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Clinical Evaluation of Gamma-Sterilized, Air-Dried Human Amniotic Membrane as a Biological Scaffold in Pterygium Surgery

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Abstract

Purpose

This study aims to evaluate the clinical outcomes of using gamma-sterilized, air-dried human amniotic membrane (AD-hAM) as a biological scaffold in pterygium treatment and to develop a sterile and safe amniotic membrane product with an extended shelf life for clinical use.

Methods

Three patients with primary pterygium undergoing excision surgery between March 2024 and March 2025 at Abdhadie Eye Clinic were included in this prospective study. After pterygium excision, a gamma-irradiated, air-dried amniotic membrane graft of 3×3 cm size was applied to cover the bare scleral bed. Preoperative and postoperative slit-lamp examinations were performed to assess surgical outcomes, including corneal clarity, graft attachment, and recurrence of pterygium.

Results

Notable postoperative improvement was observed in all patients. The amniotic membrane grafts were well-attached, and there was a noticeable reduction in vascularization and scarring. The corneal surface cleared with minimal residual opacity at the excision site, and no recurrence of pterygium was noted at the one-month follow-up. Additionally, the graft supported wound healing and reduced inflammation, indicating its potential as an effective biological scaffold in pterygium surgery.

Conclusions

The use of AD-hAM demonstrated promising results in pterygium surgery, including reduced recurrence and improved corneal healing. This technique offers a viable alternative to fresh or cryopreserved amniotic membrane, particularly in settings with limited resources. The development of a sterile, safe amniotic membrane with an extended shelf life holds promise for widespread clinical use. Further studies with larger sample sizes and longer follow-up are needed to validate these findings and assess long-term outcomes.

Introduction

Pterygium is a common ocular surface disorder characterized by fibrovascular proliferation of conjunctival tissue extending onto the cornea, often associated with chronic ultraviolet exposure, inflammation, and limbal stem cell dysfunction [1]. Its global prevalence rises with age, varying from 3% to 19.5%, with an average of 12% [2]. Surgical excision remains the primary treatment; however,

postoperative recurrence continues to represent a significant clinical challenge, particularly in the absence of adjunctive therapies [3]. Corneal growth is believed to result from the destruction of limbal tissue, supporting the use of limbal stem cells in the limbal conjunctival autograft (LCA) technique, which leads to lower recurrence rates for both primary and secondary pterygia [4]. Recently, human amniotic membranes have become more widely used in managing various ocular surface disorders, including covering conjunctival defects after pterygium excision, and even in combination with other techniques like conjunctival autograft [5]. Amniotic membrane (AM) transplantation has been widely employed in ocular surface reconstruction due to its anti-inflammatory, anti-fibrotic, and pro-epithelialization properties [6].

Fresh AM is more frequently used in developing countries, where preservation methods are not easily accessible, Unfortunately, the use of fresh AM has several drawbacks, including its limited usability timeframe, inability to utilize the membrane size for multiple tissue transplants, and an increased risk of infection transmission [7]. Traditionally, cryopreserved AM has been used; however, it requires specialized storage conditions and may not be readily available in resource-limited settings [8]. More recently, air-dried and terminally sterilized AM, particularly using gamma irradiation, has emerged as a promising alternative, offering advantages such as extended shelf life, ease of storage, and potential preservation of biological activity [9]. Nevertheless, clinical evidence evaluating its use in pterygium surgery remains limited. In this study, we present a preliminary case series of three patients with pterygium treated using gamma-sterilized, air-dried human amniotic membrane, focusing on surgical outcomes and early clinical performance.

Material and Methods

2.1. Obtaining and transporting placentas

The study was approved by the Bioethics Committee at the Libyan Biotechnology Research Center, Tripoli, Libya LBTRC (Ref No. BEC-BTRC6-2021). The protocol complied with the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects)[10]. Written informed consent was obtained from all participants. The standard operating procedure (SOP) for placenta collection, handling, and transportation was reviewed and validated by the Biosafety and Biosecurity Committee at the Libyan Biotechnology Research Center to ensure adherence to established quality assurance and biosafety standards.

2.2 Processing of h-AM

hAM was prepared according to the procedure described in our earlier publication [11] and with modifications based on previously reported protocols for amniotic membrane processing and decellularization [12]. Briefly, the membrane was separated from the chorion by blunt dissection in stainless steel pans under a Class II safety hood (Telstar, Spain). Fresh placentas were washed three times with sterile saline solution (0.9% NaCl) at room temperature. The washing and shaking steps were

repeated to remove blood clots and debris. Finally, the amnion was rinsed with sterile saline solution containing penicillin 10,000 IU/mL, streptomycin (10,000 µg/mL), and amphotericin (B 2.5 µg/mL) and stored at 4°C.

2.3 Bioburden determination

The bioburden of AD-hAM, including aerobic and anaerobic bacteria, yeasts, and molds, was assessed in accordance with ISO 11737-1 [13]. From each production batch derived from a single placenta (20–25 individually packaged sachets), one non-irradiated membrane package was randomly selected for microbiological evaluation. Following a minimum holding period of 48 h post processing, the selected package was aseptically opened. Membrane samples were aseptically sectioned into small fragments and inoculated onto trypticase soy agar plates to detect aerobic microorganisms, followed by incubation at 30°C for 14 days. Anaerobic bacteria were cultured in thioglycolate broth and incubated at 37°C for 7 days under anaerobic conditions. Fungal contamination was evaluated by culturing samples on Sabouraud dextrose agar and incubating at 30°C for 14 days. The sensitivity of culture-based detection was about 1 colony forming unit per plate.

2.4 Sterilization of AD-hAM

Vacuum-sealed AD-hAM samples were sterilized by gamma irradiation using a cobalt-60 source at room temperature at the National Center for Nuclear Sciences and Technologies (NCNST, Tunis). The membranes were exposed to an absorbed dose of 25 kGy, which is the internationally accepted standard for tissue sterilization according to ISO 11137-2-2013 [14]. The required radiation sterilization dose (RSD), corresponding to the targeted sterility assurance level (SAL), was calculated using the following equation: Required sterilization dose = D_{10} (log bioburden − log SAL) kGy [15]. After irradiation, the sterilized AD-hAM packages were stored at 4°C until further analysis.

2.5. Assessment of Microbial Barrier Function of AD-hAM

The purpose of this assay was to assess the ability of the air-dried, irradiated amniotic membrane to act as a barrier against microbial contamination, prevent bacterial growth or translocation through the membrane, and help support wound healing while reducing contamination and inflammation. Sterile air-dried, gamma-irradiated AD-hAM samples were evaluated for microbial impermeability against representative Gram-positive *Staphylococcus aureus* and *Streptococcus pyogenes*, and Gram-negative *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The bacterial strains were provided by the Microbiology Department at the Libyan Biotechnology Research Center (LBTRC). Membrane fragments were placed on MacConkey agar and plate count agar media, and bacterial suspensions were standardized to 1 × 10^6 CFU/ml. The inoculum was applied to the membrane surface, which was then incubated at 37°C for 24 hours.

2.5 Surgical Technique

This prospective study was conducted at Abdhadie Eye Clinic. The study was registered with ClinicalTrials.gov under Identifier NCT07466576 and was approved by the Bioethics Committee at the

Libyan Biotechnology Research Center, Tripoli, Libya (Ref No. BEC-BTRC9-2023). The study adhered to the provisions of the Declaration of Helsinki, and all participants provided informed consent. The sample size consisted of three patients who underwent pterygium excision between March 2024 and March 2025. Inclusion criteria were patients with primary pterygium who were suitable candidates for surgical excision. Exclusion criteria included a history of recurrent pterygium, immune-related diseases, glaucoma in the studied eye, other concurrent ocular surface pathology, ocular surface or eyelid disease, poor general health, and concurrent or anticipated enrollment in an interventional clinical trial involving either an investigational medicinal product or medical device. All surgeries were performed by two surgeons. Each affected eye underwent standard sterile preparation and draping. The procedure was conducted under peribulbar lidocaine anesthesia to ensure patient comfort throughout the surgery. Dissection began by creating a peristome approximately 3mm from the limbus. The pterygium was then dissected carefully underneath and removed en bloc using a hemostat. A 3×3 cm bare scleral bed was created after excision. Both nasal and temporal pterygium were present in this case. After excision, a neurosurgical sponge soaked in mitomycin-C (MMC) was placed on the subconjunctival opening to minimize recurrence. For this case, a gamma-irradiated AD-hAM graft of 3×3 cm size was chosen to cover the bare scleral bed. The membrane was precisely placed on the dried scleral bed, with its edges trimmed to size and tucked under the conjunctival defect to secure the graft in place and promote proper adhesion. Immediately following the procedure, a bandage contact lens was applied to protect the graft, and the eye was patched until the first postoperative day. Surgical time was measured from the injection of lidocaine to the final placement of the bandage contact lens. The bandage contact lens was removed during the first postoperative week visit.

Results

In terms of bioburden and microbial content, no germs were detected in the AD-hAM samples. Regarding the microbial barrier function, no penetration was observed, indicating the membrane's effectiveness in preventing microbial translocation. These findings are consistent with our previous study (Fawzi et al., 2025), where the air-dried, irradiated membrane also demonstrated significant resistance to microbial contamination.

Case 1

A 64-year-old Libyan male, a diabetic on oral hypoglycemics (metformin) for 2 years, with a recent HbA1c of 7.7, presented with a complaint of a large pterygium in the right eye complicated by diplopia. He had a history of pterygium excision in the left eye 5 years earlier. On examination, best-corrected visual acuity was 0.05 in the right eye (RE) and 0.2 in the left eye (LE), with a refraction of + 3.50 sphere, -3.50 cylinder in the right eye. Intraocular pressure (IOP) was 15 mmHg in both eyes. Slit lamp examination revealed a double-headed pterygium, with large nasal and temporal fibrovascular extensions invading the cornea and reaching the pupillary zone, associated with corneal vascularization and scarring (Fig. 1.A). The left eye showed a recurrent nasal pterygium. Surgical excision of the nasal and temporal pterygium in the right eye was performed, followed by amniotic membrane grafting, sutured with 4 sutures, and covered

with a bandage contact lens (Fig. 1B). At the last follow-up, the patient's visual acuity had improved to 0.3 in the right eye. Slit lamp examination revealed a clear cornea with no evidence of pterygium recurrence and resolution of central involvement (Fig. 1.C.D).

Case 2

A 74-year-old Libyan male, known to have systemic hypertension and treated for glaucoma since 2017, presented to the ophthalmology clinic with complaints of recurrent pterygium in the right eye. The patient was monocular, as the left eye had no perception of light due to advanced glaucomatous optic neuropathy. The patient had undergone pterygium excision in the right eye in 2017, which recurred one year later and was managed with repeat pterygium excision and conjunctival autografting. Three months before presentation, he underwent another pterygium excision in the same eye but noted regrowth soon afterward. On initial examination, his best-corrected visual acuity (BCVA) was 0.5 in the right eye (RE) and no perception of light in the left eye (LE). Autorefraction of the RE showed + 0.50 sphere / −2.75 cylinder × 14°. Intraocular pressure measured 13 mmHg (RE) and 16 mmHg (LE). Slit-lamp examination of the right eye revealed a large, recurrent fibrovascular pterygium extending over the nasal cornea (Fig. 2.A), in addition to cortical cataract changes and pseudo exfoliation (PEX). The optic disc appeared with a cup-to-disc ratio (CDR) of 0.4. Given the recurrence and associated fibrosis, recurrent pterygium excision with amniotic membrane grafting and release of symblepharon was planned. Intraoperatively, after excision of the fibrovascular and fibrotic tissue, a thinned corneal area was observed beneath the excised lesion. A double-layer amniotic membrane graft was applied to cover the corneal defect and another over the adjacent scleral bed, and was secured with six interrupted sutures on the temporal scleral edge. A bandage contact lens (BCL) was applied, and cycloplegic eye drops were prescribed postoperatively. At the one-month postoperative visit, a slit-lamp photograph taken at that time (Fig. 2.B) demonstrated a well-attached amniotic membrane graft, with visible sutures and mild conjunctival hyperemia. The cornea appeared clear with a faint superficial opacity at the nasal periphery, consistent with the previous pterygium site, and no evidence of recurrent fibrovascular growth

Case 3:

A 72-year-old Libyan female with a medical history of diabetes and hypertension presented with recurrent pterygium complicated by symblepharon in the left eye (Fig. 3.A). The patient had been managing her diabetes with oral hypoglycemics and hypertension with antihypertensive medications. She had previously undergone pterygium excision surgery in the left eye. However, the pterygium recurred, and she developed significant vascularization, scarring, and symblepharon, with adhesions between the conjunctiva and cornea. Due to the progression of the disease, surgery was again recommended. The patient underwent pterygium excision followed by the application of an irradiated AD-hAM as a biological scaffold. The graft was applied to the surgical site to reduce inflammation, prevent further scarring, and support the healing of the corneal and conjunctival tissues. This was the only available option to address both the recurrent pterygium and symblepharon. As seen in (Fig. 3.B). Considerable improvement is evident postoperatively. The graft is well-attached, with a noticeable

reduction in vascularization and scarring. The corneal surface has cleared, with minimal residual opacity at the previous pterygium site, demonstrating successful healing and a reduced risk of recurrence.

Discussion

Over the years, surgical techniques for pterygium treatment have advanced significantly. The primary goals of modern pterygium surgery are to close the tissue defect, prevent recurrence, alleviate ocular surface symptoms, and improve patients' quality of life [16]. In recent years, the field of ocular surface tissue regeneration has seen notable progress. Key advancements include the use of tissue replacements, as well as auto-, allo-, and xeno-grafts for limbal stem cell therapy and pterygium surgery. These approaches are used either as standalone treatments or in combination with temporary grafts such as amniotic membrane (AM) (AM)[17]. The human amniotic membrane consists of a dense basement membrane and an avascular stromal matrix. The basement membrane facilitates epithelial cell migration, enhances basal epithelial cell attachment, and supports epithelial regeneration [18]. In our study, the use of gamma-irradiated air-dried amniotic membrane (AD-hAM) in pterygium surgery yielded promising results. The grafts were well-integrated, leading to significant improvements in corneal clarity and minimal residual opacity. Okabe et al state that AD-hAM useful for covering large ocular surface defects, such as in cases of large or double-headed pterygium. Additionally, many surgeons have noted that handling AD-hAM is straightforward, and its use may result in shorter surgical times. Compared to synthetic biomaterials and animal-derived alternatives, HD-AM offers excellent mechanical properties, allowing it to be directly sutured during surgery [20]. The effectiveness of amniotic membrane (AM) in pterygium surgery is primarily due to its ability to inhibit pathological neovascularization, prevent excessive inflammation, and promote conjunctival epithelialization. Our findings are consistent with these properties, as the use of HD-AM in our study helped support these processes, leading to reduced vascularization, scarring, and minimal recurrence at the surgical site. Therefore, the use of HD-AM may contribute to these beneficial effects and help reduce the recurrence of the condition [21 22]. In addition to minimizing recurrence rates and surgical complications, it is anticipated that patients treated with AD-hAM will experience less postoperative pain and discomfort compared to those undergoing conjunctival autograft surgery. These outcomes may be attributed to the amniotic membrane’s role in covering the corneal epithelial defect and its ability to reduce inflammation, as observed in our study. Our findings are consistent with those of Benoit et al., who concluded that amniotic membrane grafting (AMG) appears to be a reasonable option to consider in primary pterygium surgery [23]. Based on our study, AD-hAM demonstrated favorable outcomes, making it a viable alternative for pterygium treatment. This study has some limitations. The sample size was small, with only three patients, which limits the generalizability of the results. The follow-up period was short (one month), and longer-term outcomes were not assessed.
Over the years, surgical techniques for pterygium treatment have advanced significantly. The primary goals of modern pterygium surgery are to close the tissue defect, prevent recurrence, alleviate ocular surface symptoms, and improve patients' quality of life [16]. In recent years, the field of ocular surface tissue regeneration has seen notable progress. Key advancements include the use of tissue replacements, as well as auto-, allo-, and xeno-grafts for limbal stem cell therapy and pterygium surgery.

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Conclusion

The use of gamma-sterilized, air-dried amniotic membrane (AD-hAM) in pterygium surgery showed promising results in our study, with improved corneal clarity, reduced vascularization, and minimal recurrence. AD-hAM demonstrated its effectiveness as a biological scaffold, promoting wound healing and reducing inflammation. This technique presents a viable alternative to traditional treatments, particularly in settings with limited resources. Further studies with larger sample sizes and longer follow-up are needed to confirm these findings and evaluate the long-term efficacy of AD-hAM in pterygium surgery.

Declarations

Data Sharing Statement

All data generated or analyzed during this study are included in this article. Further information can be obtained by contacting the corresponding author.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants. The study was registered with ClinicalTrials.gov under Identifier NCT07466576 and was approved by the Bioethics Committee at the Libyan Biotechnology Research Center, Tripoli, Libya (Ref No. BEC-BTRC9-2023).

Consent for publication

Written informed consent was obtained from patient for publication of this case report and accompanying images.

Author Contributions

All authors made substantial contributions to the work presented, including in the conception, study design, execution, data acquisition, analysis, interpretation, or in all of these areas.

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Disclosure

All authors declare no conflicts of interest in relation to this work.

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Figures

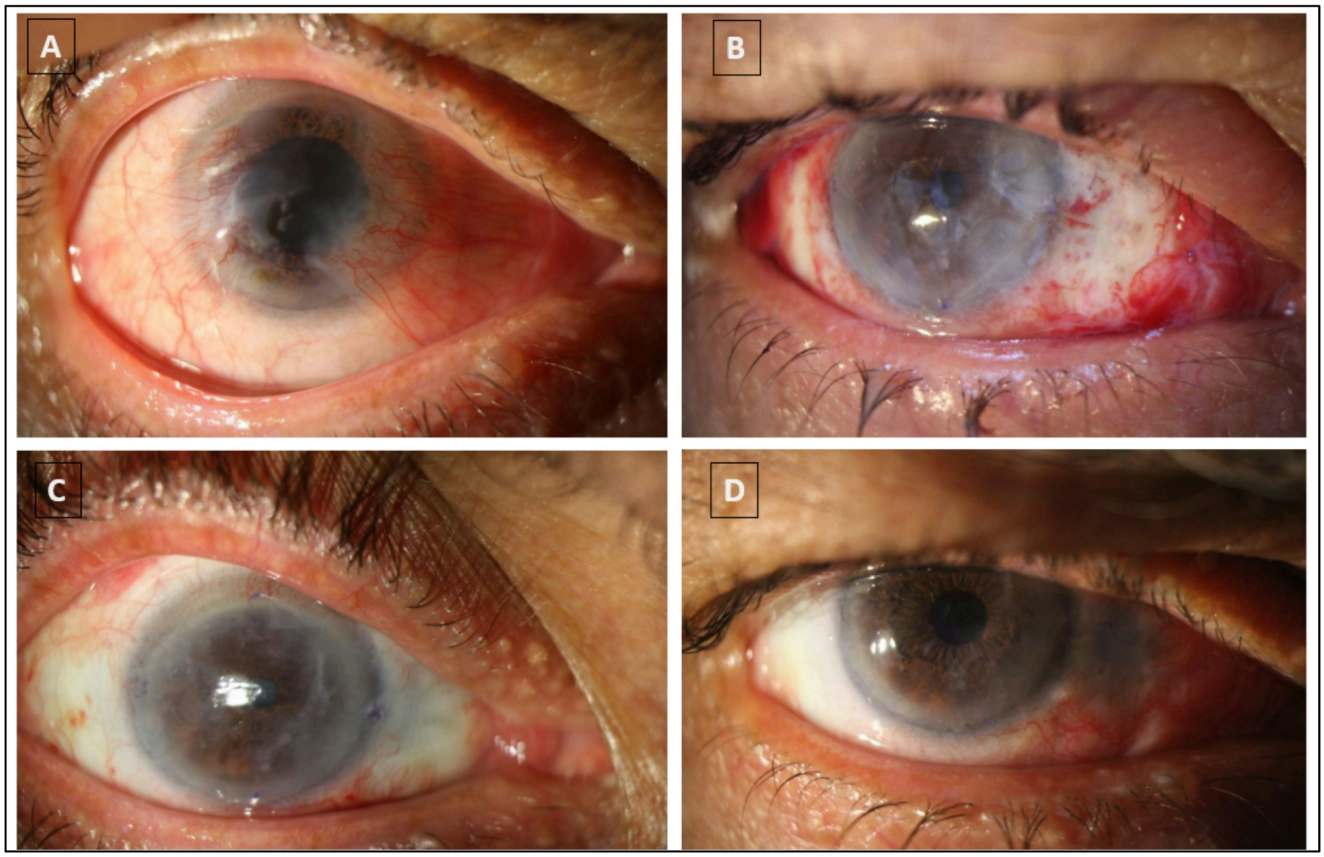


Figure 1

A. Slit lamp photograph showing a double-headed pterygium with fibrovascular growth on both the nasal and temporal sides extending onto the cornea, involving the pupillary area. B. Postoperative slit lamp image after pterygium excision and amniotic membrane grafting, showing the graft well-positioned with mild conjunctival hyperemia and residual corneal haze (1 day after surgery). C.Slit lamp photograph taken two weeks after surgery. D. Slit lamp photograph from the final follow-up visit showing a clear cornea with no recurrence of the nasal and temporal pterygium and resolution of central corneal involvement (1 month after surgery).

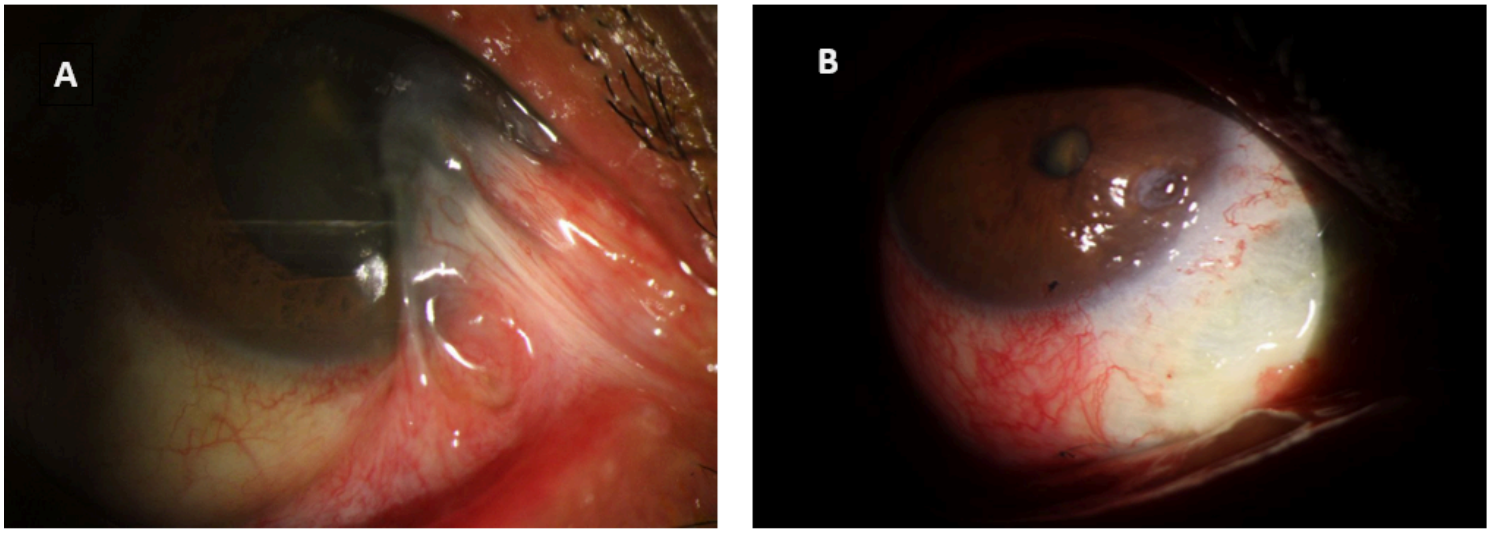


Figure 2

A.Recurrent pterygium in the right eye showing fibrovascular conjunctival tissue invading the cornea with significant vascularization and fibrosis. Figure 2. **B.** Slit-lamp image one month after pterygium excision with amniotic membrane grafting, showing a well-attached graft, mild conjunctival hyperemia, visible sutures, and a clear cornea with faint peripheral opacity at the previous lesion site, with no evidence of recurrent fibrovascular growth.

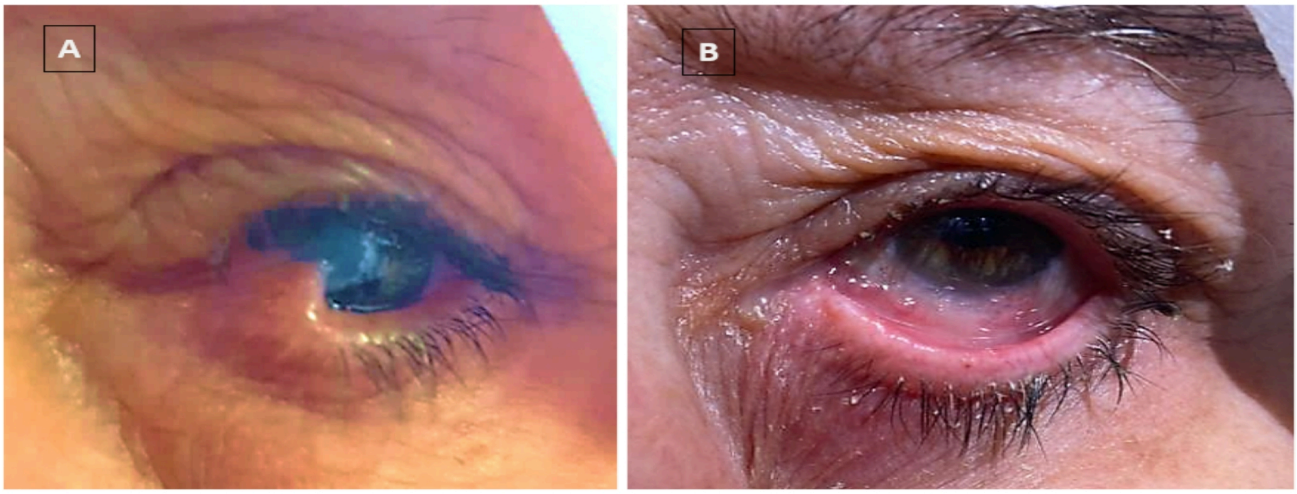


Figure 3

A.Preoperative slit-lamp photograph showing recurrent pterygium in the left eye with significant vascularization, scarring, and symblepharon, with adhesions between the conjunctiva and cornea. Figure **B.**Postoperative slit-lamp photograph showing significant improvement after pterygium excision and

treatment with a irradiated AD-hAM graft. The cornea appears clearer, with reduced vascularization and scarring, and the graft is well-attached with minimal residual opacity at the previous pterygium site.