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Amniotic membrane transplantation in ocular surface diseases

Transplante de membrana amniótica em doenças da superfície ocular

Nayara Teixeira Flügel[1] https://orcid.org/0000-0003-3474-713X
Bruna Girardi[1] https://orcid.org/0000-0002-6584-113X
Daniel Wasilewski[1] https://orcid.org/0000-0001-9681-4559

ABSTRACT

Objective: *To evaluate the clinical effectiveness of amniotic membrane transplantation for ocular surface reconstruction.* ***Methods:*** *Prospective study including 23 eyes of 21 patients who underwent amniotic membrane transplantation at Hospital de Clínicas da Universidade Federal do Paraná (HC-UFPR) and at Cirurgia e Diagnose em Oftalmologia do Paraná (CDOP) clinic, located in Curitiba, PR, Brazil, from may 2015 to july 2019. The amniotic membrane was collected from elective and term cesarean delivery, and conserved in preservation medium and glycerol 1:1, stored at -80° Celsius. The membrane was fixed on the ocular surface with 10-0 nylon, 8-0 vicryl, biological glue or a combination of these materials.* ***Results:*** *The ocular surface reconstruction was successful in 22 eyes (95.6%). Failure was observed only in 1 case (bullous keratopathy) in which the condition was maintained postoperatively. Patients' age ranged from 11-82 years, with a mean age of 37.4 years. There was a higher incidence in males (66.6%). A difference was perceived in the distribution of the affected eye (which was greater in the right eye - 65.2%). As for the previous ophthalmic surgery history, 12 of the 23 eyes had a positive history (52.2%). It was observed that all patients who had preoperative visual acuity assessed showed improvement or maintenance of corrected visual acuity. In the postoperative period, complications associated with the underlying disease were observed, although not particularly related to the amniotic membrane transplantation. There were not any cases of postoperative infection.* ***Conclusions:*** *There was an improvement in the general state of the ocular surface in almost all of the cases in which the transplant was performed. Therefore, the amniotic membrane can be considered a good alternative for reconstructing the ocular surface, as a single or supporting treatment.*

Keywords: *Amnion; Biological dressings; Transplantation; Eye injuries; Anterior eye segment*

RESUMO

Objetivo: Avaliar a eficácia clínica do transplante de membrana amniótica na reconstrução da superfície ocular. **Métodos:** Estudo prospectivo incluiu 23 olhos de 21 pacientes que realizaram transplante de membrana amniótica no Hospital de Clínicas da Universidade Federal do Paraná (UFPR) e na clínica de Cirurgia e Diagnose em Oftalmologia do Paraná (CDOP), localizados em Curitiba, PR, Brasil, no período de maio de 2015 a julho de 2019. A membrana amniótica foi captada a partir de parto cesárea eletivo e a termo, conservada em meio de preservação e glicerol 1:1 e armazenada a -80° Celsius. A membrana foi fixada na superfície ocular com fio nylon 10-0 ou vicryl 8-0 e/ou cola biológica. **Resultados:** A idade dos pacientes variou de 11-82 anos, com média de 37,4 anos. Houve maior incidência no sexo masculino (66,6%). Ocorreu diferença na distribuição do olho acometido (maior no olho direito – 65,2%). Quanto à história de cirurgia oftalmológica prévia, 12 dos 23 olhos tinham história positiva (52,2%). Observamos que nos pacientes em que foi possível a avaliação da acuidade visual pré-operatória, todos apresentaram melhora ou manutenção da acuidade visual. No pós-operatório foi observado complicações associadas à doença de base e não propriamente ao transplante de membrana amniótica. Não foram registrados casos de infecção pós-operatória. **Conclusão:** Houve melhora do estado geral da superfície ocular em quase totalidade dos casos em que o transplante foi realizado. Portanto, a membrana amniótica pode ser considerada uma boa alternativa para reconstrução da superfície ocular, como tratamento único ou coadjuvante.

Descritores: Âmnio; Curativos biológicos; Transplante; Lesões oculares; Segmento anterior do olho

[1] Department of Ophthalmology, Hospital de Clínicas da Universidade Federal do Paraná Curitiba, PR, Brazil.

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Introduction

The ocular surface is an extremely sensitive and dynamic structure, with numerous cellular processes that maintain its integrity. Any chemical, thermal or mechanical aggression induces, among others, processes of fibrosis, increased vascular permeability, exudation, and lipid deposits, causing anatomical and physiological dysfunction in the whole eye.[1-3]

A variety of donated biological tissues have been used to repair or decrease inflammation, in cases where the cornea and conjunctiva are seriously affected. The human amniotic membrane (AM) has been used in surgery since the beginning of the 20th century, although its use in ophthalmology is relatively recent. The AM, internal part of the placenta, is a translucent and viscoelastic membrane consisted of five layers, where the epithelial layer, a thick basal membrane and an extracellular stromal avascular matrix stand out. It is not vascularized and does not have direct blood supply.[2,4]

AM transplantation has been consolidated as an adjuvant in the treatment of ocular surface disorders. Its use is based on the ability to benefit the epithelialization process and reduce the inflammatory, angiogenic and cicatricial processes.[1-24]

Documented reports in literature state that AM acts by modulating levels of cytokines, growth factors, enzymes and local receptors. Membrane transplantation seems to be an excellent alternative for several ocular pathologies, with a high success rate and low complication rate, once ensured its obtention, preservation and handling conditions.[2,7-9,13,16]

The AM promotes epithelialization by acting as a basal substrate, which facilitates the migration of limbal epithelial cells, reinforces the adhesion of the epithelium to the basement layer, promotes cell differentiation and prevents cell apoptosis. It can also function as a mechanical defense against eyelid friction, inflammatory cells and tear film proteins. In some cases, AM transplantation may postpone or prevent future surgical procedures, sparing patients from a more risky intervention.[2,3,8,16]

This work aims to evaluate the clinical efficacy of AM transplantation in ocular surface reconstruction.

Methods

This study was approved by the Research Ethics Committee at HC-UFPR, under protocol 2.760.226, respecting the ethical principles of privacy and confidentiality of the collected data. All participants signed an informed consent form (ICF).

This prospective study included 23 eyes of 21 patients who underwent AM transplantation at Hospital de Clínicas da Universidade Federal do Paraná (HC-UFPR) and at Cirurgia e Diagnose em Oftalmologia do Paraná (CDOP) clinic, located in Curitiba, PR, Brazil, from May 2015 to July 2019.

After the parturient's authorization through the ICF, the AM was collected from elective and term cesarean delivery, performed at HC-UFPR. These patients were submitted to laboratory analysis for the following diseases: HIV, hepatitis B and C and syphilis; these serologies were confirmed by analysis of the umbilical cord blood after delivery. In cases of positive serology, the tissue was discarded.

The placenta was washed with 0.9% saline and preserved in a diluted solution with gentamicin antibiotic, from the time of birth to its final preparation, which never exceeded 6 hours. The AM was prepared in the outpatient surgery center of the above-mentioned hospital, in a sterile environment. After its complete separation from the placenta, the AM was extended on sterile nitrocellulose filter of various sizes, with the epithelium always facing upwards, then placed in a sterile flask containing glycerol and preservation medium at 1:1 ratio, kept in a freezer at -80° Celsius and refrigerated at +4° Celsius. Its maximum use time was 6 months when frozen and 2 weeks when refrigerated.

The surgeries were performed by ophthalmologists and residents at HC-UFPR and ophthalmologists at CDOP. During surgery, the membrane was fixed on the ocular surface with 10-0 nylon or 8-0 vicryl (in 20 cases), with simple and/or continuous stitches, or biological glue (in 8 cases, 7 of which were associated with the threads). The membrane was attached to the cornea using a 360º circular and continuous suture with 10-0 nylon thread at the extreme periphery of the cornea. The conjunctival suture was also performed continuously using 8-0 vicryl thread (in a unique way, regardless of the amount of membrane applied).

The technique for membrane transplantation varied depending on the type of trauma on the ocular surface: the membrane was placed exclusively on the cornea, on the conjunctiva or on the entire ocular surface (cornea and conjunctiva); in some cases, the membrane was used for conjunctival sacs reconstruction. In all studied cases, the membrane was placed on the ocular surface with the epithelium facing upwards. Sutures were removed according to each case; sutures with vicryl thread were usually removed between 2-3 weeks after surgery while sutures with nylon thread on the cornea were removed around 2-4 months after complete absorption of the membrane.

Postoperative topical therapy was administered to all patients in order to reduce the inflammatory process and prevent secondary infection. Antibiotic eye drops (fourth generation fluoroquinolone) were prescribed for 14 days and corticoid eye drops (prednisolone acetate 1%) in a regressive fashion until complete absorption of the membrane. In all cases, therapeutic contact lenses were used in the postoperative period, for better comfort and greater adhesion of the membrane to the ocular surface.

All patients are still on an outpatient basis and postoperative follow-up is performed according to the need of each case.

Results

As shown in table 1, among the 21 patients in this study, 5 were diagnosed with alkali eye burn (Figures of 2 of these 5 patients - Figures 1A,B and 2A,B), 1 with thermal burn, 3 with shield ulcers, 1 with ulcer perforation, 1 with Stevens-Johnson syndrome, 1 with bullous keratopathy, 2 with neurotrophic keratopathy, 3 with grade 3 or 4 recurrent pterygium (Figures of 1 of these 3 patients - Figure 3A,B), 1 with bullous epidermolysis, 1 with ocular cicatricial pemphigoid, 1 with symblepharon after excision of epidermoid carcinoma (Figures 4A,B) and 1 with limbal deficiency. Therefore, in the present study, we observed that the main indications for AM were: chemical/thermal burn (28.5%) and shield ulcer (14.3%).

The ocular surface reconstruction was successful in 22 of the 23 eyes (95.6%). Failure was observed in only 1 case (bullous keratopathy) in which the condition was maintained postoperatively. Patients' age ranged from 11-82 years, with a mean age of 37.4 years. The incidence was higher in males (66.6%). There was a difference in the distribution of the affected eye (which was greater in the right eye - 65.2%). As for the history of previous ophthalmic surgery, 12 of the 23 eyes had a positive history (52.2%).

Table 1
Demographic characteristics, affected eye, diagnosis and corrected visual acuity (CVA) pre-and postoperatively

Diagnosis	Age (years)	Sex	Affected eye	Preoperative CVA	Postoperative CVA
Alkali burn	45	Female	Right	------	20/30
(Figures 1A, B)					
Alkali burn	12	Male	Left	------	Hand Motion
Alkali burn	28	Male	Right	20/120	20/25
Alkali burn	44	Male	Right	Hand Motion	20/400
(Figures 2A,B)					
Alkali burn	39	Male	Left	Hand Motion	CountingFingers
Thermal burn	14	Female	Right	------	20/125
Shield ulcer	11	Male	Right	20/50	20/40
Shield ulcer	13	Male	Left	------	20/50
Shield ulcer	12	Male	Right	20/100	20/30
Perforated ulcer	82	Male	Right	------	20/100
Stevens-Johnson syndrome	28	Female	Both	------	20/20
20/20					
Bullous keratopathy	47	Female	Right	Counting Fingers	CountingFingers
Neurotrophic keratopathy	21	Female	Right	20/60	20/60
Neurotrophic keratopathy	34	Male	Right	Hand Motion	Hand Motion
Grade 4 recurrent pterygium	41	Male	Right	20/40	20/30
(Figures 3A,B)					
Grade 4 recurrent pterygium	39	Male	Both	20/50	20/25
in right eye and grade 3 in left eye				20/25	20/25
Grade 3 recurrent pterygium	37	Female	Left	20/160	20/50
Epidermolysis bullosa	54	Male	Left	Counting Fingers	CountingFingers
Ocular cicatricial pemphigoid	60	Male	Left	Hand Motion	Hand Motion
Symblepharon after excision of epidermoid carcinoma	72	Male	Right	Hand Motion	CountingFingers
(Figures 4A, B)					
Limbal deficiency	54	Female	Right	Counting Fingers	CountingFingers

Table 2
Postoperative complications

Postoperative complications	n(%)
Symblepharon	7 (33.3)
Corneal opacity	5 (23.8)
Conjunctivalization	5 (23.8)
Granuloma	3 (14.2)
Corneal thinning	2 (9.5)
Corneal neovessels	2 (9.5)
Trichiasis	1 (4.7)
Conjunctival sac shortening	1 (4.7)
Neurotrophic ulcer	1 (4.7)

N=21 patients

Patients who had their preoperative visual acuity assessed showed improvement or maintenance of corrected visual acuity. In some cases, this analysis was not possible due to intense discomfort or inability to open the eyes.

Table 2 shows the complications observed in the postoperative period (some patients had more than 1 complication simultaneously): corneal thinning (2 patients), conjunctivalization (5 patients), corneal opacity (5 patients), corneal neovessels (2 patients), trichiasis (1 patient), conjunctival sac shortening (1 patient), symblepharon (7 patients), neurotrophic ulcer (1 patient) and granuloma (3 patients).

Surgical reintervention with amniotic membrane re-transplantation was necessary for 2 patients (alkali burn and symblepharon) due to incomplete resolution of the first transplant condition. In 1 case (using only biological glue), the amniotic membrane was extruded from the ocular surface, but the shield ulcer was already healed, and a re-transplantation was not needed. Six cases were associated with autologous limb transplantation and 1 case was associated with autologous lip mucosa transplantation.

Discussion

The AM was used essentially as a substrate or basement membrane to promote the growth of new corneal or conjunctiva cells, utilizing different techniques to cover the defect area. In patients with extensive trauma and limbal germ cell deficiency, a single layer of AM associated with limbal transplantation was applied. In patients with deep corneal ulcers, the AM was applied in multiple layers to fill the stromal defect. This procedure is justified by the different functions performed by the AM in each technique. The greater amount of matrix available in the latter case accelerates epithelial healing due to the greater number of growth factors involved. AM transplantation in pathologies, such as persistent epithelial defects and ulcers with and without corneal perforation, can be considered an effective treatment, as it promotes epithelial growth and facilitates cicatrization.[3,4,12,17,18,20]

Maharajan et al.[21] pointed out that success must be related to the AM objective, which is to be used as an adhesive or graft, and the result of its application. Thus, a complete success was obtained when the membrane produced the desired result to which its use was proposed; a partial success when the objective was attained, but not necessarily due to the intended use of the AM, or even when not all objectives were achieved; and failure when the intended objective was not achieved.

The objectives of AM transplantation are little related to

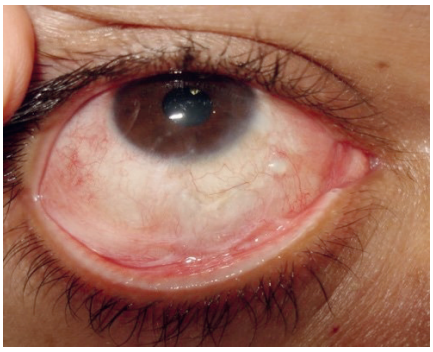

Figure 1A: Alkali burn (preoperative period)

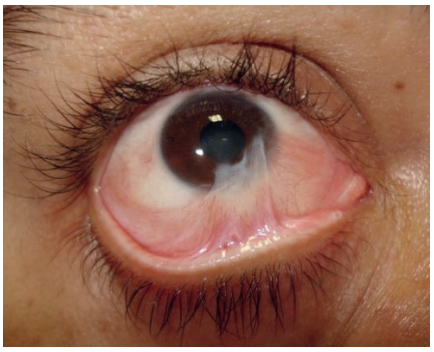

Figure 1B: Alkali burn (postoperative period – 11 months)

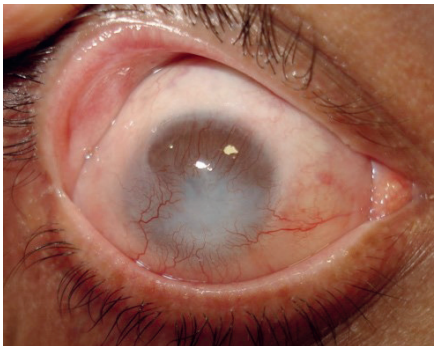

Figure 2A: Alkali burn (preoperative period)

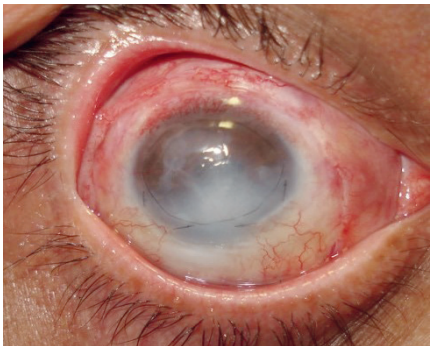

Figure 2B: Alkali burn (postoperative period – 4 months)

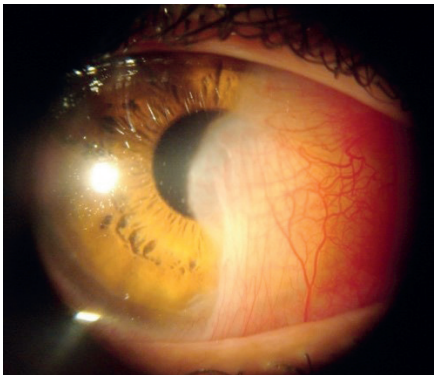

Figure 3A: Grade 4 recurrent pterygium (preoperative period)

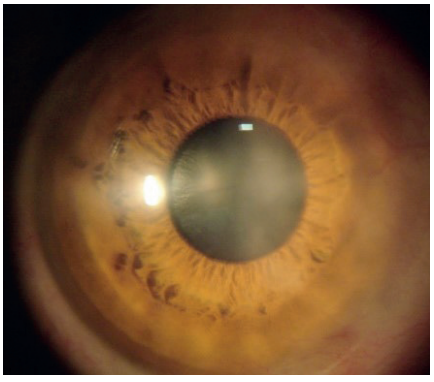

Figure 3B: Grade 4 recurrent pterygium (postoperative period – 18 months)

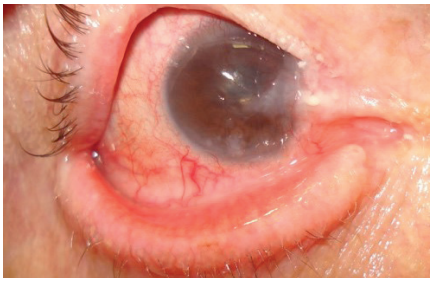

Figure 4A: Symblepharon after excision of epidermoid carcinoma (preoperative period)

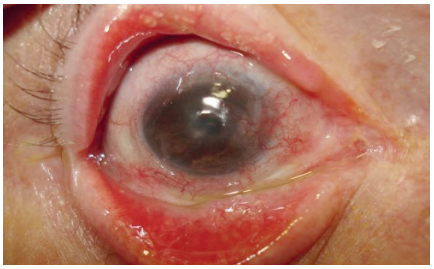

Figure 4B: Symblepharon after excision of epidermoid carcinoma (postoperative period – 4 months)

visual improvement, focusing mostly on pain relief and containment and/or correction of sequelae of other diseases or previous surgical procedures. However, the present study shows that corrected visual acuity improved or remained unchanged in all cases.

Similarly to the study by Lee et al.,[24] who reported good results with AM transplantation in 10 of 11 cases with a chronic epithelial defect of different etiologies; in this study, almost all patients undergoing AM transplantation obtained satisfactory results, with complete epithelialization being observed in the cases under analysis, which was the main objective. Except in a single case (bullous keratopathy), in which we believe that surgical success was not obtained due to the patient's specific condition:

an elderly woman, with several pro-inflammatory systemic comorbidities, who had previously performed other unsuccessful eye treatments (lubricant eye drops, corticosteroids, hypertonic and anti-inflammatory drugs, use of therapeutic contact lenses and micropuncture), and AM was indicated as an attempt to delay and/or prevent corneal transplantation.

Symptomatic bullous keratopathy is a serious pathology and it is increasingly frequent due to the growth of cataract surgeries performed by phacoemulsification. AM transplantation has been used in these cases with inconclusive results. Its effectiveness has been proven in the palliative treatment of patients with low visual acuity or as a temporary measure in patients that are intolerant to therapeutic contact lenses and indicated for corneal transplantation.[2]

The small sample of patients with bullous keratopathy undergoing AM transplantation in our service is due, in most part, to the short queue for corneal transplantation. In most cases, corneal transplantation is opted instead of membrane transplantation, aiming to improve visual acuity and pain relief.

AM has shown excellent results in cases of ocular burns, where there is partial or total damage to limbal region,[3,4,9,12] as demonstrated in 6 cases in this study, with good postoperative results (significant improvement of signs and symptoms). In the acute phase, it can be used to relieve local inflammation, promote rapid epithelialization and decrease pain. Moreover, in the chronic phase, it is used to reconstruct the ocular surface and avoid more serious sequelae, such as symblepharon.[3,4,9,12]

The use of AM in the treatment of pterygium is an effective therapy in cases where conjunctival transplantation is not advised (patients with extensive pterygiums, nasal and temporal pterygium, cicatrizing conjunctival pathologies and who need filtering surgery to treat glaucoma in the future),[5,19] with recurrence rates varying between 3% and 25%[2]. In the studied cases, AM was used only in the treatment of recurrent pterygiums, which is a hospital routine, associated with 0.02% topical mitomycin perioperatively, obtaining good results, such as the improvement of corrected visual acuity, symptoms and aesthetic aspect, for they were extensive pterygiums (grades 3 and 4).

More than half of the evaluated patients had already undergone previous ophthalmic surgery, related to the diagnosis that culminated in the transplantation of AM or another pathology. Their eyes had a severe inflammatory process and, in some cases, exacerbated areas of fibrosis and scarring. Even so, the membrane usage improved pain symptoms and foreign body sensation, besides improving or maintaining corrected visual acuity in most cases. The majority of patients in this study are young and are in an economically active phase (mean age was 37.4 years), hence the great importance in minimizing the functional and, if possible, aesthetic defects of these patients.

Crisóstomo et al.[6] evaluated AM transplantation in pediatric patients (mean age of 7 years) and attained complete success in all patients without limbal insufficiency and only 1 case of therapeutic failure (16.7%), as in the present study. An improvement was also perceived in the aesthetic aspect of all respondents. These data corroborate the results of this research, provided that the best aesthetic and functional results (CVA) were in younger patients, in comparison to older patients.

As with any human tissue, donor variations affect the composition of AM to some degree. This includes age, race, maternal health and donor diet. Additional variations depend on fetal sex, health, gestational age and labor-related specificities. [22] Furthermore, Hopkinson et al.[23] showed the relationship between handling and processing the AM, which indicates that the clinical effect of the membrane can be improved or lost, depending on the handling and processing of the amnion. What leads to the conclusion that the final result of AM transplantation not only involves the severity of the case and the age of the recipient patient but also characteristics of the donor (mother and fetus), manipulation of the placenta and membrane manufacturing process.

Up to this moment, there is not a standard for AM production, but the manufacturing process for 'synthetic membrane' already exists in laboratory. Collagen or polymers are used as matrices to incorporate growth factors, cytokines, antimicrobial peptides and other substances that are adapted to specific clinical applications. Although not yet available in surgical practice, this paves the way for a standardized product with more predictable results.

As AM is basically formed by collagen, its use to fill in fine areas allows the increase of corneal or scleral thickness, when incorporated. In cases of corneal thinning, good results were achieved, except in 2 cases where thinning persisted. These unsuccessful cases referred to patients with neurotrophic ulcer and severe alkali burn. Nevertheless, patients reported significant improvement in symptoms. Lee and Tseng[24] also obtained the same result in similar cases. The effect of AM in these cases was corneal structure restoration, inflammatory process decrease and stimulation of the epithelium due to its previously described biochemical properties.

As shown in table 2, there were postoperative complications, but we believe they are related to the underlying disease and not exactly to AM transplantation, as suggested in other studies in literature.[3-5,7,13,15,16,18-24]

In the immediate postoperative period, hematoma formation under the membrane was perceived. The blood is usually absorbed or needs to be drained, being necessary to make a small opening in the graft, if excessive. Occasionally, some cases may present a residual subepithelial membrane and inadvertently cause visual axis opacification. Calcification occurs in about 12.8% of cases. White plaques were associated with ciprofloxacin eye drops therapy.[16] We emphasize that none of these complications related to membrane transplantation occurred in this study.

In the one case performed only with biological glue, the membrane was extruded and, for that reason, we do not recommend its use without some stitches, for safety and better adhesion of MA on the ocular surface.

The incidence of postoperative infection is very low (<1.6%),[16] and no signs were observed in any case of the present analysis, proving itself to be a safe method.[2,5,7,8] Gram-positive organisms are the most frequent isolates.[16] However, we emphasize the importance of a careful choice of patients and the maintenance of aseptic conditions throughout pre-, peri- and postoperative procedures, as they are fundamental to the success of AM transplantation.

Conclusion

AM can be considered a good alternative for ocular surface reconstruction, as a sole or coadjuvant treatment. Almost all eyes in which transplantation was performed showed improvement in the general state of the ocular surface. There was not a higher incidence of complications compared to those described in literature. Studies with greater casuistry and follow-up time may be useful for a better understanding of the factors associated with the outcome.

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Corresponding author:
Nayara Teixeira Flügel
E-mail: dra.nayaraflugel@gmail.com