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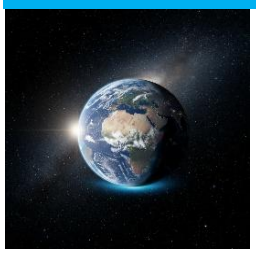

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Short Article

Human Amniotic Membrane in Ocular Surface Reconstruction: Biological Properties, Clinical Applications, and Future Perspectives

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ABSTRACT

The human amniotic membrane (HAM) is the innermost layer of the placenta and has been extensively utilized in ophthalmology due to its anti-inflammatory, antimicrobial, antiangiogenic, and pro-regenerative properties. Rich in growth factors, cytokines, and stem cells, HAM supports corneal epithelial healing and modulates inflammatory responses, making it a valuable therapeutic option in the management of ocular surface disorders. Established clinical indications include chemical and thermal burns, corneal ulcers of various etiologies, corneal perforations, bullous keratopathy, and limbal stem cell deficiency. Conventional amniotic membrane transplantation remains a well-established surgical technique, with multiple application methods tailored to the depth and extent of corneal damage.

In addition to sutured grafts, sutureless cryopreserved and dehydrated amniotic membrane devices mounted on ocular rings have expanded outpatient treatment options. More recently, amniotic membrane extract eye drops (AMEED) have emerged as a minimally invasive therapeutic approach, with preliminary studies demonstrating improvements in tear film parameters, ocular surface integrity, and symptoms in conditions such as dry eye disease and ocular chronic graft-versus-host disease. Despite promising clinical outcomes, significant challenges remain, including the lack of standardized extraction, preservation, and quality-control protocols for amniotic membrane derivatives. Variability in biological composition related to donor characteristics and processing methods may influence therapeutic efficacy. Further well-designed controlled clinical trials, standardization of manufacturing procedures, and long-term safety assessments are required to fully validate and integrate amniotic membrane–based therapies into routine ophthalmic practice.

Keywords: keywords: amniotic, membrane, amniotic membrane extract, eye drops, ocular surface disease, serum

1. Introduction

The human amniotic membrane (HAM) constitutes the innermost layer of the placenta and has been utilized for several decades across multiple medical specialties, including plastic surgery, otolaryngology, regenerative medicine, and ophthalmology. In ophthalmic surgery, it is widely employed due to its broad spectrum of biological properties [1]. HAM represents a source of stem cells and, owing to its high content of growth factors, supports tissue repair processes, making it an effective biological dressing frequently applied in ocular surface disorders [2].

The amniotic membrane is rich in bioactive molecules, including platelet-derived growth factors (PDGF-AA, PDGF-BB), hepatocyte growth factor (HGF), transforming growth factor beta (TGF-β), basic fibroblast growth factor (bFGF), interleukin-10 (IL-10), tissue inhibitors of metalloproteinases (TIMP-1, TIMP-2, TIMP-3, and TIMP-4), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF). These components contribute to its anti-inflammatory and pro-regenerative effects [3–5]. The absence of major histocompatibility complex antigen expression enables the use of HAM in ocular surface reconstruction without the risk of graft rejection or the need for systemic immunosuppression [6].

Additionally, HAM contains antimicrobial substances such as lysozyme, bacteriocins, immunoglobulins, and transferrin, conferring antibacterial activity. The presence of nerve growth factor (NGF) provides a promising therapeutic avenue for corneal pathologies, including persistent epithelial defects and neurotrophic keratopathy [7,8].

One of the principal indications for amniotic membrane transplantation (AMT) is its application as a biological dressing in chemical and thermal ocular surface burns. In such cases, AMT promotes corneal epithelial regeneration, reduces pain, and inhibits neovascularization, particularly in grade II and III burns according to the Roper–Hall classification [10,11]. Additional indications include closure of corneal perforations, treatment of corneal ulcers of diverse etiologies (bacterial, viral, autoimmune), and symptomatic relief in bullous keratopathy, manifested by ocular pain, epiphora, and photophobia.

2. Conventional amniotic membrane transplantation

The first clinical application of human amniotic membrane dates back to 1940, when De Roth used AMT combined with chorion for the treatment of conjunctival epithelial defects and symblepharon. Subsequently, dehydrated amniotic membranes separated from the chorion were introduced [9].

HAM is obtained from donors undergoing elective cesarean section following informed consent. Donors are screened for infectious diseases, including hepatitis B and C, human immunodeficiency virus (HIV), and *Treponema pallidum*. Tissue procurement is performed under sterile conditions. The amnion is separated from the chorion and thoroughly rinsed to remove blood clots. It is then carefully spread epithelial side up, without folds or tears, onto sterile nitrocellulose membranes with a pore diameter of 0.22 μm and trimmed to the required dimensions. The prepared graft is stored in Dulbecco's Modified Eagle Medium (DMEM) at –80°C [13,14].

Several surgical techniques are currently used in ophthalmology, depending on the type and depth of corneal injury. The "sandwich" technique is applied in cases involving stromal defects with concurrent epithelial damage; the "inlay" technique is used for isolated stromal defects; and the "overlay" technique is reserved for superficial epithelial lesions [12]. Depending on the clinical scenario and availability, the membrane may be secured with sutures or tissue adhesive [15].

3. Amniotic membrane in the form of a sutureless ocular device

Commercially available products utilizing amniotic membrane in alternative formats have been introduced. One such product is ProKera® (Bio-Tissue, Inc., Miami, FL), a cryopreserved, sutureless amniotic membrane device resembling a contact lens. The membrane is mounted on a retaining ring that maintains its position on the ocular surface and ensures adherence to the affected area. Several variants are available, including ProKera Slim (with a thinner ring), ProKera Plus (with a double membrane), and ProKera Clear (featuring a central aperture to

preserve visual acuity; however, it is not suitable for central corneal pathology) [16,17]. The device is typically removed within seven days.

Other alternatives include the lyophilized product XcellerEYES, applied in conjunction with a bandage contact lens, and the dehydrated biological dressing Omnigen, which can be delivered using the dedicated OmniLenz ocular device. The lens-based format allows outpatient treatment and may be particularly useful in patients who are not candidates for surgical suturing of the membrane. However, its effectiveness may be limited in individuals with excessive eye rubbing or abnormal blinking patterns [18,19].

4. **Amniotic membrane eye drops**

Given the increasing number of patients requiring ophthalmic care, there is a need for therapeutic modalities suitable for outpatient use. In recent years, reports have described amniotic membrane extract eye drops (AMEED), although they are not yet widely available commercially. These preparations may represent an alternative to conventional treatments in dry eye disease, chemical burns, and in patients ineligible for surgical intervention [20].

To date, only two completed clinical trials investigating AMEED have been registered on ClinicalTrials.gov, both conducted in Tehran, Iran, addressing limbal stem cell deficiency and corneal healing, respectively [21,22]. A clinical study evaluating amniotic membrane–derived cytokines for dry eye disease has also been completed; however, its results have not been published [23].

Various preparation methods for amniotic membrane extract (AME) have been described. Bonci et al. utilized cryopreserved AMT homogenized with antibiotics and balanced salt solution (BSS) [24]. Baradaran et al. processed cryopreserved AMT with antibiotics, followed by freezing, grinding, and centrifugation [25]. Sabater-Cruz et al. produced a lyophilized extract from fresh amniotic membrane, allowing room-temperature storage prior to reconstitution [26]. Cheng et al. employed eye drops containing human amniotic epithelial stem cells (hAESCs) for the treatment of ocular chronic graft-versus-host disease (ocGVHD) [27]. In that study, adult patients with stable systemic GVHD but refractory ocular manifestations received two drops four times daily for six weeks. Clinical assessments were conducted at regular intervals up to

six months. Improvements were observed in tear secretion, reduction in Ocular Surface Disease Index (OSDI) scores, and decreased corneal epithelial damage.

Manufacturing processes for AME remain heterogeneous and non-standardized. Preservation methods such as dehydration, irradiation, or cryopreservation may reduce biological activity through structural and functional alterations of biomolecules. There is a lack of consensus regarding optimal extraction techniques, storage conditions, and standardized assays for assessing composition and bioactivity. Variability in growth factor concentrations depends on donor characteristics and processing protocols. Rigorous microbiological safety procedures and comprehensive donor screening documentation are essential. The clinical efficacy of AME and AMEED requires confirmation in controlled trials, including comparative studies with autologous blood-derived preparations used in ocular surface disease. Although no significant adverse events have been reported to date, long-term data in larger patient cohorts are needed. Standardization of production methods, improved preservation strategies, and further clinical research are crucial for broader clinical implementation [28].

5. **Conclusion**

Human amniotic membrane is a valuable therapeutic modality in ophthalmology due to its anti-inflammatory, antimicrobial, antiangiogenic, and epithelial regenerative properties. Its high concentration of growth factors, cytokines, and stem cells enables broad application in ocular surface diseases, including chemical and thermal burns, corneal ulcers, perforations, bullous keratopathy, and limbal stem cell deficiency. The absence of major histocompatibility antigen expression enhances graft safety by eliminating the need for immunosuppression.

Conventional AMT remains an established surgical technique. Sutureless, lens-based systems provide a viable outpatient alternative. Amniotic membrane extract eye drops represent an emerging, less invasive therapeutic strategy, particularly in dry eye disease, chemical burns, and refractory ocular chronic graft-versus-host disease. Preliminary clinical findings indicate improvement in tear film parameters and corneal epithelial integrity; however, the number of completed studies remains limited.

A major challenge lies in the lack of standardized production and preservation protocols for AME. Differences in processing techniques may influence biological composition and activity,

and growth factor variability is dependent on donor and preparation methods. The absence of unified quality-control assays and comparative trials with autologous blood-derived therapies further limits clinical translation.

Amniotic membrane and its derivatives demonstrate substantial therapeutic potential in ocular surface disease. Well-designed clinical trials, standardized manufacturing procedures, and long-term safety evaluations are essential for full validation and broader integration into routine clinical practice.

Author Contributions

A – conceptualization , A. K-K

B – methodology , A. K-K

C – check, DD

D – formal analysis, DD

E – investigation, A. K-K

F – resources, A. K-K

G - data curation, A. K-K,

H - writing - rough preparation, A. K-K

I – writing - review and editing ,A.K-K

J – visualization, DD

K – supervision, DD

L - project administration - A. K-K

M – receiving funding - Not applicable

Conflict of interest

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

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