O MUNDO DA SAÚDE

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Simple Limbal Epithelial Transplantation (SLET): case reports

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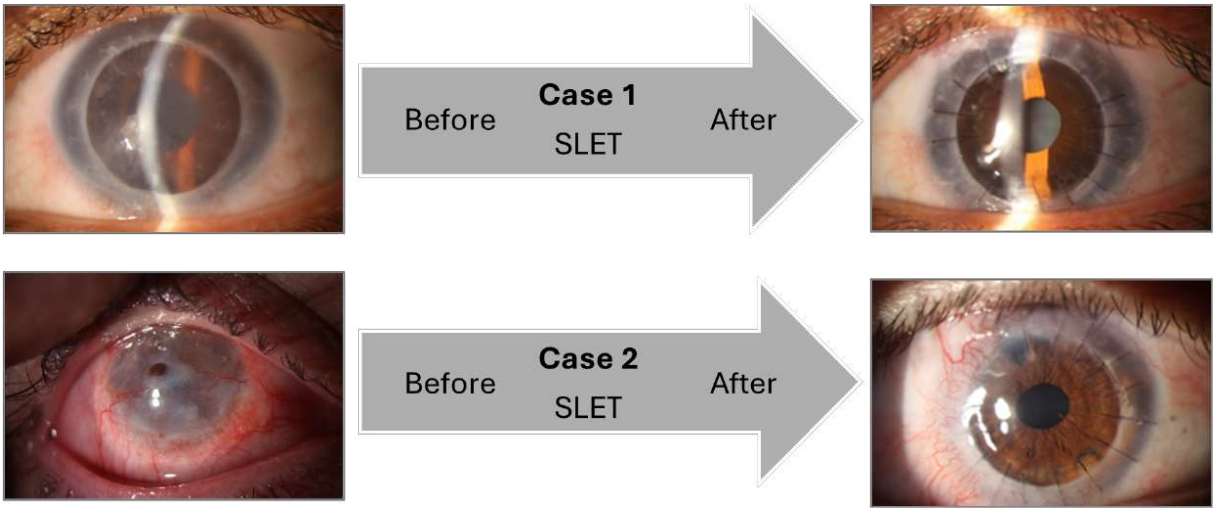
Graphical Abstract



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Abstract

The limbus is a transition zone between the cornea and the conjunctiva of the eye, housing epithelial stem cells responsible for maintaining and ensuring the proper functioning of the cornea. Circumstances that damage these cells lead to a condition known as "limbal stem cell deficiency" (LSCD). Simple Limbal Epithelial Transplantation (SLET) is a technique that involves transferring healthy cells from the limbus of the contralateral eye to the injured corneal region. This study aimed to describe two cases of patients undergoing the SLET technique for limbal insufficiency repair, as well as to evaluate their outcomes with this recent surgical technique. A descriptive, qualitative clinical case report study was conducted through the analysis of medical records of two patients affected by unilateral LSCD. The two patients presented with different etiologies and classifications of the conditions. Case 1 resulted from mechanical trauma, while Case 2 was due to chemical burns. Both underwent the same surgical technique, showing good clinical progression over the follow-up period, with a clear and epithelialized cornea, as well as improved visual acuity and quality of life, without significant complications. In conclusion, the SLET technique demonstrated favorable outcomes in the repair of limbal insufficiency in both cases. However, further studies are required to confirm its potential efficacy and safety in ocular surface reconstruction.

Keywords: Cornea. Corneal Limbus. Corneal Transplantation. Limbal Stem Cells. Case Reports.

INTRODUCTION

The limbus, a transition zone between the cornea and the bulbar conjunctiva, contains epithelial stem cells responsible for maintaining and ensuring the proper functioning of the cornea, as well as the ocular surface. Thus, the limbus plays a critical role in maintaining ocular health, and when partially or completely impaired, it leads to a spectrum of corneal surface abnormalities characterized by conjunctival epithelial overgrowth (conjunctivalization), neovascularization, and chronic inflammation—a condition known as limbal stem cell deficiency (LSCD)[1,2]. In cases of LSCD, the corneal epithelium is unable to maintain its integrity, resulting in an irregular corneal surface and causing symptoms such as reduced visual acuity and chronic ocular irritation.

In this context, three different surgical approaches have been developed and refined to treat this condition and promote corneal epithelialization using autologous limbus: Cultivated Limbal Epithelial Transplantation (CLET), which requires an ex vivo cultivation system for epithelial cells, limiting its use due to cost and availability; Conjunctival-Limbal Autograft (CLAU), which poses a significant risk to the healthy donor eye due to the relatively large margin of donor tissue required; and, more recently, Simple Limbal Epithelial Transplantation (SLET), which has emerged as a new approach to restore limbal epithelial integrity, combining the advantages of existing techniques while addressing some of their limitations and challenges[3].

The discovery of stem cells in the limbal region of the eye initially led to the development of the CLAU technique. However, to reduce the risk of LSCD in the donor eye, the CLET technique was conceived, utilizing a smaller area of donor tissue expanded into a transplantable epithelial sheet[3]. First described in 2012, SLET is a procedure that combines the CLAU and CLET techniques. However, it involves transplanting a small area of healthy limbal tissue from the patient or a compatible donor to the affected area, stimulating stem cell regeneration and restoring the ocular surface. For this reason, unilateral LSCD is one of the primary indications for autologous SLET[3,4]. Furthermore, in cases of severe corneal opacification, patients may also require penetrating keratoplasty (PK), which can be performed simultaneously with SLET, particularly if patients wish to avoid a second operation[5]. The limitations of the SLET technique include the necessity of amniotic membrane, which is difficult to obtain in Brazil, as well as the use of fibrin glue, increasing the cost of the procedure. Another

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limitation is that SLET can only be performed in cases of unilateral burns.

Among the causal factors of LSCD, burns and ocular trauma represent significant public health concerns, as these conditions are often associated with occupational hazards and accidents, particularly in industries such as construction, metallurgy, and agriculture. Studies show that the primary cause of LSCD is ocular burns, with chemical burns being more common in males and typically caused by alkaline substances, which are widely used in various industries. Patients with LSCD may experience discomfort and ocular pain, foreign body sensation, photophobia, reduced vision, and even blindness—potentially debilitating symptoms that can significantly impact quality of life[6].

As a recent and innovative ophthalmological procedure, SLET represents a significant advancement in the approach and treatment of severe diseases affecting the ocular surface, particularly those causing damage to the corneal limbus. This study highlights an important role in the field of innovation that directly impacts clinical practice, as the outcomes demonstrate clear corneas, which, in addition to improving patients' vision, underscore the progress of regenerative ophthalmology. Furthermore, as a unique aspect of this case report, one of the patients presented with an uncommon etiology for limbal insufficiency (trauma).

This study aimed to describe two cases of patients undergoing the SLET technique combined with penetrating keratoplasty for the repair of limbal insufficiency, as well as to evaluate their outcomes with this recent surgical technique for the treatment of monocular trauma and chemical burns.

The repair of the corneal epithelium in both patients was performed as originally described, in a single procedure by a single surgeon, using a small 2x2 mm area of healthy limbus from the contralateral eye or a compatible donor, without the need for cultivation. In this technique, the donor tissue is divided into eight to ten smaller pieces and fixed with fibrin glue onto the amniotic membrane, which is previously placed on the diseased ocular surface around the center of the cornea[3]. In comparison, the CLAU technique requires a relatively large margin of donor tissue, posing a significant risk of iatrogenic LSCD to the healthy donor eye, while CLET necessitates an ex vivo cultivation system, limiting its use due to cost and availability[3]. These factors make the SLET technique more advantageous in these aspects. The study was approved by the Research Ethics Committee (CEP) under the Opinion Number: 6.178.356. The guidelines used for the preparation of this report followed the CARE standards.

Case 1 Description

A 46-year-old female patient, an office worker, with no previous health issues. She underwent penetrating keratoplasty (PK) in the left eye (LE) in 2007 due to an injury sustained in a car accident. One year prior to the initial consultation, the transplanted cornea increased in thickness and lost its transparency. The patient had been continuously using a soft contact lens (SCL) to alleviate symptoms.

At the initial ophthalmological examination on July 26, 2017, the patient presented uncorrected visual acuity (VA) of 20/20 in the right eye (RE) and 20/500 in the left eye (LE). Slit-lamp examination of the RE showed no abnormalities; however, the LE displayed an opaque cornea, vascularized limbus across the entire periphery, and good adaptation to the soft contact lens (Fig. 1a). Intraocular pressure (IOP) measured with a Goldmann tonometer was 14 mmHg in the RE and 16 mmHg in the LE. Fundoscopy revealed no abnormalities in either eye. LE LSCD was suspected, and a diagnostic impression cytology test confirmed it as partial. Based on the results, a new PK was indicated for the LE, performed two months later without complications, using a donor cornea with a cell count of 2,800 cells. Immediate postoperative (PO) slit-lamp examination showed a clear corneal graft with mild edema (+/4), which resolved within 16 days, resulting in a fully transparent graft. The patient showed good progression up to six months post-transplant, with uncorrected VA of 20/100 and IOP of 16 mmHg.

Ten months after the new transplant, the patient experienced significant vision loss in her left eye (LE), with visual acuity reduced to cou-

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nting fingers at 50 centimeters, accompanied by edema and diffuse epithelial irregularity associated with keratitis. A new PK was indicated, this time combined with an epithelial limbal transplant from the contralateral eye using the SLET technique, as a simple corneal transplant would not replace the limbal stem cells.

The surgery was performed two months later, in September 2018, involving PK combined with the SLET technique, with no intraoperative complications. The patient progressed without major issues, reporting only mild discomfort in the left eye when blinking during the first postoperative week. One month after the transplant, the amniotic membrane (AM) had been absorbed, and the cornea showed good transparency (Fig. 1c). In subsequent follow-ups, the patient continued to progress without complications, achieving improved visual acuity (20/40). However, after the last consultation in March 2020, six months post-procedure, the patient was lost to follow-up.

Table 1 - Timeline of Case 1. Joaçaba, Santa Catarina, 2023.

Timeline	Description of the Consultation and Follow-up
DAY 1	Patient with visual acuity of 20/20 in the right eye (RE) and 20/500 in the left eye (LE). Biomicroscopy of the RE showed no abnormalities, while the LE presented an opaque cornea, well-adapted soft contact lens (SCL), and vascularized limbus across the entire periphery. Intraocular pressure (IOP) was 14 mmHg in the RE and 16 mmHg in the LE. Pachymetry measured 580 microns in the RE and 793 microns in the LE. Diagnostic impression cytology was requested.
MONTH 2	A new PK was performed in the LE using a cornea with 2,800 cells, successfully. The corneal graft was clear with edema. Moxifloxacin 0.5% and Prednisolone 1% eye drops were prescribed every 6 hours, along with an antiglaucoma eye drop combining Brimonidine Tartrate 0.2% and Timolol Maleate 0.5% every 12 hours.
MONTH 8	LE cornea epithelialized, sutures intact, no signs of limbal failure. Visual acuity (uncorrected) in LE: 20/100; with +2.0/-1.50x70°: 20/40. Patient using Prednisolone 1% eye drops every two days and maintaining antiglaucoma eye drops.
MONTH 12	The patient presents with vision of counting fingers at 50 centimeters, significant epithelial edema, diffuse irregular epithelium associated with diffuse keratitis, and corneal edema; LE IOP: 16 mmHg. Limbal failure in the LE was diagnosed, and a new PK combined with SLET was recommended.
MONTH 14	PK combined with SLET performed in the LE. The superior nasal limbus showed no abnormalities, and the cornea was unremarkable. The patient was using Moxifloxacin 0.5% and lubricating eye drops in both eyes, as well as an antiglaucoma eye drop combining Brimonidine Tartrate 0.2% and Timolol Maleate 0.5% in the LE.
MONTH 15	The patient shows good progression without complications. RE: donor area and cornea unremarkable; LE: Amniotic Membrane absorbed by more than 80%, with only 20% remaining in the central area, intact limbal fragments. SCL removed.
MONTH 18	Good progression without complications. LE: cornea fully epithelialized, with one loose suture. LE IOP: 12 mmHg. The patient is using Prednisolone on alternate days in the LE and lubricating eye drops as needed.

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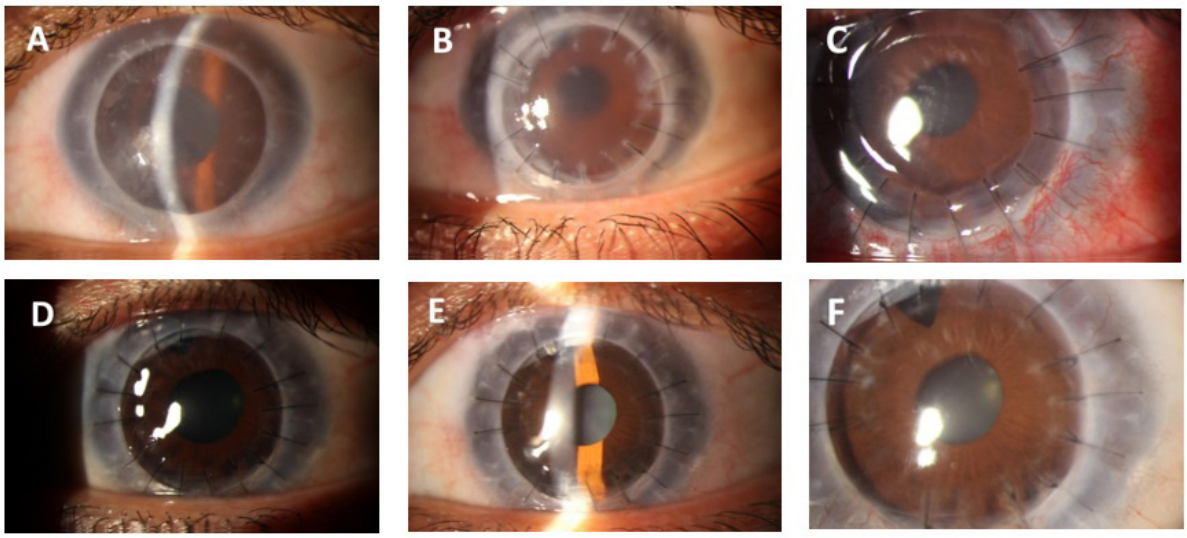
...continued - Table 1.

Timeline	Description of the Consultation and Follow-up
MONTH 26	Good progression. Visual acuity in RE: 20/20 uncorrected; in LE: 20/100 uncorrected. IOP in RE: 12 mmHg; in LE: 14 mmHg. RE: limbal donor area unremarkable; LE: epithelialized cornea, no keratitis. The patient is using Prednisolone 1% eye drops on alternate days in the LE and lubricating eye drops as needed.
MONTH 32	Eighteen months post-transplant with the SLET technique, the patient showed good progression without complications. The patient was using Prednisolone 1% eye drops on alternate days in the LE. Refraction: RE plano, VA 20/20; LE: +2.0/-0.75x180°, VA 20/40; Addition +1.0 (J1). RE: donor area unremarkable; LE: clear graft, intact sutures. IOP: RE 14 mmHg; LE 16 mmHg.



A) Opaque and thick cornea, limbus vascularized across the entire periphery, with an apparent diagnosis of limbal failure. B) Tenth month post-LE PK without SLET, showing diffuse irregular epithelium, associated with diffuse keratitis and an edematous cornea. C) First month post-new PK combined with SLET, presenting a transparent and fully epithelialized cornea with mild stromal edema. D) Fifth month of progression post-SLET, showing a fully epithelialized cornea. E) Epithelialized cornea, no keratitis, one year after PK combined with SLET. F) Intact sutures and epithelialized cornea without abnormalities 18 months post-SLET.

Figure 1 - Progression of the left eye in case 1 during the follow-up period. Joaçaba, Santa Catarina, 2023.

Case 2 Description

A 35-year-old male patient, a general laborer, with no prior ophthalmological comorbidities. He sustained a chemical burn to the right eye (RE) caused by quicklime approximately six months before the first consultation and had been using therapeutic soft contact lenses (SCL) and topical Moxifloxacin 0.5% every six hours continuously.

At the initial ophthalmological examination on June 29, 2022, slit-lamp examination of the RE showed corneal neovascularization

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and conjunctivalization across the entire surface, associated with significant stromal opacity, central descemetocele, and total limbal failure (Fig. 2a). The therapeutic SCL was maintained, Moxifloxacin 0.5% eye drops were prescribed every 12 hours, and lubricating eye drops were recommended as needed.

The patient was placed on the urgent PK waiting list due to the imminent risk of ocular perforation and, after five days, underwent the procedure combined with the SLET technique without complications. On the following day, the patient reported intense discomfort in the right eye (RE) when blinking, which improved within approximately ten days. Prednisolone 1% and Moxifloxacin 0.5% eye drops were prescribed every six hours, along with an antiglaucoma eye drop combining Brimonidine Tartrate 0.2% and Timolol Maleate 0.5% every 12 hours.

The progression occurred without complications or complaints from the patient. The amniotic membrane was gradually absorbed, accompanied by total corneal epithelialization. The contralateral donor area showed good recovery. Fourteen months after the surgery, slit-lamp examination of the RE revealed a clear and epithelialized cornea, although neovascularization was present in the superior temporal region, without progression during the follow-up period (Fig. 2f). Slit-lamp examination of the left eye (LE) showed an unremarkable limbal donor area. Uncorrected visual acuity was 20/60 in the RE and 20/20 in the LE.

Table 2 - Timeline of Case 2. Joaçaba, Santa Catarina, 2023.

Timeline	Description of the Consultation and Follow-up
DAY 1	RE: cornea with neovascularization across the entire surface, associated with stromal opacity, central descemetocele, and total limbal failure. Registered on the urgent transplant waiting list due to the imminent risk of ocular perforation. Using therapeutic soft contact lens (SCL), Moxifloxacin 0.5% every 12 hours, and lubricating eye drops as needed.
DAY 5	Underwent penetrating keratoplasty combined with the SLET technique in the RE without complications.
DAY 6	The patient reports intense discomfort when blinking. RE: opaque cornea, adhered amniotic membrane (AM), intact limbal fragments, and SCL without abnormalities. LE: limbal donor area in recovery.
DAY 12	Using Prednisolone 1% and Moxifloxacin 0.5% every six hours, Brimonidine 0.2% combined with Timolol Maleate 0.5% every 12 hours in the RE. The patient reported reduced discomfort when blinking. Opaque cornea, adhered amniotic membrane in absorption, intact limbal fragments, and SCL without abnormalities. Limbal donor area unremarkable. Topical medication regimen maintained.
MONTH 3	Good progression. RE: clear and epithelialized cornea, firm and well-positioned sutures. Amniotic membrane fully absorbed. Presence of large neovessels at the donor/recipient junction in the superior temporal region. LE: limbal donor area unremarkable. Visual acuity (VA) of RE: 20/150 uncorrected; VA of LE: 20/20. Intraocular pressure (IOP): RE 12 mmHg; LE 12 mmHg. Using Prednisolone 1% every 12 hours in the RE and lubricating eye drops as needed. Two sutures were removed.
MONTH 4	Paciente não apresenta queixas. OD: córnea clara e epitelizada, neovasos em região temporal superior sem progressão. OE: área doadora de limbo sem alteração. AV de OD: 20/80, sem correção. PIO de OD: 12 mmHg. Em uso de Prednisolona uma vez ao dia em OD e colírio lubrificante se necessário.
MONTH 10	The patient reports no complaints. RE: clear and epithelialized cornea, neovessels in the superior temporal region without progression. LE: limbal donor area unremarkable. Visual acuity (VA) of RE: 20/60 uncorrected; VA of LE: 20/20. Intraocular pressure (IOP) of RE: 12 mmHg. Using Prednisolone 1% eye drops every two days in the RE and lubricating eye drops as needed.

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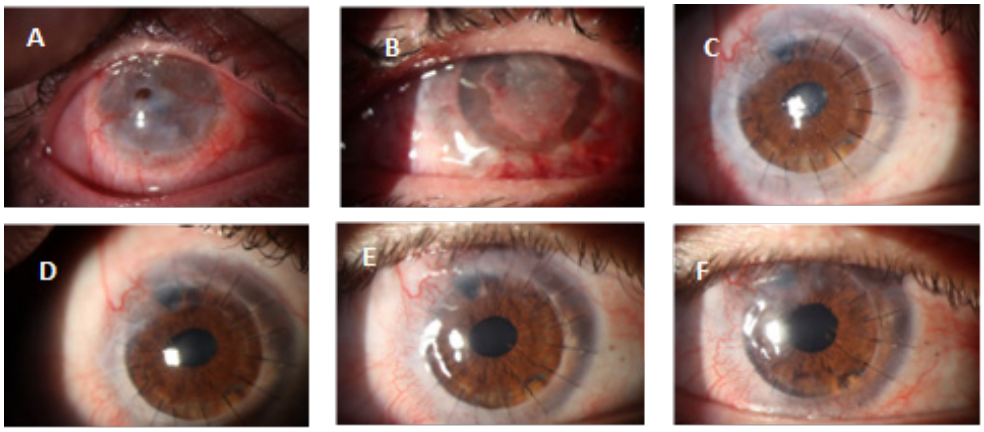
Timeline	Description of the Consultation and Follow-up
MONTH 14	Good progression. RE: clear and epithelialized cornea, neovessels in the superior temporal region without progression. LE: limbal donor area unremarkable. Visual acuity (VA) of RE: 20/60 uncorrected; VA of LE: 20/20. Intraocular pressure (IOP) of RE: 12 mmHg. Using Prednisolone every two days in the RE and lubricating eye drops as needed.



A) Neovascularization across the entire corneal surface, associated with stromal opacity, central descemetocele, and total limbal failure. B) Seventh postoperative (PO) day showing amniotic membrane in absorption. C) Second PO month presenting a clear and epithelialized cornea with large neovessels at the donor/recipient junction in the superior temporal region. D) Fourth PO month presenting a clear and epithelialized cornea, with neovessels showing no progression. E) Clear and epithelialized cornea, with neovessels without progression after the tenth month post-SLET. F) Clear and epithelialized cornea, with neovessels showing no progression after 14 months post-SLET.

Figure 2 - Progression of the left eye in case 1 during the follow-up period. Joaçaba, Santa Catarina, 2023.

DISCUSSION

Both cases successfully underwent treatment using the SLET technique with limbal cells from the contralateral eye, resulting, over an average of 16 months of follow-up, in a healthy cornea and good visual acuity. The etiology, identified as limbal stem cell deficiency (LSCD), differed between the two patients, as did the classification and severity of the condition. The patient in Case 1 had no well-defined etiology, primarily resulting from ocular trauma to the left eye (LE), with a final diagnosis of partial limbal stem cell deficiency confirmed through impression cytology. In contrast, the patient in Case 2, following an alkaline chemical burn to the right eye (RE), presented with total limbal stem cell deficiency, diagnosed based on signs, symptoms, and clinical examination, which revealed an imminent risk of ocular perforation, requiring the earliest possible surgical intervention.

Although exact epidemiological data on LSCD are currently unavailable, a significant number of patients with this condition have ocular burns as the primary etiology. Furthermore, at least two-thirds of cases undergoing stem cell transplantation are caused by LSCD[6]. In a pilot study using the SLET technique, the procedure was performed on six patients with unilateral LSCD caused by

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chronic ocular burns, yielding good postoperative results. The follow-up period averaged nine months, leading to the conclusion that the technique was promising[3]. In another significant study conducted later, 30 SLET procedures were performed on eyes of patients in whom the CLET technique had failed. Success was achieved in 80% of cases after two years of follow-up, leading the authors to recommend this technique as a replacement for CLET[7]. Among chemical injuries, alkaline burns appear to be the most common, as they cause more significant tissue damage than acidic burns. This is due to the saponification reaction of ocular membranes, which leads to deeper penetration into the limbal tissue, destroying the stem cells and their niche[6].

The severity of LSCD among the participants in this study varied. The patient in Case 1 initially presented with an opaque cornea and vascularized limbus, with symptoms limited to reduced visual acuity, requiring impression cytology for a more accurate diagnosis. Conversely, the patient in Case 2 exhibited conjunctivalization and neovascularization across the entire corneal surface, with symptoms of irritation and discomfort in addition to reduced visual acuity, making the clinical diagnosis more evident and precise. Thus, it is evident that patients with this condition can present differently—either asymptomatic at first or with debilitating symptoms such as discomfort, pain, irritation, reduced visual acuity, foreign body sensation, photophobia, and even blindness, among others[6].

The SLET technique has a significant impact on both cost reduction and minimizing risks associated with more complex ocular surgeries. By using a small donor limbal area, the risk of iatrogenic LSCD in the healthy contralateral cornea is reduced. Furthermore, the harvesting and transplantation are performed in a single operation, making the technique less invasive and easily replicable by corneal surgeons. Additionally, the direct transfer of limbal stem cells eliminates the need for specialized laboratory culture, drastically reducing the procedure's cost. In comparison, CLAU requires a larger donor tissue margin (ranging from 90 to 300 degrees of the corneal area), posing a higher risk of iatrogenic damage, while the cost and availability of the ex vivo system used in CLET limit its applicability[3,5,8].

Regarding the average follow-up period of 16 months in the present study, it aligns with most reports on the subject. A study published in 2016 followed 68 eyes of 68 patients who underwent SLET for unilateral LSCD, with an average follow-up of 12 months, achieving a completely epithelialized and avascular corneal surface in 83.8% of cases[9]. According to authors[3], most therapeutic failures after limbal cell transplantation occur within the first six months postoperatively.

As an abnormal progression, the appearance of neovessels around two months postoperatively in Case 2 is noteworthy. However, these remained stable and did not interfere with the patient's quality of life during the following 12 months of follow-up. This effect may be related to both the severity of the patient's initial condition and the possible angiogenic influence of the conjunctiva on the corneal surface during this period, as conjunctival epithelium is highly vascularized and consists of poorly organized cellular layers[10].

It is also noteworthy that the amniotic membrane showed rapid absorption in both cases, being fully absorbed approximately five weeks postoperatively, revealing a completely transparent cornea. Its use may be a critical factor for the success of SLET, as the amniotic membrane contains anti-inflammatory cytokines, growth factors, and provides a substrate that facilitates the incorporation of stem cells, promoting corneal epithelialization[5,11].

The primary determinant of successful outcomes in the SLET procedure is the health of the donor eye's limbus[4]. In the two cases reported in this article, the material was collected in a small amount from the contralateral side of the injured eye, which exhibited an intact surface and physiological limbal anatomy, following a thorough preoperative inspection of its viability. Subsequently, the donor eye in both patients achieved full recovery within a short period, with no asso-

ciated complications.

Two of the limitations of this study relate to the sample size and the follow-up duration, as 14 and 18 months are still limited periods for evaluating the efficacy and safety of a new surgical technique, even though most failures occur within the first six months postoperatively. Nonetheless, the description of these cases contributes to expanding knowledge about SLET and provides evidence regarding its application, practicality, and advantages. Moreover, it is worth noting that studies like this are scarce in Brazilian literature, given the recency of the technique, which enhances the relevance of this report.

CONCLUSION

The post-Simple Limbal Epithelial Transplantation (SLET) progression was favorable in both cases, showing transparent, epithelialized corneas without significant complications during follow-up. Conventional corneal transplantation had been ineffective in the patient with partial limbal stem cell deficiency (Case 1), highlighting the importance of SLET in the success of the second procedure, whose cause was not ocular burns. SLET, particularly in settings lacking cell culture resources, emerges as an attractive option for treating LSCD. In conclusion, the SLET technique was effective in repairing limbal insufficiency in the two cases presented.

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Author CRediT statement

Conceptualization: Silva, JPG; Marquezan, MLS; Stock, RA. Methodology: Silva, JPG; Marquezan, MLS; Stock, RA. Validation: Silva, JPG; Marquezan, MLS; Stock, RA; Bonamigo, EL. Formal analysis: Silva, JPG; Marquezan, MLS; Stock, RA; Bonamigo, EL. Investigation: Silva, JPG; Marquezan, MLS. Resources: Stock, RA. Writing-original draft preparation: Silva, JPG; Marquezan, MLS. Writing-review and editing: Silva, JPG; Marquezan, MLS; Stock, RA; Bonamigo, EL. Visualization: Silva, JPG; Marquezan, MLS; Stock, RA; Bonamigo, EL. Supervision: Stock, RA; Bonamigo, EL. Project administration: Stock, RA.

All authors have read and agreed to the published version of the manuscript.

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