operation (11.0 mmHg) was significantly different from that of pre-operation in the conjunctiva overlap group. The IOP fluctuated from 14.1-13.8 mmHg at 1-6 months post-operation, which did not differ significantly from that of pre-operation.

Filtering morphology

ADM group: At 1 week and 1 month post-operation, a distinct filtering bleb with no conjunctival neovascularization was observed. AS-OCT showed that the filtering bleb was diffused with low reflection inside. At 3 months post-operation, the filtering bleb turned flat, and the conjunctival neovascularization crawled to the center of the bleb. At 6 months post-operation, the filtering bleb began to shrink with the conjunctival neovascularization crossing the bleb (Figure 1A). AS-OCT also showed that the bleb turned flat post-operation and the low reflection could be identified, indicating it as functional blebs (Figure 1B).

AM group: At 1 week post-operation, only a filtering bleb, not conjunctival neovascularization, was observed. AS-OCT showed that the filtering bleb was diffused with micro cysts. At 1 month post-operation, the filtering bleb turned flat, and at 3 months, it almost vanished with the conjunctival neovascularization crossing

| Table 1. The IOP comparison of the three groups preoperative and postoperative (mmHg) |
|-----------------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Group                        | Pre-operation 1 day (N=16) | Post operation 1 week (N=16) | Post operation 1 month (N=12) | Post operation 3 months (N=8) | Post operation 6 months (N=4) |
| ADM group                     | 13.5±2.1            | 8.3±2.6             | 11.0±3.1            | 9.2±3.3             | 11.4±1.3            | 11.8±0.7            |
| AM group                      | 14.9±2.7            | 7.4±3.2             | 10.8±3.9            | 13.1±2.1            | 13.0±2.1            | 13.0±3.0            |
| Conjunctiva overlap group     | 14.4±2.2            | 7.8±3.5             | 11.0±3.2            | 14.1±2.1            | 12.4±2.1            | 13.8±1.0            |

Data are presented as mean ± SD.
Efficiency and safety of HADM for repair of leaking filtering blebs

the bleb; the filtering bleb completely vanished at 6 months (Figure 2A). AS-OCT also showed that the bleb turned flat post-operation with an increased inside reflection (Figure 2B).

Figure 1. The clinical and histopathological characteristics of the acellular dermal matrix (AMD) group. A. Slit lamp examination of different time-points after surgery (1 week, 1 month, 3 months, and 6 months). B. Anterior segment optical coherence tomography (AS-OCT) (upper iris-anterior chamber module, down high solution cornea module) at different time-points after surgery (1 week, 1 month, 3 months, and 6 months). C. Hematoxylin and eosin (H&E) staining of different time-points after surgery (1 week, 1 month, 3 months, and 6 months); upper bar 200 μm and lower bar 100 μm, arrowhead: ADM patch. D. Picrosirius red staining of different time-points after surgery (1 week, 1 month, 3 months, and 6 months); bar 100 μm.

 Conjunctiva overlap group: At 1 week post-operation, distinct filtering bleb with severe conjunctival neovascularization was observed. AS-OCT showed that the bleb was localized with low reflection. At 1 month post-operation, the filtering bleb turned flat with the conjunctival neovascularization crawled to the center of the bleb. At 3 months post-operation, the filtering bleb almost vanished with the conjunctival neo-
vascularization crossing the bleb, while at 6-months, it completely vanished. (Figure 3A). AS-OCT showed that the bleb turned flat post-operation with a high reflection subconjunctiva at 3 months after the operation, which indicated conjunctival and subconjunctival proliferation (Figure 3B).

**Patch tissue and cornea**

ADM group: at 1 week post-operation, the ADM patch was semi-transparent with visible iridectomy. AS-OCT showed that the ADM patch crossed the limbus, covered the trabecular meshwork excision with the front part located...
in the transparent cornea stroma, and stretched to the scleral surface. H&E staining also established the localization of the ADM patch. The ADM patch contacted with the aqueous humor at trabecular meshwork excision region. A large number of inflammatory cells were small and round. The cells also surrounded the AMD patch, and a few of them were inside the patch. The fibrils inside the ADM patch were regularly arranged with only a few of them dissolved.

Subsequently, the ADM patch turned transparent with visible iridectomy. The cornea around the ADM clarified, and the limbus neovascular-

**Figure 3.** The clinical and histopathological characteristics of the conjunctiva overlap group. A. Slit lamp examination at different time-points after surgery (1 week, 1 month, 3 months, and 6 months). B. AS-OCT (upper iris-anterior chamber module, down high solution cornea module) of different time-points after surgery (1 week, 1 month, 3 months, and 6 months). C. H&E staining of different time-points after surgery (1 week, 1 month, 3 months, and 6 months); upper bar 200 μm, lower bar 100 μm; arrowhead: scleral flap removal location. D. Picrosirius red staining of different time-points after surgery (1 week, 1 month, 3, and 6 months); bar 100 μm.
 Efficiency and safety of HADM for repair of leaking filtering blebs

The boundary blurred partially, suggesting that the dissolution had initiated. A large number of inflammatory cells could be identified by the AM patch. At 3 months post-operation, most of the AM was absorbed with only some remaining fragments. At 6 months post-operation, the AM patch was almost absorbed with only few fragments remaining. However, the inflammatory cells could still be found at the operation site (Figure 2C). Picrosirius red staining showed that the AM graft crimped and dissolved after the operation. In the dark field, the AM was strip-shaped, uniform, and coarse with reddish color at an early stage after the operation. With the passage of time, it also crimped and fragmented, and could not be identified at 6 months after the operation. In the light field, the AM was intact and regularly arranged with a clear boundary visible at the early stage of operation. Subsequently, the AM fragmented, and the boundary became blurry; the patch disappeared at 6 months post-operation (Figure 2D).

H&E staining showed that from 1 week to 6 months post-operation, the conjunctiva overlap group altered only slightly as all the images showed thinning sclera and a few inflammatory cells infiltrating at the operation region (Figure 3C). Picrosirius red staining also showed that the scleral tissue turned thinner in the operation region. The thin scleral fibrils were thick and exhibited a red or bright yellowish color with some fine greenish fibrils under the conjunctiva and connected to the sclera (Figure 3D).

Discussion

The current study demonstrated that the ADM patch could repair the filtering bleb leakage safely and effectively in the rabbit eye model. The filtering bleb leakage ceased after the ADM patch was covered on the leaking region without obvious intraocular inflammation or rejection. Simultaneously, other obvious bleb morphologies, better-controlled IOP, and later scar healing in the ADM group were observed than the control groups. The ADM patch showed adequate histocompatibility that could retain the filtering function for at least 6 months. Such results might imply that ADM contributed towards better IOP control after repair of the bleb leakage. Thus, it is can efficiently and safely repair the filtering blebs leakage. To the best of our knowledge, this is the first study to repair the filtering bleb leakage with ADM.
In our study, the clinical and histopathological observation identified the good histocompatibility of ADM. Slit lamp and AS-OCT images both showed that the ADM patch contacted aqueous humor; however, the inflammatory reaction was even less than AM group without any rejection. Although the histopathology showed a dissolution process after the operation, the infiltration of the inflammatory cells was slight. The good histocompatibility of ADM could be attributed to its acellular characteristic. ADM indicates that the cells, antigen, lipids, and soluble proteins of the tissue were all removed; however, the matrix components including collagen, elastin, non-collagen glycoprotein, proteoglycan, glycosaminoglycan were reserved [27]. Thus, the rejection could be avoided because the cellular components that could trigger the immunological reaction were removed. Therefore, ADM was speculated to repair the filtering blebs leakage safely.

In the current study, the IOP values identified the efficiency of ADM for repairing the bleb leaking. We identified that the duration of low IOP was longer in the ADM group than the control groups. The IOP was lower than that of pre-operation than at 3 months post-operation in ADM group but 1 month in the control groups. The filtering blebs turned flat at 3 months post-operation in the ADM group but 1 month in the control groups. The neovascularization occurred later in the ADM group than that in the control groups. The bleb morphology and later scar healing in ADM group were optimal than that in the control groups.

Thus, we could conclude that ADM could not only repair the filtering blebs leakage effectively but also postpone the scar formation of filtering blebs and maintained functional blebs, which were crucial for clinical doctors as only the functional blebs contributed towards IOP control.

The functionality of the ADM patch in maintaining functional blebs could be explained by our surgical techniques and the biomechanical characteristics of ADM. Herein, we fixed the front part of the ADM patch and laid the posterior part on the sclera. The thickness of the ADM patch could act as filtering media. The ADM patch served as the loose acellular connective tissue with three-dimensional space structure of derma [28], and the gaps among it could also contribute to filtering. ADM could act as a supportive material after implantation, which separated the conjunctiva from the surface of the sclera. The aqueous humor could flow through the ADM patch, and one of the pivotal characteristics of aqueous humor was inhibiting proliferation. The AM was extremely thin, and without sufficient support, it dissolved rapidly; thus, the healing process was faster than the ADM group. In the case of the conjunctiva overlap group, the scleral flap was removed, but the conjunctiva contacted directly with the sclera; thus, the healing in this group was also rapid.

Picrosirius red staining is a specific method for collagen, which could distinguish collagen type I and type III. Several researchers speculated that the thick red or yellowish lines under the microscope were collagen type I while the fine greenish lines were collagen type III or the fragments of type I [29]. Our study showed that the ADM patch turned from thick, red, and yellowish fibrils to red and greenish cross fibrils that suggested slow dissolution post-operation. Such components remaining in the ADM patch had intact morphology and ultra-microstructure [27]. The tissue reconstruction was processed with host cells and finally replaced by the autogenous tissue [30].

The physiological cause due to which the ADM patch could maintain functional blebs after repair of filtering blebs leakage was laid in its component that consisted of several arginine-glycine-aspartic acid (three-peptide) sequences in ADM. The domain effect of such sequence was to guide the adherence of the cells between the matrix and cells. After the ADM had been implanted, it acted as a stent. The fibroblasts migrated to the stent by the interaction of adhesion sites and proteinase activity. The fibroblasts synthesize the extracellular matrix and cytokines to increase the activity of the stent while on the other hand, the fibroblasts could release collagenase to dissolve the matrix. This mechanism might explain the underlying reason of ADM to be recognized as autogenous tissue with dissolving, remodeling, and regenerating, as well as, why ADM patch could maintain the functional blebs after repair of filtering blebs leakage.

Nevertheless, there are some limitations of this study. First, the sample was limited. Second,
Efficiency and safety of HADM for repair of leaking filtering blebs

there was a trend of proliferation of rabbit eyes, which is different from the human eyes. The ADM was an allograft material for rabbit eyes that may explain why the filtering blebs almost disappeared at 6 months post-operation. If the scar healing is desired to be postponed, other treatments such as anti-cicatricial treatment might be essential. Third, the ADM was a commercial material, and the variance induced during production should be considered.

In summary, we found a distinct bleb morphology and better-controlled IOP duration in the ADM group than the control groups. The conjunctiva neovascularization occurred later in the ADM group than the control groups. There is good histocompatibility of the ADM patch. ADM could repair the bleb leakage safely and effectively, and thus, might be a better material than those used currently, which is critical for the treatment of filtering bleb leakage.

Disclosure of conflict of interest

None.

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Efficiency and safety of HADM for repair of leaking filtering blebs


