DOI: 10.5281/zenodo.4070120
UDC: 617.713-089.843:611.013.83

EFFICIENCY OF AMNIOTIC MEMBRANE TRANSPLANTATION IN THE MANAGEMENT OF LIMBAL STEM CELL DEFICIENCY

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Abstract
Objectives. This paper aims to examine the efficacy, safety, and long term outcomes of amniotic membrane transplantation for corneal surface reconstruction, in cases of limbal stem cell deficiency.
Material and methods. A systematic literature search was performed on PubMed, for papers published up to February 2020, using the following combined search terms: "limbal stem cell deficiency", "amniotic membrane", "limbal transplant". Only clinical trials with human subjects were selected for analysis. We collected the data on amniotic membrane properties and mechanisms of action, processing, preservation and transplantation techniques, and clinical outcomes of different treatment methods.
Results. The surgical approach for treating limbal stem cell deficiency depends on the extent of the disease. Isolated amniotic membrane transplantation appears to have a limited beneficial effect on limbal stem cells, whereas amniotic membrane transplantation, combined with certain types of limbal stem cell transplantation, provides long-term biological and mechanical support for the donor tissue explants. Combined with simple limbal epithelial transplantation, the amniotic membrane has shown excellent results in the surgical management of limbal stem cell deficiency.
Conclusions. Preliminary results of amniotic membrane use in limbal transplantation show quite satisfactory data, but the lack of high-level randomized controlled studies makes it difficult to assess the comparative efficacy of amniotic membrane transplantation in limbal stem cell deficiency surgical management.
Keywords: amniotic membrane, cornea, limbal stem cell deficiency, limbal graft

Introduction

Limbal stem cells (LSC) have the function of regenerating corneal epithelial cells, as well as maintaining the integrity of the corneal epithelium [1]. Various ocular pathologies can affect the limbal region, causing LSC dysfunction. Limbal stem cell deficiency (LSCD) is a condition characterized by a number of pathological signs: delayed or incomplete regeneration of epithelial lesions, recurrent epithelial erosions, scarring and stromal opacity, prolonged corneal edema after cataract surgery, rejection of the corneal graft, etc. [2] Among the causes of LSCD are pathologies such as: chemical and thermal burns, severe microbial infections, chronic cicatricial inflammation (Stevens-Johnson syndrome and bullous pemphigoid), incorrect wearing of contact lenses, trauma, etc. [3]

LSCD treatment is not an easy task, and attempts to restore the integrity and functionality of LSC are often in vain. It should be noted that corneal transplantation cannot treat LSCD and usually fails after surgery. Therefore, LSCD management requires a complex approach and, most of the time, the combination of several treatment methods. Medical therapy has a limited success rate and is only appropriate in mild cases [4]. All cases of moderate to severe LSCD require surgical management. The main directions of LSCD surgical treatment are: direct limbal tissue transplantation and ex vivo/in vivo expanded LSC transplantation [5]. The amniotic membrane (AM), used alone or as a substrate for LSC, can be incorporated into LSC transplantation in almost all surgical approaches. This review aims to investigate the role of AM and its effectiveness in the surgical management of LSCD.

Material and methods

In this paper, we reviewed the literature in order to analyze current data on applications of amniotic membrane in surgical management of limbal stem cell deficiency and summarized the results of different surgical approaches. A systematic literature search was performed on PubMed for papers published up to February 2020, using the following combined search terms: "limbal stem cell deficiency", "amniotic membrane", "limbal transplant". Only clinical trials with human subjects were selected for analysis. We collected the data on amniotic

membrane properties and mechanisms of action, processing, preservation and transplantation techniques, and clinical outcomes of different treatment methods.

Results

A. Structure, properties and mechanisms of action of the amniotic membrane

AM is a biological material of the human placenta, constituting the inner wall of fetal membranes [6]. It has a fetal part - the chorion, and a maternal component - the decidua. Histologically, it consists of a monolayered epithelium, rich in collagen basement membrane, and stroma. Amniotic epithelial cells have qualities that make them a very good source of stem cells. Thus, studies have shown that these cells have "stem-like" characteristics, as they express surface cell markers associated with embryonic stem cells (such as SSEA 3 and SSEA 4), TRA 1-60 and TRA 1-81. It should also be noted that these cells express transcription factors specific to pluripotent stem cells, such as Oct-4 ("octamer binding transcription factor"), which suggests the pluripotent ability to differentiate [7].

The amniotic membrane transplantation (AMT) can be used either as a graft, to replace damaged tissue on the eye surface, or as a tissue patch to prevent the spread of inflammation. The mechanisms of action are: anti-inflammatory, antiangiogenic, facilitator of epithelialization. Also, through the biological similarity between the conjunctiva and the amniotic basement membrane, the latter can be a matrix for the development of epithelial cells, supporting their differentiation and nutrition and prolonging their lifespan. The AM functions as a substrate for stem cell expansion, with implications in the treatment of LSCD of various etiologies and in facilitating the epithelialization of severe corneal ulcers with epithelial and stromal destruction [8].

Restoring ocular homeostasis after a severe disease is a challenge, due to its complexity, which requires both reconstructive surgery and the modulation of cellular mechanisms of inflammation, scarring and tissue regeneration in vitro.

AM has many beneficial properties:

• anti-inflammatory: AM produces natural inhibitors of metalloproteinases and suppresses the pro-inflammatory cytokines IL1-alpha and IL1-beta [9]; AM, also, induces apoptosis of inflammatory cells in chemical burns of the ocular surface [10].

• antimicrobial: AM produces beta defensins and proteinase inhibitors, adhering tightly to the damaged surface and constituting a barrier against the external environment [11];

• antifibrotic and antiadhesive: AM secretes the tissue inhibitor of metalloproteinase, which inhibits protease activity, with the reduction of tissue fibrosis; also, by inhibiting TGF beta, the inactivation of fibroblasts is obtained, which prevents the formation of adhesions between the damaged tissue surfaces [12];

• immunological inertia and low level of antigenicity: these translate into very low expression of histocompatibility antigens HLA, B, CDR, beta-2-microglobulin and unique surface and biochemical properties of placental membranes, which do not express the MHC II class of histocompatibility antigens [13];

• analgesic: rapid pain reduction, by effectively covering the nerve endings [14];

• antiangiogenic: AM epithelial cells express antiangiogenic molecules, such as thrombospondin-1 and endostatin; metalloproteinase inhibitors have also been isolated in amniotic epithelial cells: type 1, 2, 3, 4; at the level of the amniotic basement membrane was identified the pigment epithelial derived factor (PEDF), with strong anti-angiogenic properties; recent studies have shown that the antiangiogenic effect of the AM may be influenced by the presence of inflammation and the type of pathology [9, 15];

• promoter of cell differentiation and deionization, through the content of type IV, V and VII collagen [16];

• favoring epithelialization, through several mechanisms: the amniotic basement membrane represents the support for the growth of epithelial cells and maintaining their functionality; at the level of AM, the migration and differentiation of epithelial cells is facilitated; collagen-rich amniotic basement membrane is a very good substrate for re-epithelialization; at the level of AM are expressed several growth factors: KGF (keratinocyte growth factor – present in the epithelialization phase of wound healing, when keratinocytes cover the wound); bFGF (basic fibroblast growth factor – involved in angiogenesis, wound healing, embryo development); HGF (hepatocyte growth factor – that regulates cell growth, motility and morphogenesis); TGF-beta (transforming growth factor beta – that controls cell proliferation and differentiation) [16, 17].

B. Limbal stem cells - anatomy, physiology and pathology

The ocular surface is an anatomical assembly, consisting of three contiguous structures: conjunctiva, limbus and cornea. The limbus is the anatomical barrier between the transparent and avascular cornea and the sclera, covered by the richly vascularized conjunctiva. It represents the transition area between the corneal epithelium and the epithelium of the bulbar conjunctiva. Basically, there are three ways in which the limbus can be described: anatomical, histological and surgical [18]. The limbal epithelium is a transitional epithelium between two biologically different areas: the conjunctival area, vascularized and rich in lymphoid elements, and the corneal, avascular and richly innervated area [19]. At this level, the epithelium and conjunctival stroma are organized into papillary formations, similar to dermal papillae, radially oriented and called "Vogt palisades" (0.5 mm x 2-4 mm). Fine blood vessels, nerves, and lymphatic vessels pass through the palisades, inside which are the corneal stem cells [20].

The regeneration of the corneal epithelium depends on these stem cells [14]. The term was introduced by Till and McCulloch [15], and defines cells with self-regenerative and differentiation potential in all cell types [16]. The limbal stem cell theory has crucially changed the therapeutic approach to ocular surface pathologies in humans, allowing the destruction of the ocular surface of various etiologies (inflammation, burns, trauma) to be treated by auto- or allografts of limbal stem cells, all on AM support [17]. Limbal stem cells prevent the invasion of the corneal epithelium by the conjunctival one, ensuring the transparency of the cornea. The corneal epithelium contains keratin 3 and 12 (K3/K12) and the cells of the conjunctival epithelium contain keratin 7 and keratin 19 (K7/K19) [17]. Specific cellular markers can assess the degree of cell differentiation, and these markers can be used to demonstrate limbal stem cell failure syndrome. Detection of MUC5AC transcription in corneal epithelial cells, by polymerase chain reaction, is a method of molecular diagnosis of limbal stem cell failure. In the case of destruction of all limbal stem cells, the conjunctival epithelium would replace the corneal one, the cornea becoming opaque.

This demonstrates that the limbus is essentially involved in regulating the differentiation of the corneal epithelium [19]. Regulatory dysfunction of cell differentiation in limbal stem cell insufficiency leads, either to corneal epithelial metaplasia (in which case the corneal epithelium can "conjunctivalize"), or to its dysplasia with carcinogenesis [21].

Conjunctiva and cornea respond very differently to the aggressions to which they are exposed (mechanical, microbial, toxic): the conjunctiva is immunologically hyperactive, whereas the cornea benefits from the so-called immune privilege and inhibition of inflammatory reactions, in order to preserve its transparency. Immunological reactions, at the ocular surface level, are performed through a complex network of inflammatory cells, cytokines and chemokines. There is a dual unitary system: innate and acquired, which works complementarily and/or intricately [22].

LSCD is a complex ophthalmic disorder that accompanies a number of congenital or acquired pathological conditions, resulting in the total or partial dysfunction of these cells. The clinical resonance of LSCD derives from this dysfunction, with the most severe effects on the integrity of the cornea and the entire ocular surface [14, 16]. The etiology of LSCD is varied; from chemical, thermal and mechanical aggressions of the limbus (burns, multiple surgeries, antimitotic agents, dry eye syndrome, etc.), to infections (recurrent ulcer), chronic inflammation, neoplasms and destruction of the corneal matrix (neurotrophic keratitis) [23]. Chronic corneal ulcer and corneal neovascularization are the main complications of LSCD.

AMT acts in LSCD through several mechanisms:

a) prolongs the lifespan and maintains the clonogenicity of the progenitor epithelial cells;

b) favors the differentiation of epithelial cells, avoiding the differentiation into goblet cells;

c) favors the differentiation of goblet cells in the presence of conjunctival fibroblasts;

d) inhibits inflammatory cells with antiprotease activity;

e) suppresses the molecular signaling system of TGF-beta and differentiation of normal fibroblasts [24].

The AM is a tissue, unique in its biochemistry. In terms of lack of immunogenicity – it is the ideal transplant; in terms of the richness of growth factors and cytokines – it is a tissue that facilitates healing; in terms of harvesting and transplantation technique – it is affordable. All these qualities argue for the use of the AM in severe pathology of the ocular surface.

C. Sampling, processing and preserving the amniotic membrane

The potential donor is assessed before birth and after expressing full consent. AM should be obtained aseptically, after cesarean section, in a full-term pregnancy. It is excluded to take the AM after vaginal birth, because it can be contaminated with the saprophytic flora of the birthways. Diseases of the urogenital tract, other diseases of the donor or fetus, that may present a risk to the recipient, include: significant local bacterial, viral, parasitic or fungal infections of the genital tract, especially chorioamnionitis; (known) malformations of the newborn; premature rupture of membranes; endometritis; meconium ileus; tuberculosis, syphilis, HIV/AIDS; viral hepatitis B, C, D [25].

Taken samples are processed to facilitate longer storage periods until transplantation. Fetal membranes should be rinsed several times, the amnion and the chorion should be mechanically separated, and blood residues should be removed. The amnion must be placed separately on a suitable support membrane (e.g. nitrocellulose), where it must be divided into smaller pieces (50x30 mm) [26].

There are several methods for preparing and preserving AM.

Cryopreservation

The AM can be cryopreserved in a cryoprotective medium (10% dimethyl sulfoxide), using a suitable container (bags or cryotubes) and transferred to liquid nitrogen tanks (vapor phase, below -130°C). However, when AM is stored in a sterile glycerol medium (and nutrient medium), the storage temperature is usually below -75°C [27].

Thermally dried AM

The tissue is dried overnight in an oven at 40°C ± 2°C, and then sterilized with radiation. The membrane loses many of its biological properties due to the high temperature, so the AM kept this way is usually employed as a biological dressing for the management of burns.

Preservation of the AM with dry air

After the AM is separated and washed under sterile conditions, it is dried overnight with dry air in a laminar flow hood. It can then be packaged and sterilized with radiation. Although high temperatures are not applied using this method, some properties of the amnion are lost or altered, due to dehydration. AM prepared this way can be used for dressings. Air-dried AM must be transported at room temperature [28].

Lyophilized AM

AM can be cut into pieces and quickly frozen at -50°C to -80°C. It is then dried under vacuum, using a freeze-drying device. The water from the tissue is extracted by sublimation, until a final water content of 5-10% is reached. The tissue can then be packaged and sterilized by irradiation. This type of preparation induces minimal changes in the properties of the amniotic membrane, and the product can be stored at room temperature. Freeze-dried AM should be transported at room temperature. AM preserved by this method is mainly used for wound management [29].

Preservation in cold glycerol

Glycerol has long been used as a cryoprotective agent. Due to its high osmotic potential, it can extract interstitial water from AM. 80% glycerol is normally used to store the AM. Under such conditions, AM can be stored at 2-8°C for a long period, although it loses some of its biological properties. AM kept this way is used as a biological dressing for burns.

AM soaked in antibiotics

After separation, the AM is placed overnight in a solution consisting of various types of broad-spectrum antibiotics, an antifungal agent and a nutrient medium. It is then frozen at -80°C. The resulting AM is convenient for the treatment of infected wounds in combustion, by ensuring an adequate concentration of antibiotics on the wound surface [30].

D. Principles and surgical methods of amniotic membrane implantation

Determination of AM orientation on the ocular surface

The indication for which the AM is used and the desired final effect, determine the orientation with which it is applied to the ocular surface. Histopathological analysis showed that, after the application of AM, repopulation of the ocular surface by the host epithelium (e.g. the host corneal or conjunctival epithelium)

occurs preferentially on the basement membrane side of the epithelium [31], although, Seitz et al. [32] demonstrated that corneal epithelial cells also have the ability to grow on the stromal side of the membrane. When the membrane is used to secure the conjunctival or corneal cells with a substrate on which they could grow, AM is applied with the epithelial/basal face up. On the other hand, the stromal matrix of AM has the ability to capture inflammatory cells and induce their apoptosis, thus regulating the inflammatory response [33]. Thereby, in the presence of acute inflammation, the membrane can be used to protect the ocular surface from the harmful effects of cells and proinflammatory mediators. In this case AM is applied with the epithelial face down, so that the stromal face is oriented towards the eyelids.

The AM, provided on the nitrocellulose filter paper, is usually oriented with the epithelial side up, with the stromal face in direct contact with the paper. The stromal surface can be identified by the presence of vitreous-like strands that can be lifted with a sponge or fine forceps. Depending on the indication for which it is used, there are three surgical techniques by which AM can be applied to the ocular surface.

Inlay technique ("graft")

In this technique, AM is intended to act as a substrate for epithelial cell growth. The AM is placed with the epithelial/basement membrane facing up and cut to fit the size of the underlying epithelial or stromal defect. It is usually secured to the cornea with a 10-0 nylon suture or to the episclera and conjunctiva, using a 9-0 or 10-0 vicryl thread. It is preferable to keep the epithelium up in this technique, as the amniotic basement membrane acts as an excellent substrate for the growth of progenitor epithelial cells, by prolonging their lifespan, maintaining clonogenicity and preventing apoptosis [34]. Approximately 1-2 mm of the surrounding epithelium of the host cornea is debrided. This ensures that the regenerated epithelium grows over the basement membrane of the AM and, therefore, the amniotic stroma becomes embedded in the host tissue (graft). Depending on the depth of the underlying defect, this technique can use a single layer of AM (single-layer graft) or several (multi-layer graft). In the second case, several layers of unsutured AM are placed in the ulcer crater, over which a last layer of AM sutured to the edges of the ulcer is placed, after a prior deepithelization and debridement of the area around the corneal defect. The epithelium is to grow over the top layer of this multilayer graft. Layering can be done either by cutting the AM into several pieces and placing them on top of each other, or using a larger piece of folded AM.

Overlay technique ("patch")

In this case, a fragment of AM, larger than the underlying defect, is sutured to the ocular surface so that the host epithelium is completely covered by the membrane. The AM is secured to the perilymbal conjunctiva using 9-0 vicryl or 10-0 nylon suture. The membrane can be placed with both the epithelial side and the stromal side up, as the host epithelium is to regenerate under the membrane, which, in this case, acts as a therapeutic contact lens or a biological bandage, designed to protect the new and fragile epithelium from the forces of friction generated by eyelid movements. In this situation, the AM either decomposes, or is removed after a certain period.

Combined technique ("sandwich")

In this technique, two or more layers of AM are used - the inner layer or layers serving as a graft and the larger outer layer serving as a patch. Also, known as the "sandwich" technique, this method involves combining single-layer or multi-layer graft techniques ("inlay") with biological patching ("overlay"). In this case, the epithelium is expected to grow under the bandage, but over the graft.

The availability of fibrin glue for ophthalmic use has, in many cases, replaced the application of sutures, and the AM can be adhered to the ocular surface using recombinant fibrin glue. This reduces the time of intervention, but also increases patient comfort [36, 45].

E. Limbal stem cell deficiency - surgical treatment using amniotic membrane

1. Isolated AMT

AMT can be used in the treatment of chemical burns and thermal injuries, due to its properties of epithelium healing [35]. It also seems to be beneficial in managing the Stevens-Johnson Syndrome, thanks to its anti-inflammation properties [36]. Here, the AM is acting as a temporary patch, protecting the ocular surface, promoting epithelial healing and preventing cicatricial sequelae. That being said, controlled clinical trials failed to show any definitive long-term benefits of AMT alone, in comparison to medical therapy, when it comes to visual outcome, ocular surface integrity and corneal vascularization [37]. AMT has also been used for treating partial LSCD [38]. In this case, as histological findings show, the effect of AM is believed to reside in its biological properties rather than mechanical ones [39].

Surgical technique

After fibrovascular pannus was surgically debrided and the inflamed tissue was removed, AM was taken out from the container with a suitable storage environment and placed over the freshly denuded cornea and limbus. In most studies, AM was placed with the epithelium facing up [40, 41, 42]. AM was secured either to the cornea, with 10-0 nylon sutures [40, 43], or to the perilimbal conjunctiva using 9-0 Vicryl sutures [24,

Table 1

Outcomes of AMT alone, in patients with LSCD

Year of publication	Author	No of eyes	Mean age	Re-epithelialization (weeks)	Stable cornea (%)	VA improvement (%)	Follow-up (months)
2015	Chugh JP [41]	30	48.9±16.3	2	-	55	6
2013	Konomi K [38]	16	57.4±16.4	1.5	31.2	31	52.3±26.3
2008	Kheirkhah A [48]	11	32.4±18.4	2.5	72.7	-	14.2±7.7
2005	Lopez-Garcia JS [47]	14	37	2	-	60	24
2005	Ivekovic R [42]	5	31.6±12.3	3	60	80	18±4.3
2003	Gomes JA [46]	4	34.5±26.3	3	-	-	17.5±5.1
2001	Anderson DF [40]	17	42.3±4.6	2.5	58.8	29	25.8±2.5

44]. In the latest studies, there were reports that fibrin glue can prevent suture-related complications [36, 45].

Results

We selected 7 studies [38, 40-42, 46-48] that reported results of isolated AMT in the treatment of partial LSCD (Table 1). Complete corneal and conjunctival re-epithelialization usually occurred in 1.5-3 weeks [38, 40, 46]. Stable epithelial surface was maintained during a period of 12-25 months after surgery [40, 42, 47]. Visual acuity (VA) has improved in 29-80% of operated eyes [38, 40-42, 46, 48]. Nonetheless, the success rate of AMT alone, in patients with LSCD, was only 31% at over a 50 months follow-up period [38].

2. Transplantation of limbal tissues combined with AM

There are three methods of direct limbal tissues transplantation: conjunctival limbal autograft transplantation (CLAU), conjunctival limbal allograft transplantation (CLAL) and keratolimbal allograft transplantation (KLAL). Because there is no consistent data on KLAL, we didn't include these studies into the analysis. Instead, we focused mainly on the techniques, indications and effectiveness of CLAU and CLAL combined with AMT.

Surgical technique

Inlay technique: In this case, AM was placed on the ocular surface and sutured, then the limbal graft was secured to the recipient limbal area [24, 28]. This way, AM is believed to alleviate inflammation and scarring after surgery. Additionally, the combination of AMT may improve the regeneration of LSCs [49] and decrease the risk of induced LSCD in the donor's eye.

Overlay technique: After the application of limbal tissues, AM was placed as a temporary patch over the grafts, covering the entire ocular surface [50]. In some cases, AM was placed first under and then over the transplanted limbal grafts – the so called "sandwich" technique [49]. Here, the AM plays the role of a contact lens, providing mechanical protection and relieving postoperative pain and discomfort.

Results

A total of 15 studies [42, 46, 51-63] were selected for the analysis of the outcome of CLAU or CLAL (Table 2). The follow-up period in these studies was at least 12 months. One study [42] compared the re-epithelialization times in three distinct groups: after AMT (24 days), CLAU (14 days), and CLAU plus AMT (15 days). No notable difference was reported between CLAU and CLAU combined with AMT, but there was a greater re-epithelization time when AMT was used alone. Another study [51] showed that, even though the graft success rate was somewhat similar between the tested groups regardless of the use of AMT, the re-epithelialization was considerably slower in the group with AMT. However, the lack of sufficient comparative studies and the difference between study designs and population structure means that there is insufficient data to demonstrate the effectiveness of combining AMT with CLAU or CLAL [46, 52-55, 62]. Nonetheless, AMT is being actively used as a common procedure in direct limbal tissue transplantation for its healing properties and to increase graft survival rate [56, 57].

Table 2

Outcomes of AMT+CLAU/CLAL in patients with LSCD

Year of publication	Author	No of eyes	Mean age	Re-epithelialization (weeks)	Stable cornea (%)	VA improvement (%)	Follow-up (months)
2017	Arora R [55]	10	18±8	2.5	90	33	6
2015	Moreira PB [58]	28	40.3	4	-	-	24.8
2014	Barreiro TP [51]	15	36.3	2	73.3	66	19.7±5.6
2012	Baradaran-Rafii [56]	34	27.3±9.4	3.5	-	58	17.2±6.3
2012	Eberwein P [59]	20	44	1.5	-	-	22.4
2011	Han ES [60]	24	39.4±17.4	4	66.6	62	47.3±22
2010	Miri A [57]	27	-	2	-	48	38±35.9
2008	Scocco C [54]	39	33.6±18.9	1.5	69.2	-	48.7±30.6
2008	Shi W [61]	39	-	2	-	38	32
2006	Maruyama-Hosoi F [62]	85	52.5±19.5	2	69.4	-	46.6
2005	Santos MS [53]	33	35±16	3.5	-	51	33±12
2005	Lopez-Garcia JS [47]	14	47	2	78.5	60	24
2005	Ivekovic R [42]	4	27.8±7.8	3	100	-	12.8±1.7
2004	Shimazaki J [63]	21	43.2±19.1	2.5	-	80	15
2003	Gomes JA [46]	16	42.3±11.2	3	87.5	62	18.3±6.1

3. Transplantation of ex vivo expanded LSC on AM

This method of treatment is used in cases of bilateral, complete limbus injury, or when there is not enough healthy limbal tissue in the contralateral eye to collect sufficient LSCs. There are two main surgical procedures: cultivated limbal epithelial transplantation (CLET) and cultivated oral mucosal epithelial transplantation (COMET) [64]. The source of cells can be either autologous, or allogenic. The main advantage of these techniques is the small size of donor tissue (<1 mm2) [65], while, also, presenting a low risk of damaging the donor eye. AM here serves as a carrier for cultured cells. De-epithelialized AM is preferred over the intact AM, as it better facilitates the migration of LSCs. It has also been reported that AM provides a beneficial stromal microenvironment for LSCs expansion and preservation. Furthermore, it is believed that AM protects cultured LSCs from apoptosis [66].

Cultivation on AM

One method of cultivation is chopping the tissue into small

fragments and subsequently placing it on the epithelium side of the AM [67]. Another method consists in obtaining single cell suspension by incubating the tissue with trypsin and then seeding the cells on AM [64, 68].

The minimal size of live limbal tissue, required to obtain enough cells for expansion and transplantation, is 0.3 mm^2 (0.5 mm^2 for cadaveric limbal tissue) [69]. For oral mucosal epithelium, a specimen of 3 mm^2 is required [70].

Surgical technique

The fibrovascular pannus is surgically debrided and the perilimbal scarring tissue is removed at least 2 mm behind the limbus. The cultivated epithelial cells, together with the AM substrate, are then placed on the cornea, with the epithelial side facing up, and secured with sutures to the ocular surface.

Results

A total of 23 studies [71-93] were selected for the analysis of the outcome of CLET and COMET combined with AM substrate (Table 3). The rejection rate of CLET was relatively low, even when allogenic tissue was used, thanks to the small size of transplanted tissue [94], and the rate of success was stable even after one year [68, 71]. Similarly, the successful rate of COMET was stable two years after surgery [72]. The result of immunostaining and PCR showed that although oral mucosal epithelial cells expanded on AM became phenotypically similar to limbal and corneal epithelium, they did not undergo a veracious differentiation [73].

Table 3

Outcomes of CLET/COMET on AM substrate in patients with LSCD

Year of publication	Author	No of eyes	Mean age	Re-epithelialization (weeks)	Stable cornea (%)	VA improvement (%)	Follow-up (months)
2017	Parihar JK [74]	25	46±6	3	88	40	12
2017	Cheng J [75]	80	42.4±13.7	2.5	-	-	26.4±13.6
2016	Scholz SL [76]	61	48.9±17.5	4	-	-	50.8±32.7
2016	Prabhasawat P [73]	20	48.2±15.5	2	75	38	31.9±12.1
2015	Ramirez BE [77]	20	51.6±14.2	1.5	-	-	36
2015	Ganger A [71]	62	14.7±10	3	82.2	42	21.4±17.8
2015	Dobrowolski D [90]	17	31.1±11.5	3	-	44	16±2.2
2014	Zakaria N [78]	18	40.7±19.4	4	72.2	-	23.7±13.3
2014	Vazirani J [79]	70	24±12.5	3	72.8	38	17.5±7
2013	Subramaniam SV [80]	40	16.8±9.3	3	-	51	33.4±29.2
2013	Sejpal K [81]	107	7.5±3.72	2.5	68.2		41.2±26
2013	Qi X [82]	42	38±14.7	3.5	73.8	39	17.8±3.8
2012	Prabhasawat P [83]	19	44.7±15.2	4	-	-	26.1±13.5
2012	Basu S [84]	50	20.7±11.4	3	74	55	27.6±16.8
2012	Basu S [85]	28	27.9±17.4	2	-	38	58±33.6
2012	Hirayama M [91]	16	58.4±17.7	2.5	75	-	35±17.6
2011	Sharma S [86]	50	14.5±10	4	24	-	13.8±2.9
2011	Sangwan VS [87]	200	24.1±9.9	3	64.5	59	36±19.2
2011	Satake Y [92]	40	58.5	3.5	-	75	25.5
2011	Nakamura T [72]	19	54±21	1.5	63.1	-	55±17
2010	Pauklin M [88]	44	47.4±20.1	4	-	80	28.5±14.9
2010	Meller D [89]	30	47.4±20.1	2.5	73.3	62	28.9±15.5
2006	Inatomi T [93]	15	48.4±22.3	3	-	-	20±11

4. Transplantation of in vivo expanded LSC on AM

This new surgical technique, called Simple Limbal Epithelial Transplantation (SLET), is usually employed in the management of unilateral LSCD. It consists in the in vivo expansion of limbal tissue pieces on AM. Because it is relatively cheap, easy to perform and requires very low amounts of donor tissue, it combines the advantages of both CLAU and CLET techniques.

Surgical technique

AM is secured on the ocular surface with the epithelial side up and the donor limbal tissue is then placed on the AM [55, 95]. There are, also, modified versions of SLET. Instead of placing the limbal explants on the AM, they can also be placed directly on the cornea, using the AM to cover both the grafts and the entire ocular surface [96]. Another modification, called "sandwich technique", consists in placing the donor tissue between two layers of AM [97].

Results

A total of 4 [95, 98-100] studies were selected (Table 4). Although the follow-up period was relatively short (up to 18 months), SLET showed excellent results in the treatment of LSCD. Re-epithelialization was achieved in 3-4 weeks after surgery, while stable avascular cornea was reported in 80% cases at 12 months and in 76% cases at 18 months [95, 99].

Table 4

Outcomes of SLET in patients with LSCD

Year of publication	Author	No of eyes	Mean age	Re-epithelialization (weeks)	Stable cornea (%)	VA improvement (%)	Follow-up (months)
2017	Iyer G [98]	18	-	4	100	-	10.3±6.7
2017	Arora R [55]	10	15.2±10.8	3	100	80	6
2016	Vazirani J [99]	68	22	3	80	61	12
2016	Basu S [95]	125	-	4	76	58	18

Conclusions

1. The ocular surface is one of the targets of biological and bio-artificial regeneration technologies. The AM, due to its composition and unique properties, explains the special interest of researchers especially through its implications in regenerative medicine.

2. Studies on laboratory animals highlight the beneficial properties of the AM; anti-inflammatory, antimicrobial, antifibrotic and antiadhesive, analgesic and antiangiogenic. In addition, the AM is a promoter of cell differentiation and adhesion, with a low level of antigenicity through epithelial cells in the amniotic epithelium that have stem-like qualities.

3. Used in tissue engineering, the AM is an exoskeleton with a particular pattern of extracellular matrix components, having the capacity to modulate wound healing by promoting tissue reconstruction. For this reason, the AM has been employed as a substrate in numerous surgical techniques, particularly in those used for treating partial or total LSCD.

4. The approach in treating LSCD depends on the extent of the disease. Isolated AMT appears to have modest results in the long term. Although studies have consistently reported favorable outcomes of different LSC transplantation techniques combined with AMT, currently, it is unclear what is the role of AM specifically and to what extent it impacts the final result.

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Received – 09.08.2020, accepted for publication – 20.09.2020
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Conflict of interest Statement: The authors report no conflicts of interest in this work.
Funding Statement: This research was supported in part by the Ministry of Education, Culture and Research of the Republic of Moldova, under the Grant #20.80009.5007.20, and the Laboratory of Tissue Engineering and Cell Cultures from University of Medicine and Pharmacy "Nicolae Testemițanu", Chișinău, Republic of Moldova. The funders had no role in study design, data collection and analysis, decision on publishing or preparation of the manuscript.

Citation: Procopciuc V., Mihaluța V., Ignatov O., Cușnir V., Nacu V. Efficiency of amniotic membrane transplantation in the management of limbal stem cell deficiency. Arta Medica. 2020;76(3):100-109.