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Systematic Review

Effects of Amniotic Membrane on Limbal Stem Cell Transplantation on Ocular Trauma in Animal Models: A Systematic Review

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Received: November 11, 2021; **Published:** January 31, 2022

Abstract

Objectives: Limbal stem cell deficiency (LSCD) is a condition which limbal stem cells (LSC) are weakened and the ability to reproduce is lost. Without the use of limbal epithelial stem cells, amniotic membrane transplantation may help restore LSCD corneas. The aim of this study was to see how amniotic membrane transplantation affected LSC transplantation in animal models of ocular trauma.

Materials and Methods: Regardless of time limitations, we searched databases such as PubMed, Scopus, Springer, Science Direct, and Cochrane. Animals with LSCD, ocular-surface injury, and epithelial wound healing met the eligibility requirements, which included descriptive and observational studies in English.

Results: In animal models, we discovered that using Amniotic membrane on limbal stem cell transplantation for ocular trauma could successfully regenerate corneal surfaces.

Conclusion: All of these characteristics of the amniotic membrane, as well as the fact that it is the only epithelium transplanted, indicate that the amniotic membrane is critical in preventing immunologic rejection of the transplanted epithelial cells.

Keywords: *Amniotic Membrane; Ocular Trauma; Limbal Stem Cell Transplantation*

Introduction

The cornea is a translucent, avascular tissue protected by non-keratinized stratified epithelium that keeps the ocular surface smooth for normal vision and acts as a buffer against environmental and external stress. Corneal, limbal, and conjunctival epithelial cells cover the entire ocular surface, maintaining its integrity with the help of a healthy pre-ocular tear film. Corneal scarring and opacity are the fifth leading cause of blindness in the world, accounting for 5.1 percent of all blindness cases [1].

Corneal epithelial stem cells, which are responsible for the renewal of the corneal epithelium, are found in the corneoscleral limbus [2,3]. Corneal epithelial stem cells are adult somatic stem cells that are found at the limbus and are the origins of the clear corneal epithelium [3,4]. These stem cells are essential for maintaining the health of the corneal surface. Limbal stem cell deficiency (LSCD) is caused by

diseases that kill these stem cells or their stromal microenvironment [4]. LSCD is an important cause of corneal scarring. It may be caused by a number of congenital or acquired factors that are infectious, immunologic, oncologic, or iatrogenic, and results in severe ocular surface dysfunction. Stevens-Johnson syndrome, ocular cicatricial pemphigoid, aniridia, chemical or thermal burns, contact lenses, a number of microbial infections, long-term use of topical (including anti-glaucoma) drugs, irradiation, tumors, multiple surgical procedures, and ocular surface cryotherapy may all cause LSCD [5]. Thousands of people in North America suffer from LSCD [6]. When these epithelial stem cells are damaged, LSCD develops [7,8]. Limbal stem cell loss may be partial or complete. Patients with LSCD experience blurred vision, discomfort, photophobia, and watering eyes [9,10]. Progressive invasion of conjunctival epithelial cells into the cornea, superficial vascularization, squamous metaplasia of corneal epithelial cells, involvement of goblet cells, and degradation of the corneal basement membrane with fibrous tissue deposition and chronic inflammatory cell infiltration characterize LSCD histopathologically [11].

Since ocular trauma is an unplanned but potentially preventable occurrence, serious and sight-threatening eye injuries are of particular public health concern. Ocular trauma epidemiology receives a steady stream of publications each year. Simultaneously, the published evidence has limitations due to differences in the definition of trauma, data sources, and service delivery [12-17].

Corneal damage may result from a variety of clinical conditions, including physical ones, anorexia, Stevens-Johnson syndrome, chemical, mechanical, or thermal injury, or immunological trauma, and permanent damage to the corneal epithelial stem cells leads to limbal stem cell deficiency (LSCD). Furthermore, primary aniridia and congenital red skin keratosis can lead to limbal stem cell deficiency or dysfunction, resulting in corneal epithelial cell loss of proliferation and a reduction in the limbal barrier, resulting in continuous corneal epithelial defects, recurrent epithelial erosion, corneal conjunctival epithelial ingrowth, and corneal neovascularization [18,19]. Both the eye limbus and central epithelia can be lost in a serious injury, which may be followed by inflammation, and neovascularization in the patient's eye. These pathological characteristics are important factors in vision loss, corneal blindness, and corneal transplant failure [4,20-22]. The level of deficiency in limbal epithelial stem cells (LSCs) determines the prognosis of corneal damage in many cases [4,21].

While just five layers thick, the human adult cornea performs critical sensory and defensive functions. The transparent tissue allows light to pass through and acts as a refractive lens, allowing light to pass through to the light-sensitive retina. The cornea also acts as a physical shield to external insults, protecting the inner ocular tissue [23]. The human corneal epithelium is made up of 5 - 7 epithelial cell layers, each of which contains three types of cells: superficial cells, wing cells, and columnar basal cells [24,25]. The corneal epithelium's main functions are to provide a smooth corneal surface by contact with the tear film and to serve as an effective shield against pathogenic species, chemical agents, and trauma through complexes of cellular junctions [24,25].

The extreme ocular surface disease develops when the limbus is destroyed. Epithelial defects, repeated erosions, recurrent stromal inflammation, corneal vascularization, and conjunctival epithelial ingrowth are all symptoms of this condition, which may contribute to corneal blindness [4,21]. The transplantation of limbal autografts containing limbal stem cells collected from the contralateral eye is used to treat this disease [26].

Amniotic membrane transplantation is an alternative treatment for LSCD (AMT). The human amniotic membrane is the placenta's innermost layer, and it has shown promise in treating ocular surface reconstruction [18,19], first as a conjunctival graft in symblepharon repair by A. de RÖTTH, and Kim et al. [27,28] defined the epithelialization of the corneal surface with cells expressing corneal type keratin to recreate the rabbit ocular surface in a chemical burn model. In bilateral trauma, this approach was combined with allograft limbal transplantation to reconstruct the ocular surface. Allograft limbal transplantation, on the other hand, requires immune response modulation [29,30].

Aim of the Study

The aim of this systematic review was to determine the effect of amniotic membrane on limbal stem cell transplantation on ocular trauma in animal models including rabbits and mice, based on a previous study that demonstrated that AMT could help restore LSCD corneas without the use of limbal epithelial stem cells.

Methods

Search strategy

The current research is a systematic review of the effects of the amniotic membrane on limbal stem cell transplantation on ocular trauma in animal models. Documents were analyzed using databases such as PubMed, Scopus, Springer, Science Direct, and the Cochrane Library, with no time limitations and among international magazines. The publishing language was English. Amniotic membrane, limbal stem cell, ocular trauma, animal models, and rabbit model were among the keywords. In MeSH, keywords were standardized and provided in a likely combination.

Selection criteria

To begin, a list of titles was compiled, and abstracts of all papers were found by searching internal and external databases. Three researchers worked independently on this project. Interventional studies to refer the impact of Amniotic membrane on limbal stem cell transplantation on ocular trauma in animal models were included in the study. The papers with duplicate titles were then deleted.

In the case of duplicate articles, the review was based on the first published study. Studies were obtained from databases that met the predetermined selection criteria and were independently evaluated by two authors. If the authors disagreed, they worked out their differences through discussion or consultation with another author.

Non-relevant articles on the type of study and subject of research, low-quality studies focused on the Critical Appraisal Skills Programme (CASP), and studies with insufficient information were among the exclusion criteria.

Quality evaluation of articles

Two researchers assessed the papers' quality using the CASP checklist. The intervention study's final CASP checklist includes ten items.

Data extraction

PICOS parameters were used to extract data. Population, intervention, comparison, results, and study design are some of these elements. The following information was extracted from the articles: the first author's name, the country in which the study was conducted, the publication date, the type of animal used, the sample size, and the study's location and population (Table 1).

	First author	Date of publication	Country	Animal	Sample	Place
128	Jae Chan Kim	1995	USA	Rabbit	23	Florida
26	Noriko Koizumi	2000	Japan	Rabbit	8	Kyoto Prefectural University of Medicine conducts animal research.
18- m	H-M Woo	2001	Korea	Rabbit	40	Seoul, Korea
16- m	Seng-Ei Ti	2002	USA	Rabbit	52	The University of Miami's Animal Research Committee
23	Samantha herretes	2006	USA	Mice	30	The animal treatment and use committee at Johns Hopkins University
126-m	H. A. Shahriari	2008	Iran	Rabbit	30	Al Zahra Eye Center, Zahedan, Iran
140	Jin A Choi	2011	Korea	Rat	30	Seoul, Korea
45	Qing Zhou	2014	Chine	Rabbit	48	The Experimental Animal Center and Ethics Committee

Table 1: *Studies on the effects of amniotic membrane on limbal stem cell transplantation on ocular trauma in different animal models.*

Result

Figure 1 shows a flow chart we extracted from relevant articles. A total of 160 papers were identified in the first stage by searching databases and bibliographies. 125 papers were not included because they did not meet the inclusion requirements. The full text of the selected papers was reviewed; as seen in figure 1, the systematic review included 8 articles.

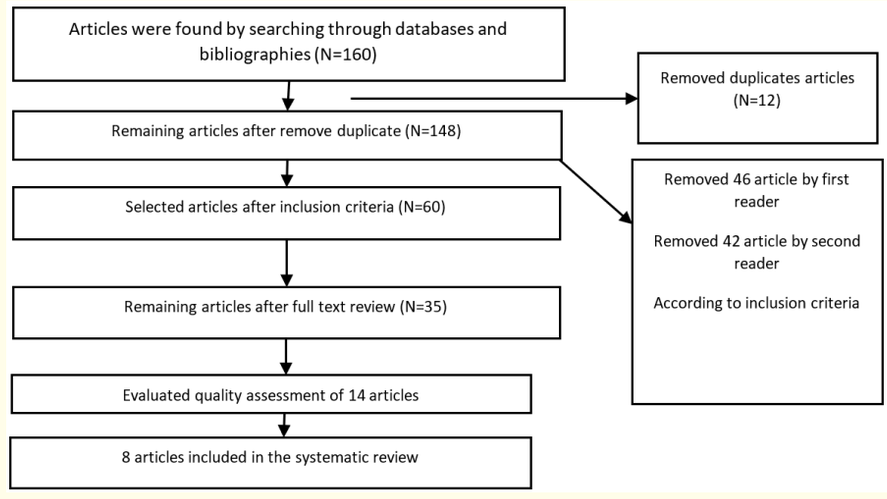

Figure 1: *Flow chart of the process of study entering into a systematic review study.*

In intervention studies, the sample size was 261 animals in the eight studies, and the details of the articles were shown in table 1. Rabbits, mice, and rats were included in the experiments. These studies looked at diseases including limbal stem cell deficiency, epithelial wound healing, chemical burns, alkali injuries, ocular-surface injuries, and total corneal epithelium removal (Table 2).

	First author	Follow-up	Nature of injury	Conclusion
128	Jae Chan Kim	3 months	Removal of the total corneal epithelium	Steps taken to facilitate epithelialization while preventing host fibro vascular ingrowth onto the amniotic membrane, according to this report, could make this procedure clinically useful for ocular surface reconstruction.
26	Noriko Koizumi	1 month	Ocular-surface injury	Using an acellular amniotic membrane as a transporter, autologous transplantation of cultivated corneal epithelium is possible.
18-m	H-M Woo	8 weeks	Epithelial wound healing	The use of the AM as a dressing on a corneal wound created by excimer laser surgery, where severe haze is required, can result in rapid epithelial healing and a reduced inflammatory response. The AM can prevent abnormal stromal collagen synthesis, which is linked to corneal haze.
16-m	Seng-Ei Ti	1 year	Totally limbal stem cell deficient	This study shows that after transplantation of autologous, *ex vivo* LSC grafts expanded on AM, rabbits can have a long-term follow-up of more than a year. The success of this model can be attributed to the use of rabbits as a culture substrate and transplant carrier rather than human AM.
23	Samantha herretes	2 weeks	Chemical burn, Acute ocular alkai injuries	Preterm/term with a current theme In this animal model, HAF was a successful topical treatment for reducing eye damage after acute alkali burns.
126-m	H. A. Shahriari	1 week	Central corneal alkali wound	This study found that amniotic membrane suspension heals alkali-injured corneal epithelial wounds faster than autologous serum or preservative-free artificial tears.
140	Jin A Choi	----	Alkali burn	In alkali burns, the suspension shape of the amniotic membrane aided epithelial healing and decreased corneal opacity and NV.
45	Qing Zhou	-----	Limbal deficiency	This research suggests that corneal epithelium reconstruction can be accomplished using HAECs-rabbit corneal stroma tissue engineering cornea grafts.

Table 2: *Follow-up period, diagnosis and results of eight studies entered to review systematically.*

Only the amniotic membrane was studied in eight of the eight articles included in this systematic review. The findings of eight papers are discussed separately in the following parts:

1. In 1995, Jae Chan Kim., *et al*. evaluated 23 rabbit eyes for conjunctival epithelial ingrowths, vascularization, and chronic inflammation, all of which are symptoms of LSCD. A complete keratectomy was performed on 10 control eyes, and the glycerin-preserved human amniotic membrane was transplanted into 13 experimental eyes. After a three-month follow-up, all control corneas were vascularized; however, 5 corneas were transparent, while 8 corneas developed cloudier stroma and vascularization. This study found that amniotic membrane transplantation helps to restore the healthy normal cornea epithelium by repairing the basement membrane [20].

2. Amniotic Membrane as a Substrate for Cultivating Limbal Corneal Epithelial Cells for Autologous Transplantation in 8 Rabbits was studied by Noriko Koizumi., *et al*. in 2000. They signified eyes that had been grafted with an amniotic membrane containing epithelial cells that had been cultured. However, up to 5 days after surgery, they were all successfully epithelialized [31].

3. In a study published in 2001, H-M Woo., *et al*. discovered that most ablated eyes in the AM group were almost completely re-epithelialized 72 hours after surgery, while defective epithelium existed in eight of ten eyes in the control group. The AM group's areas shrank far more quickly than the control groups. We found a significantly shorter latent phase and more active epithelial migration in AM-coated corneas than in the control group, particularly from 6 to 24 hours [32].

4. In 2002, Seng-Ei Ti., *et al*. examined the left eyes of 52 rabbits who had their entire corneal epithelium debrided with n-heptanol, resulting in complete limbal stem cell deficiency. They claim that the human AM patch serves a function other than simply offering protection from exposure. Furthermore, the results of this study showed that rabbit AM culture can be used to expand LSCs *ex vivo*. Total LSCD-affected corneal surfaces can be successfully reconstructed using extended LSCs. This animal model can be used to examine culturing variables that affect epithelial stemness in order to successfully regenerate corneas with complete LSCD [33].

5. Samantha Herretes., *et al*. investigated 30 mice with acute ocular alkali injuries in 2006. In comparison to topical isotonic saline solution, they found that topical preterm and term human amniotic fluid reduced corneal opacity, scarring, and promoted re-epithelization [34].

6. Shahriari., *et al*. found that amniotic membrane suspension was effective in treating alkali injures in both control and experimental models in 30 rabbits in 2008. Amniotic membrane suspension treats epithelial wounds quicker than autologous serum or artificial tears. The suspension of the amniotic membrane returns a smooth corneal surface with minimal neovascularization and opacity [35].

7. In 2011, Jin A Choi., *et al*. investigated the effects of amniotic membrane suspension in the rat alkali burn model, finding that both the AM suspension and serum eye drop groups experienced substantially faster healing than the control group in terms of re-epithelialization. In inflammatory corneal wounds, the AM suspension was found to improve epithelial healing and decrease corneal opacity and NV [36].

8. In 2014, Qing Zhou., *et al*. conducted a study of 48 rabbits suffering from limbal deficiency. It is concluded that HAECs can be induced to differentiate into corneal epithelial cells and that HAECs-rabbit corneal stroma tissue-engineered cornea grafts are ideal for corneal epithelium reconstruction in LSCD cases [37].

Discussion and Conclusion

We searched articles in a variety of databases for this systematic review. The abstracts and text of the papers were thoroughly examined from a variety of perspectives. The content of 14 publications was assessed, and six papers were then included in the systematic review.

As a result, this review included eight separate design experiments with a total of eight articles. There was no way to meta-analyze studies because there was no standard index for determining the function of amniotic membrane on limbal stem cell transplantation on ocular trauma in different animal models.

Due to the challenges associated with the function of amniotic membrane in limbal stem cell transplantation, we decided to see if transplanting human and animal amniotic membrane as well as autologous sheets of limbal epithelium could be used to restore the ocular surface. It's still uncertain if a cornea made from rabbit, rat, and mouse corneal stroma and amniotic membrane has normal function and can achieve ocular surface reconstruction. Therefore, this systematic review study aimed to determine the impact of amniotic membrane on limbal stem cell transplantation on ocular trauma in rabbit, rat, and mouse models.

In animal models, we discovered that using amniotic membrane on limbal stem cell transplantation for ocular trauma can successfully reconstruct corneal surfaces damaged by total LSCD, is an effective topical therapy for limiting the damage after acute alkali burns of the eye, and can reconstruct the corneal epithelium [36,38]. This is the first systematic review to examine at the impact of the amniotic membrane on limbal stem cell transplantation in rabbits, mice, and rat [14,16,22,23,25,27,28,33,34,37-39]. Some researchers believe that intact HAM promotes limbal stem cell preservation and growth, while denuded HAM promotes a corneal epithelial phenotype [40,41].

The corneal basement membrane stimulates cultured conjunctival epithelial cells to express K12 keratin, according to Jae Chan Kim., *et al* [28]. This was first hypothesized by Rodriguez., *et al*. [42] but confirmed by Kurpakus., *et al*. [43], who showed that corneal basement membrane stimulates cultured conjunctival epithelial cells to express K12 keratin. The amniotic basement membrane has been shown to replace some properties of the corneal basement membrane by Jae Chan Kim., *et al* [28].

We conclude that sheets of limbal epithelial cells could be obtained and used as grafts over a new basement membrane given by the amniotic membrane based on these findings [44,45]. The amniotic membrane is a thick basement membrane that secretes a variety of cytokines that can help epithelial cells expand by creating an ideal microenvironment [46,47]. The amniotic membrane, according to Marcel Avila., *et al*. shielded these cells from rejection [39]. The amniotic membrane is used with epithelial grafts to prevent cultivation. As seen in previous studies, the use of the second amniotic membrane as a dressing patch (epithelial face down) allows for re-epithelialization underneath this membrane [48,49]. Several studies have shown that human amniotic fluid has unusual properties that reduce wound contraction and are involved in a variety of processes that lead to scarring [50-52]. Tissue engineering techniques, especially those involving amniotic membrane, have shown promise in treating corneal injuries in recent years [53-58].

Only a few studies on the effects of amniotic membrane on limbal stem cell transplantation on ocular trauma in animal models were available, which limited our research.

All of these characteristics of the amniotic membrane, as well as the fact that it is the only epithelium transplanted, indicate that the amniotic membrane is essential for the transplanted epithelial cells to remain immune-free. Amniotic membrane transplantation with autologous limbal cells opens up new possibilities for ocular surface reconstruction, especially in bilateral ocular surface lesions.

Acknowledgments

We appreciate faculty member of Mashhad University of Medical Sciences (MUMS).

Conflict of Interest

The authors declare no conflict of interest.

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