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The application of human amniotic membrane in the surgical management of limbal stem cell deficiency

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Abstract

The application of human amniotic membrane (AM) has a wide spectrum of indications in the treatment of ocular surface disorders. Transplantation of AM has been incorporated routinely as a component of ocular surface reconstruction in a variety of ocular pathologies. The application of human AM can be combined with nearly all types of limbal transplantation in treating limbal stem cell deficiency (LSCD). AM provides support and possible protection to the transplanted limbal tissues and limbal stem cells owing to its mechanical and biological properties, and these properties are thought to enhance the success rate of LSC transplantation. This paper reviews the current literatures on the applications of AM in the surgical management of LSCD and summarizes the outcome of different surgical approaches. The current literature contains mostly low-level evidences in supporting the role of AM. The efficacy of AM in LSC transplantation needs to be confirmed by randomized controlled clinical trials.

I. Introduction

Limbal stem cells (LSCs) are responsible for the regeneration of corneal epithelial cells and the maintenance of the integrity and transparency of the corneal epithelium.[1] The destruction to LSCs and/or the stem cell niche leads to dysfunction or deficiency of LSCs. Limbal stem cell deficiency (LSCD) is characterized with impaired epithelial wound healing, recurrent epithelial erosions, and scarring and opacity of corneal stroma. It is one of the causes of corneal blindness. The common etiologies of LSCD include chemical/thermal burn, contact lens wear, congenital abnormalities, iatrogenic trauma, severe microbial infection, and chronic cicatricial inflammation such as Stevens-Johnson syndrome and mucous membrane pemphigoid.[2]

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The treatment of LSCD is challenging because corneal transplant cannot treat LSCD and will fail after the surgery. Medical treatment has limited success. Only a few mild LSCD cases are reversible by medical treatments.[3] Surgical management is usually performed in cases of moderate to severe LSCD. The surgical treatment of LSCD to restore a stable ocular surface can be divided into three groups: direct transplantation of limbal tissues, transplantation of ex vivo/in vivo expanded LSCs, and transplantation of cultivated oral mucosal epithelium. Transplantation of AM has been incorporated into the LSC transplantation in nearly all surgical approaches. AM can be used alone, or as a substrate and cell carrier of LSCs. Therefore, we performed a systematic review to investigate the application of AM in the surgical management of LSCD.

II. Search Method

We performed a systematic literature search on PubMed and Medline for the papers published before December 31, 2017. The following combined search terms were used: “limbal stem cell deficiency”, “amniotic membrane”, “surgical treatment”, “limbal transplantation”, “cultivated limbal epithelial transplantation”, “simple epithelial transplantation”, “cultivated oral mucosal epithelial transplantation”, “conjunctival limbal autograft”, “conjunctival limbal allograft” and “keratolimbal allograft”. Only human studies with 15 or more cases are included in the outcome evaluation. Literature reviews, correspondence, notes, editorials and conference abstracts were excluded in the outcome evaluation. Neither language filter nor limitation of publication time was applied during the literature search. The non-English articles were translated to English to obtain the needed information. We also reviewed the references from retrieved studies manually to identify relevant articles. The data on the preparation and preservation of AM, indications of the surgery, surgical techniques, and clinical outcomes were collected.

III. Results

A. Property and preparation of human amniotic membrane

1. Structure—Amniotic membrane, which is semi-transparent, is the innermost layer of the placenta. It is composed of three layers: a monolayer of epithelium, a thick basement membrane and the avascular stroma. The basement membrane of AM, one of the thickest membranes found in human, is similar to the basement membrane of human corneal and conjunctival epithelium in composition.[4] The structural integrity of this layer does not alter after current cryopreservation techniques.[5]

2. Properties—Human AM has multiple functions in the reconstruction of ocular surface. Mechanically, its toughness and elasticity provides mechanical support and protection to the epithelial cells. Biologically, it could promote the adhesion and migration of limbal epithelial cells and retain their in vivo properties.[6,7] Moreover, it has the properties of anti-fibrosis, anti-inflammation, anti-angiogenesis, and anti-bacteria.[8] Several recent studies show that a novel matrix component termed heavy chain-hyaluronan/pentraxin 3 (HC-HA/PTX3) purified from cryopreserved AM is the active component responsible for the aforementioned AM's biological properties.[9,10] HC-HA/PTX3 complex also uniquely maintains limbal niche cells to support the quiescence of LSCs.[10] In addition, AM has low

immunogenicity because there is a lacking expression of human leukocyte antigen-A, B, or DR antigens.

3. Preparation, sterilization and preservation

a. Preparation: The method of AM preparation was first described by Tseng.[11] In brief, the donors of placenta are selected by serological tests to exclude hepatitis B virus, hepatitis C virus, human immunodeficiency virus (HIV), and syphilis. The placenta is washed by a sterile antibiotic solution, which contains 50μg/ml of penicillin, 50μg /ml of streptomycin, 100μg g/ml of neomycin, and 2.5μg /ml of amphotericin B. Then the AM is separated from the rest of the chorion by blunt dissection, and placed on the nitrocellulose filter paper (pore size: 0.45μm) with the stromal side facing down. The filter paper and the adherent AM was then cut into pieces with the approximate size of 3 cm × 4 cm. This method are used by many study centers with some minor modifications in different studies.[12-15]

b. Sterilization: The cryopreserved AM is usually treated with antibiotics and antimycotics as mentioned above to prevent microbial infection from contamination during processing. Alternatively, the freeze-dried or air-dried AM is usually sterilized either by 25 kGy gamma irradiation,[16-18] or by peracetic acid/ethanol mixture.[19] It is also reported that supercritical carbon dioxide can be used to sterilize AM tissue grafts with good preservation of their biological features.[20]

c. Preservation: Although non-preserved AM was used in some studies,[21,22] it is generally recommended that AM is preserved for at least 4-6 months before the confirmation of the HIV negative status of the donor by repeated serology. [18,23,24] The most common method of preservation is cryopreservation. The AM is mounted on a nitrocellulose filter paper is stored at −80 °C in a sterile vial containing Dulbecco modified Eagle medium and glycerol at the ratio of 1:1 (v/v). The cryopreservation of a suspension containing homogenized amniotic membrane was also reported in a small clinical trial.[25]

AM can also be preserved under a freeze-dried (lyophilized) [26] or air-dried [16] condition. Only one small study compared fresh and dried AM in the treatment of partial LSCD. The outcomes at 24 weeks were similar between these two preservation methods.[27]

d. Removal of epithelium: According to different clinical purposes, AM (cryopreserved, lyophilized or dry) might be used as intact (with intact epithelium) or denuded (epithelium is removed). Denuded AM has been shown to have less immunogenicity, support the proliferation of LSCs better and preserve a higher clonogenicity.[12,28] Removal of the epithelium could be accomplished by NaOH, urea (5M) treatment or mechanically scraping by using a cell scraper with or without the combination of trypsin, EDTA, dispase, or thermolysin.[13,14,29-32]

e. Effect of AM preparation, sterilization and preservation on its biological properties: A laboratory study [12] compared the effect of different methods of epithelial removal (intact, partial denuded, fully denuded), sterilization (peracetic acid sterilized, nonperacetic acid sterilized) and cryopreservation (DMEM/glycerol, glycerol only, no glycerol) on AM and its impact on the cultured LSCs. The findings showed that complete

removal of epithelium facilitated the migration and confluence of LSCs and did not affect the biological properties of LSCs. However, the use of glycerol as a cryoprotectant seemed to impair the function of AM to support the growth of LSCs, leading to a poorer morphology of LSCs and a lower percentage of cells expressing LSC biomarkers. Moreover, sterilization by gamma irradiation has been shown to cause a significant decrease of growth factors and the structural alteration of basement membrane.[33,34] The optimal method to prepare, sterilize and preserve AM still needs to be investigated to optimize the function of AM for different applications in ophthalmology.

B. Application of AM in surgical treatment of LSCD

1. Transplantation of AM alone

a. Indications: AM transplantation (AMT) is widely used in the treatment of acute phase of chemical burn, thermal injury or Stevens-Johnson Syndrome to promote epithelium healing, and alleviate ocular surface inflammation which might rescue the residual LSCs.[21,22,35-37] In these cases, AM is serving as a temporary overlay patch to mechanically protect the ocular surface, promote normal epithelial wound healing and prevent intermediate-term ocular cicatricial sequelae.[37] However, prospective, randomized, controlled clinical trials showed that no definite long-term advantage of AMT alone over medical therapy in terms of final visual outcome, appearance of symblepharon and corneal vascularization.[38-40]

AM transplantation also have been used to treat partial LSCD.[15,24,27,41-44] Although AM is believed to serve as the permanent graft in these cases and to provide a surrogate basement membrane for the regenerated epithelium, the histological study confirmed the complete integration of AM with corneal stromal tissue,[45] which suggests the effect of AM was more through its biological properties than mechanical properties.

b. Surgical technique: After debridement of fibrovascular pannus and removal of scarring and inflamed tissue, AM is removed from the storage medium, and placed over the denuded cornea, limbus and conjunctiva (Figure 1A). In a majority of studies, AM was placed with the epithelium/basement membrane side facing up.[24,27,35-37,41,43,45-48] The placement of AM with the stromal side facing up was only used in only two studies.[35,41] However, some studies did not specify the orientation of the basement membrane.[15,49-51] AM was then secured to the cornea with 10-0 or 11-0 nylon sutures [24,48] or/and to the surrounding conjunctiva with 9-0 or 10-0 Vicryl sutures.[35,49] Recent studies showed that fibrin glue could be used to avoid suture-related disadvantages and complications.[37,42]

Occasionally sectorial sequential conjunctival epitheliectomy (SSCE) combined with AMT is used in the treatment of partial LSCD.[44,50] It is a surgical procedure in which the abnormal conjunctival epithelium on the cornea is removed by mechanical superficial debridement. The denuded corneal and limbal surface could be re-epithelialized by corneal epithelial cells that migrate from the unaffected area of cornea and limbus.[52] The limitation of SSCE is that it could cause persistent epithelial defect and pain from the epithelial debridement. Multiple treatments are often required to achieve satisfactory outcome in

successful cases. The combined AMT might reduce bleeding, pain and promote epithelialization.

c. Outcome: As shown in Table 1, a total of 8 studies reported the outcome of AMT alone in the treatment of partial LSCD. After AMT for the treatment of partial LSCD, the mean time of complete corneal and conjunctival re-epithelialization is usually 2-3 weeks.[15,24,43] The mean time of the maintenance of a stable corneal epithelial surface is 14-25 months after surgery, along with less stromal opacity and vascularization.[24,46,47] Visual improvement is found in 25%-81% eyes. [15,24,27,42,43,46,49] However, the long term success rate of AMT following superficial keratectomy in cases with partial LSCD is only 40%–54% at an average follow-up period of 52 months.[43]

2. Direct transplantation of limbal tissues with AM

a. Indications: Direct LSCs transplantation includes conjunctival limbal autograft transplantation (CLAU), conjunctival limbal allograft transplantation (CLAL) and keratolimbal allograft transplantation (KLAL). Keratolimbal autograft transplantation (KLAU) has only been published by two case reports[53,54] because of the requirement of large graft size (around 180 degree) on the donor eye and the need to reconstruct the conjunctiva in LSCD eyes with abnormal conjunctiva.

CLAU is usually performed in unilateral total LSCD cases, while CLAL and KLAL are considered in bilateral LSCD cases. All of these procedures can be performed with the combination of AMT.

b. Surgical technique

(1) Under the limbal tissue in the recipient eye (inlay): After the removal of conjunctival and dermal-like epithelium covering the cornea, the dissection of fibrous tissues and the releasement of existing symblephon, AM was placed on the denuded ocular surface and secured with suture or fibrin glue. Then the limbal graft is sutured to the original limbal area (Figure 1B).[15,49,50,55-72] In these cases, AM is thought to reduce postoperative inflammation and scarring in the underlying stroma. Moreover, many researchers thought that a combination of AMT might secure an environment favorable for the regeneration of LSCs, [58,64-66] thus reducing the requirement of graft size and decreasing the risk of iatrogenic of LSCD in the donor eye.

(2) Covering the limbal tissue in the recipient eye (overlay): After the fixation of limbal grafts, AM was used as a temporary patch to cover the limbal grafts and the entire ocular surface at the end of the surgery (Figure 1C). [63,68,73-77] In some studies, AM are placed both under and over the limbal grafts, which is called “Sandwich” technique (Figure 1D). [58,61,63-65,67,78] The role of AM in this condition is similar to the contact lens, which provides mechanical protection to the limbal grafts and regenerated epithelium from external insults, and relieves ocular symptoms such as pain, photophobia and discomfort after surgery.

(3) Serving as the patch in the donor eye: The efficacy of the transplantation of 2 clock hours (60°) of donor limbus for a permanent and stable epithelialization of the cornea has been reported.[58,69] However, it is generally presumed that at least three to four clock hours (90°–120°) of a conjunctival–limbal graft is usually required to obtain enough amount of LSCs in the graft, either from the healthy contralateral eye (CLAU) or from an eye of a living relative (lr-CLAL).[15,46,66,79] Therefore, there is a risk of developing LSCD in the donor eye. In these cases, AM used as a temporary patch in the donor eye [58,64] may be helpful to reduce the risk of iatrogenic LSCD after graft removal because AM is thought to provide support for restoring the remaining functional LSCs.[36,64,68] However, all these reports are retrospective uncontrolled studies. There is no high-level data demonstrating the advantage of AMT in the donor eye.

(4) CLAU combined with AM-assisted SSCE: It is recently reported that a modified AM-assisted SSCE, named as amnion-assisted conjunctival epithelial redirection, could be combined with CLAU.[80,81] It was advocated that AM might play a role in redirecting conjunctival epithelium and preventing admixtures of conjunctival epithelial cells and limbal explant-derived corneal epithelial cells on to the corneal surface.

c. Outcome

(1) Conjunctival limbal autograft/conjunctival limbal allograft: A total of 17 studies reported the outcome of CLAU/CLAL with or without combined AMT after the follow-up of ≥12 months. Among them, only two studies directly compared the outcome with or without the use of AM in CLAU/CLAL. Ivekovic et al[46] compared the time required to re-epithelialize after AMT, CLAU, and CLAU combined with AMT. The mean re-epithelialization time was 24.6 days, 14 days and 15.3 days in each group, respectively. There was no difference between CLAU and CLAU+AMT, both of which were shorter than AMT only. However, Barreiro et al[59] reported that although the final graft survival rate was similar between groups with or without the use of AMT, re-epithelialization time was significantly longer in the group using AMT.

The other studies are non-comparative studies. They only focused either on CLAU/CLAL with AMT,[15,62,65-67,70,75,78,82] or CLAU/CLAL without AMT. [83-88] Although a higher or similar successful rate with AMT (Table 2) was reported in the majority of studies , the study designs and patient populations were quite different (Table 1). Therefore, there is insufficient evidence to support the advantages of combined use of AMT in CLAU/CLAL either to promote epithelial healing or to increase the graft survival, even though AMT is used as a routine procedure in many cases of CLAU/CLAL.

(2) Keratolimbal allograft: A total of 16 studies reported the outcome of KLAL after the follow-up of ≥12 months, 10 studies with AMT, [49,55,56,63,65,71,72,76,77,89] and 6 without AMT. [88,90-94] No comparative studies have been performed yet. The successful rate of KLAL, no matter AMT is used or not, has a similar decreasing tendency with the prolongation of follow-up. Table 2 showed that AM played a minor role in the graft survival after KLAL. Although the authors suggested that the application of AM could reduce the

postoperative inflammation and complications in these cases, the function of AM in KLAL needs to be investigated by further comparative studies.

3. Transplantation of ex vivo cultured cells on AM

a. Indications and presumed function of AM: For patients who have bilateral total limbus damage without residual LSCs, or those who do not have enough healthy limbal tissue in the other eye to harvest sufficient amount of LSCs, transplantation of ex vivo cultured and expanded cells is one of main approaches for the treatment of LSCD to restore the structural and functional integrity of corneal surface. The most commonly used cell sources for transplantation are human limbal epithelium[95] and oral mucosal epithelium.[30] The procedure is called “cultivated limbal epithelial transplantation (CLET)” and “cultivated oral mucosal epithelial transplantation (COMET)” respectively. The applications of human bone marrow mesenchymal stem cells,[96] human conjunctival epithelial cells,[97] and human nasal mucosal epithelial cells [98] have also been reported. The cell source can be taken either from the patient (autologous), or from an eye of a living relative or cadaveric tissue (allogenic). The biggest advantages of this technique is the minimal need of donor tissue (less than $1mm^2$)[99,100] and the lowest risk for the donor eye.

Many materials such as AM,[95,100,101] fibrin sheet, [99,102,103] contact lenses,[104] and nylon sheet[105] have been reported to serve as the substrate and carriers of cultured LSCs or oral mucosal epithelial cells. Among them, AM is still most commonly used. AM usually serves as a surrogate basement membrane for cultured cells and the substrate as a cell carrier in CLET or COMET. Although both de-epithelialized (denuded) and intact AM can be used, de-epithelialized AM is better than intact AM because it preserves the properties of LSCs better and facilitates the migration and confluence of LSCs.[12] Moreover, it has been reported that some limbal epithelial stem cells underwent epithelial-mesenchymal transition and invaded the limbal stroma when cultured on intact AM.[28]

It has been shown that AM preferentially preserves and expands limbal epithelial cells that retain their in vivo properties of slow cycling, putative marker expression, and an undifferentiated state.[6,106-114] The maintenance of a limbal epithelial phenotype indicates that AM provides a unique stromal microenvironment beneficial to the preservation and expansion of LSCs. AM also prevent cultured LSCs from undergoing apoptosis through interleukin-1 receptor antagonist.[115]

b. Methods of cultivation on AM: After the biopsy of limbus or oral mucosa, careful removal of excessive tissue, and rinsing with culture medium containing antibiotics, there are two methods to culture cells on AM. One is chopping the tissue into small pieces and then placing the explant on the epithelium/basement membrane side of AM.[6,32,95,100,108-113,116-120] The orientation of limbal explant on AM, either epithelial side or stromal side facing up, does not influence tissue adhesion and cell expansion.[100] The other method is incubating the biopsy tissue with trypsin, EDTA and dispase to obtain single cell suspension first. Then these single cells are seeded on AM with or without the presence of irradiation- or mitomycin C-treated 3T3 feeder cells.[30,96,107,110,121-131] These two methods do not have differences regarding the cell growth and phenotype.[110]

A minimum size of 0.3mm^2 live limbal tissue or 0.5mm^2 cadaveric limbal explant is required to achieve sufficient cells for expansion and transplantation.[100] Limbal explant takes more time to reach a linear growth phase if it is retrieved from corneo-limbal rings or discs with a longer duration of organ culture.[132] As for oral mucosal biopsy, at least a specimen with the size of 2 ~3 mm^2 is needed.[122] The successful rate of ex vivo cultured and expanded cells on AM is reported to be 96.2%-98.5%.[6,112]

c. Surgical technique: Corneal fibrovascular tissue and perilimbal subconjunctival scarring tissue are dissected and removed to the bare sclera at least 2 to 3 mm behind the limbus. Symblepharon are released if necessary. Then cultured epithelial cell sheet, together with the amniotic membrane substrate, is placed on the cornea with the epithelial side up. The graft is secured with either suture or fibrin glue.

d. Outcome

(1) CLET: Owing to the small size of tissue needed for ex vivo culture and the fact that antigen presenting cells do not survive during culture,[133] the rejection rate of CLET is relatively low even in allogenic cases. The overall successful rate of CLET is stable after one year postoperatively.[29,32,93,110,113,114,117-120,134-142,143,144] Nevertheless, the successful rate is influenced by many factors including age, donor source, and cell quality.[99,117,120,134,136,137] it should be noted that the clinical outcomes of the transplantation of LSCs cultured on AM and fibrin are similar, as shown in Table 3. Fibrin is easier to be standardized, but AM has a wider accessibility, especially in the developing countries.

(2) COMET: The overall successful rate of COMET is stable after two years postoperatively.[123,126,127,129,145] Although Kim[146] and Hirayama[147] reported that the transplantation of substrate-free oral mucosal cell sheet achieved better clinical outcomes (87.5% and 62.5%, respectively) than AM group (44%), the mean follow-up was only one year after surgery, as shown in Table 3. Its midterm and long-term outcome needs to be evaluated by further studies.

The result of immunostaining and RT-PCR showed that the oral mucosal epithelial cells cultured on AM expressed putative markers of progenitor stem cells, namely p63 and ABCG2, and markers of epithelial differentiation such as CK3 and connexin 43.[123-125,127,128,148,149] However, neither CK12, the corneal epithelium-specific marker, nor Pax6, an eye-specific transcription factor, was expressed in these transplanted oral mucosal cells.[150] These results suggest that although oral mucosal epithelial cells cultured on AM achieved a similar phenotype of limbal and corneal epithelium, they do not undergo a true transdifferentiation.

4. Transplantation of in vivo expanded LSCs on AM

a. Indications: A novel surgical technique named as “simple limbal epithelial transplantation (SLET)” was firstly described by Sangwan.[151] It allows the in vivo expansion of small pieces of limbal biopsy on AM, combining the advantages of CLAU (low cost, single staged, no requirement of clinical-grade laboratory) and CLET (using minimal

donor tissue). Both fresh AM and cryopreserved AM are applicable.[151,152] This technique is mainly used in the treatment of unilateral LSCD.

b. Surgical technique: AM is considered to provide a suitable substrate and create a nourishing ocular surface microenvironment, allowing in-vivo expansion of LSCs from the donor tissue explants. In most SLET cases, AM is placed over the bare ocular surface and donor limbal lenticule is secured on AM with the epithelial side up (Figure 2A).[75,151,153-155] Instead, Vasquez-Perez [156] and Vazirani [157] described a modified SLET. Donor tissue explants were placed on the bared cornea surface and AM is used to cover the grafts and entire corneal surface (Figure 2B). The authors believed that placing the AM either above or below the donor tissue explants is equally effective and safe. Amescua et al [152] reported another modified SLET named as “sandwich technique” in which the limbal biopsy explants were placed between the two layers of AM with the intention of replicating a fetal environment for the stem cells (Figure 2C). This technique provides protection to the graft and stem cell niche without negative effect on the clinical outcome.

c. Outcome: SLET has an excellent outcome in the treatment of partial and total LSCD. The longest follow-up has been only 18 months (Table 1). Complete epithelialization is usually achieved within four weeks after surgery.[155] A stable and avascular corneal surface is found in 100% eyes at 6 months and 9 months, in 80% eyes at 12 months, and in 76% eyes at 18 months.[75,153,154] AMT is used in all reported cases of SLET and the actual function of AMT in SLET is unknown.

IV. Conclusions

The surgical approaches to treat LSCD vary depending on the severity of LSCD. The transplantation of AM alone seems to have limited long term effect. AMT combined with various types of LSC transplantation is commonly performed based on the presumption that AM provides biologically and mechanically support, and protection to the transplanted tissues and cells. High level studies are lacking to support the efficacy of AMT in LSC transplantation. Future randomized controlled clinical trials are needed to demonstrate the efficacy of AMT in the treatment of LSCD.

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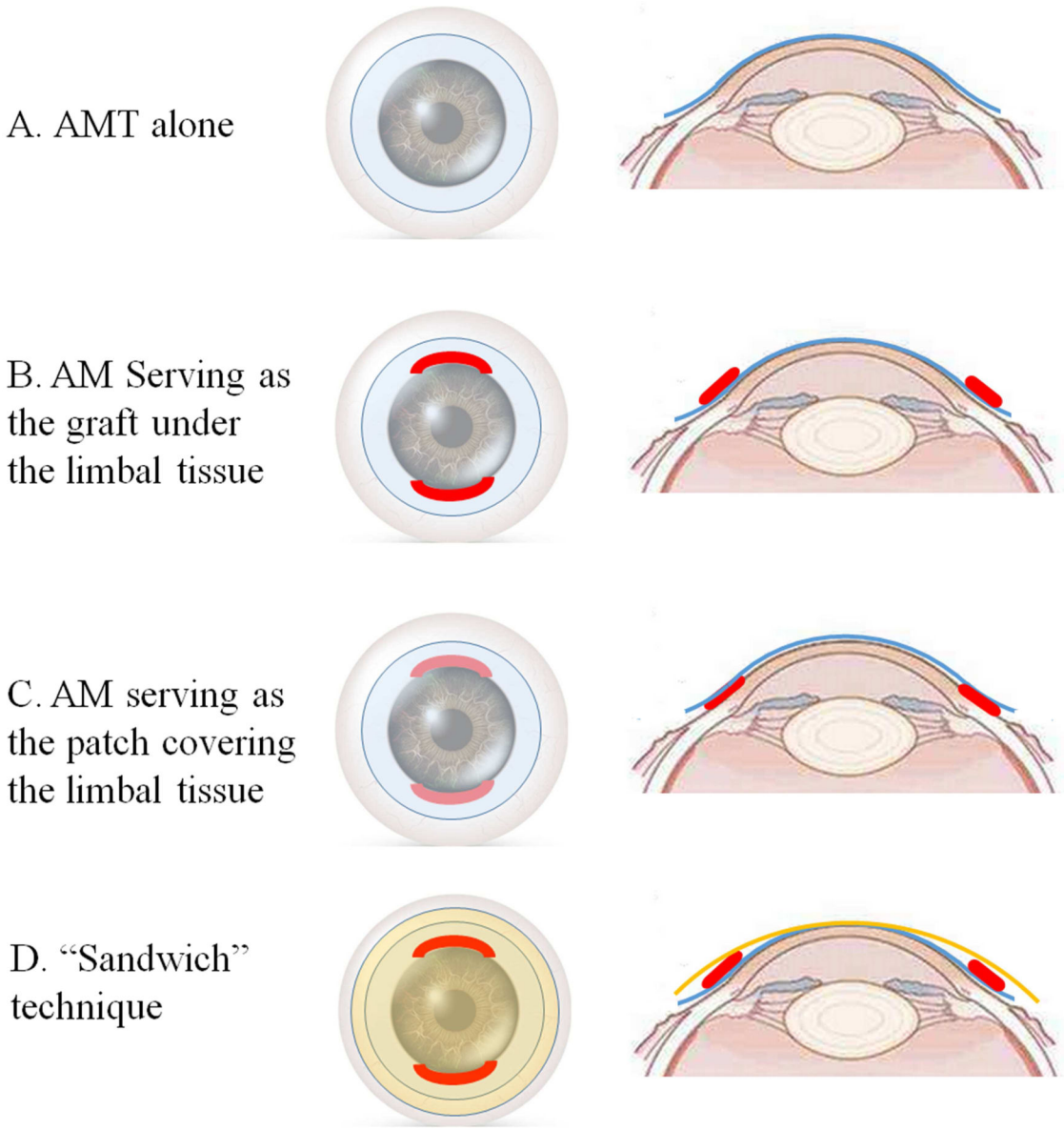

Figure 1.

Schematic diagram of amniotic membrane transplantation (AMT) alone (A) and combination of direct limbal transplantation with AMT (B to D). In AMT alone procedure, AM depicted in blue is placed over the denuded cornea, limbus and conjunctiva (A). When combined with limbal stem cell transplantation, AM is either serving as a graft under the limbal tissues depicted in red (B), or as a patch covering the limbal tissues (C). In "Sandwich" technique, AM is placed both beneath and on top of the limbal grafts. The AMs beneath and on top of the limbal tissues are labeled as blue and orange, respectively (D).

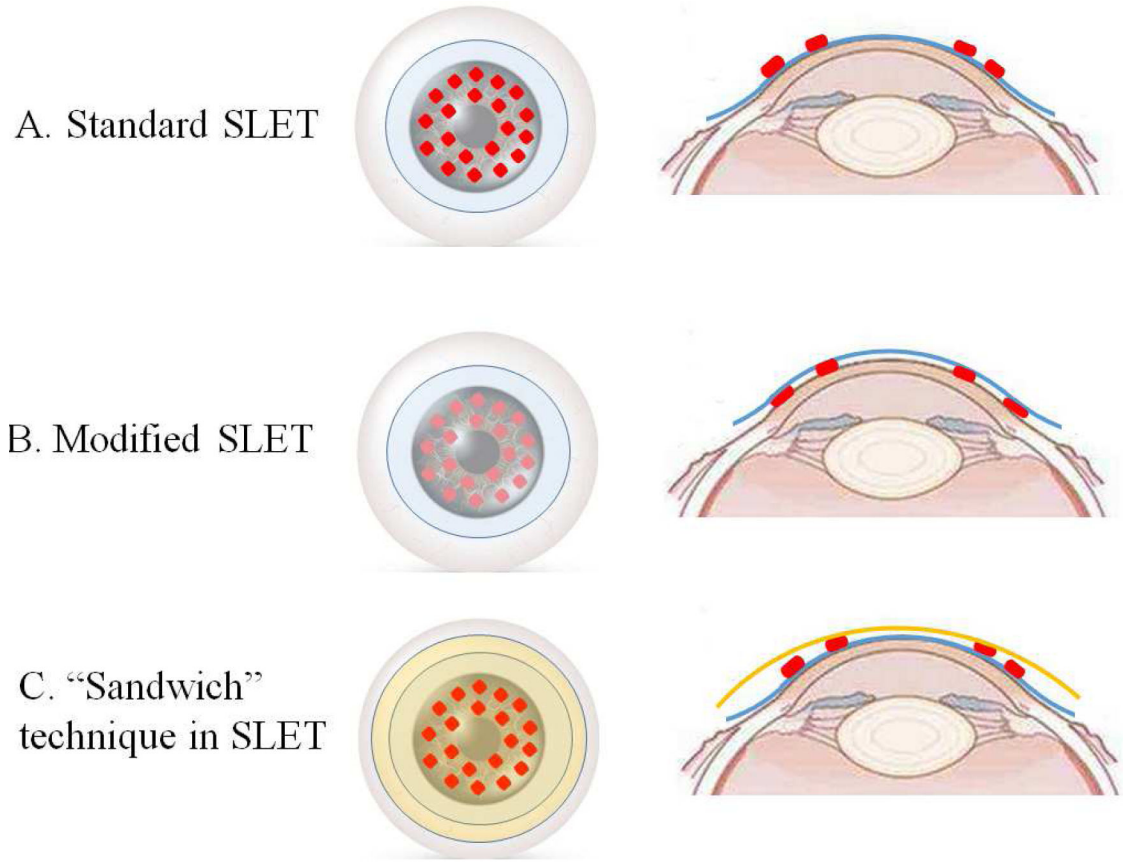

Figure 2.

Schematic diagram of different surgical techniques of SLET

In a standard SLET (A), AM depicted in blue is placed on top of the ocular surface and donor limbal biopsy explants depicted in red are secured on AM. In a modified SLET (B), donor limbal biopsy explants are placed on the bared cornea surface and AM covers the limbal grafts and the entire corneal surface. The technique in which AMs are both used beneath (blue) and on top of (orange) the limbal tissues is called "Sandwich" technique (C).

Table 1

Demographic characteristics of studies with AMT involved in the treatment of limbal stem cell deficiency

Author (Year)	Study Type	No. of Eyes	Gender		Mean Age	Etiology			Range of LSCD		Follow-up in Months
			Male	Female		Chemical/ Thermal Injury	chronic cicatricial inflammation (SJS/OCP)	Others	Total	Partial	
AMT alone											
Chung JP. 2015	P	30	15	15	48.9±16.3	3	7	30	0	30	6
Konomi K. 2013	R	16	9	7	57.4±16.4	2	2	12	0	16	52.3±26.3
Kheirkhah A. 2008	R	11	5	6	32.4±18.4	3	2	6	0	11	14.2±7.7
Lopez-Garcia JS. 2005	P	14	ND	ND	37	0	0	14	ND	ND	24
Ivekovic R. 2005	ND	5	3	2	31.6±12.3	5	0	0	ND	ND	18±4.3
Gomes JA. 2003	P	4	4	0	34.5±26.3	4	0	0	0	4	17.5±5.1
Anderson DF. 2001	R	17	9	8	42.3±4.6	8	0	9	0	17	25.8±2.5
Tseng SC. 1998	R	10	4	6	36.8±8.4	4	0	6	ND	ND	12.3±9.3
CLAU/CLAL+AMT											
Arora R. 2017	P	10	ND	ND	18±8	10	0	0	3	7	6
Moreira PB. 2015	R	28	19	9	40.3	20	3	5	ND	ND	24.8
Barreiro TP. 2014	R	15	13	2	36.3	15	0	0	15	0	19.7±5.6
Baradaran-Rafii. 2012	P	34	32	2	27.3±9.4	34	0	0	ND	ND	17.2±6.3
Miri A. 2010	R	27	19	8	ND	14	0	13	27	0	38±35.9
Scocco C. 2008	R	39	ND	ND	33.6±18.9	12	15	12	ND	ND	48.7±30.6
Santos MS. 2005	P	33	26	5	35±16	22	11	0	33	0	33±12
Lopez-Garcia JS. 2005	P	14	ND	ND	47	7	2	5	14	0	24
Ivekovic R. 2005	ND	4	4	0	27.8±7.8	4	0	0	ND	ND	12.8±1.7
Shimazaki J. 2004	R	11	11	1	40.2±14.3	11	0	0	11	0	15
Gomes JA. 2003	P	16	15	1	42.3±11.2	16	0	0	16	0	18.3±6.1
KLAL+AMT											
Baradaran-Rafii. 2013	R	45	41	4	26.7±8.7	41	4	0	ND	ND	26.1±11.8
Eberwein P. 2012	R	20	13	7	44	8	6	6	20	0	22.4
Han ES. 2011	R	24	17	5	39.4±17.4	8	8	8	ND	ND	47.3±22
Shi W. 2008	R	39	33	5	ND	39	0	0	28	11	32

Author (Year)	Study Type	No. of Eyes	Gender		Mean Age	Etiology			Range of LSCD		Follow-up in Months
			Male	Female		Chemical/ Thermal Injury	chronic cicatricial inflammation (SJS/OCP)	Others	Total	Partial	
Maruyama-Hosoi F. 2005	R	85	38	40	52.5±19.5	17	43	25	85	0	46.6
Shimazaki J. 2004	R	21	18	3	43.2±19.1	21	0	0	21	0	15
Solomon A. 2002	R	39	21	10	40.1±14.6	16	11	12	39	0	34±21.5
Ilari L. 2002	R	23	12	8	45	8	9	6	ND	ND	60
Tsubota K. 1999	ND	43	26	13	49±23	29	14	0	ND	ND	38.7
Tseng SC. 1998	R	7	4	3	54.3±17.6	2	3	2	ND	ND	11.3±4.6
CLET (AM as the substrate)											
Parihar JK. 2017	P	25	14	11	46±6	15	6	4	20	5	12
Cheng J. 2017	R	80	73	7	42.4±13.7	80	0	0	57	23	26.4±13.6
Scholz SL. 2016	R	61	46	11	48.9±17.5	34	0	27	ND	ND	50.8±32.7
Ramirez BE. 2015	P	20	12	8	51.6±14.2	7	4	9	12	8	36
Ganger A. 2015	R	62	41	13	14.7±10	60	1	1	ND	ND	21.4±17.8
Zakaria N. 2014	P	18	11	7	40.7±19.4	7	0	11	15	3	23.7±13.3
Vazirani J. 2014	R	70	56	14	24±12.5	64	1	5	ND	ND	17.5±7
Subramaniam SV. 2013	R	40	3	9	16.8±9.3	36	0	4	ND	ND	33.4±29.2
Sejpal K. 2013	R	107	ND	ND	7.5±3.72	107	0	0	92	15	41.2±26
Qi X.2013	R	42	ND	ND	38±14.7	42	0	0	42	0	17.8±3.8
Prabhasawat P. 2012	P	19	12	7	44.7±15.2	13	1	5	11	8	26.1±13.5
Basu S. 2012	R	50	35	15	20.7±11.4	50	0	0	50	0	27.6±16.8
Basu S. 2012	R	28	17	3	27.9±17.4	18	3	7	28	0	58±33.6
Sharma S, 2011	P	50	40	10	14.5±10	47	2	1	ND	ND	13.8±2.9
Sangwan VS. 2011	R	200	159	41	24.1±9.9	200	0	0	200	0	36±19.2
Pauklin M. 2010	P	44	27	11	47.4±20.1	22	0	22	32	12	28.5±14.9
Meller D. 2010	R	30	22	6	47.4±20.1	16	0	14	18	12	28.9±15.5
Shimazaki J. 2007	R	27	18	9	50.2±20.7	9	17	1	27	0	31.8
Sangwan VS. 2006	R	88	74	12	21.1±12.5	78	0	10	61	27	18.3±11.2
Schwab IR. 2000	P	14	11	3	49.4±14	6	1	7	ND	ND	11.5±6.6
COMET (AM as the substrate)											
Prabhasawat P. 2016	P	20	7	11	48.2±15.5	7	10	3	15	5	31.9±12.1

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Author (Year)	Study Type	No. of Eyes	Gender		Mean Age	Etiology			Range of LSCD		Follow-up in Months
			Male	Female		Chemical/ Thermal Injury	chronic cicatricial inflammation (SJS/OCP)	Others	Total	Partial	
Dobrowolski D. 2015	P	17	3	10	31.1±11.5	0	0	17	14	3	16±2.2
Hirayama M. 2012	R	16	11	5	58.4±17.7	6	10	0	16	0	35±17.6
Satake Y. 2011	R	40	22	14	58.5	11	21	8	40	0	25.5
Nakamura T. 2011	R	19	7	10	54±21	1	15	3	19	0	55±17
Inatomi T. 2006	R	15	9	6	48.4±22.3	6	8	1	15	0	20±11
SLET											
IyerG. 2017	R	18	8	9	ND	18	0	0	ND	ND	10.3±6.7
Arora R. 2017	P	10	ND	ND	15.2±10.8	10	0	0	7	3	6
Vazirani J. 2016	R	68	51	17	22	62	0	6	46	22	12
Basu S. 2016	P	125	82	43	ND	125	0	0	107	18	18

AM: amniotic membrane; AMT: amniotic membrane transplantation; CLAL: conjunctival limbal allograft transplantation; CLAU: conjunctival limbal autograft transplantation; CLET: cultivated limbal epithelial transplantation; COMET: cultivated oral mucosal epithelial transplantation; KLAL: keratolimbal allograft transplantation; ND: not documented; OCP: ocular cicatricial pemphigoid; P: prospective; R: retrospective; SLET: simple limbal epithelial transplantation; SJS: Stevens-Johnson Syndrome

Table 2

Comparisons on the outcome among CLAU/CLAL/KLAL with or without combined use of AMT

		AMT not used/mentioned	with AMT
Reepithelization time (Days)			
CLAU/CLAL		6.4-35.6 [46,59,83,88]	5.6-23.8 [15,46,59]
KLAL		8.4-12.7 [88,94]	
Successful rate			
CLAU			
	1Y	75% [83]	43%-91% [65,67]
	1.5Y	77%-81% [59,84,85]	67%-92% [15,59,78]
	2Y		67% [82]
	3Y	76% [86]	33% [67]
CLAL			
	1Y	53%-70% [87,88]	38%-85% [15,66,70]
	1.5Y	7.1%-40%[59,83,84]	67% [59]
	2Y		33%-71% [15,62,66]
	3Y	39%-59% [86,87]	23%-67% [67,70]
KLAL			
	1Y	40%-83% [87,90,93,94]	33%-83% [49,65,72,76]
	2Y	59%-86% [90,91]	33%-73% [72,77,89]
	3Y	74% [92]	27%-54% [55,56,72,76]
	4Y	58% [90]	33%-66% [63,71]
	5Y	51% [90]	21%-47% [56,72]

AMT: amniotic membrane transplantation; CLAL: conjunctival limbal allograft transplantation; CLAU: conjunctival limbal autograft transplantation; KLAL: keratolimbal allograft transplantation; Y: year

Table 3

Comparisons on the outcome between AM and fibrin as the substrate in CLET and COMET

substrate		AM	Fibrin
Reepithelization time (Day)			
CLET		5-13.7 [138,144]	
COMET		5.2 [127]	
Successful rate			
CLET			
	1Y	60%-91% [29,93,113,118-120,136,138,142-144]	62%-80% [102,103,158]
	2Y	56%-81% [32,110,114,117-120,135-138,140,141]	
	3Y	47%-75% [119,120,134,136,138]	77% [99]
	4Y	45% [118]	
	5Y	64%-75% [136,139]	
	8Y		66% [159]
COMET			
	1Y		63%-88% (substrate free)[146,147]
		44%-65% [123,127,129,147]	
	2Y	59%-79% [127,129]	64% (fibrin) [160]
	3Y	53%-71% [126,129,145]	

AM: amniotic membrane; CLET: cultivated limbal epithelial transplantation; COMET: cultivated oral mucosal epithelial transplantation;