Conjunctival-limbal autograft in total unilateral limbal stem cell deficiency

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Key words:
limbal stem cell deficiency; autologous limbal stem cell transplantation; conjunctival-limbal autograft; amniotic membrane; cornea

Cite as:
Zdrav Vestn. 2017; 86:266–75.

Received: 21. 4. 2016
Accepted: 6. 4. 2017

Abstract

Background: The corneal epithelium is renewed by stem cells (SCs) that reside in the corneal limbus. Absence or dysfunction of limbal SCs or their niche leads to the ocular surface disease called limbal stem cell deficiency (LSCD), clinically characterized by corneal conjunctivalization, vascularization, persistent epithelial defects, chronic inflammation, and loss of vision. Total unilateral LSCD is treated by autologous transplantation of limbal epithelial stem cells (LESC) obtained from the healthy other eye. We describe the treatment of choice for unilateral LSCD, i.e. the combination of autologous limbal transplantation, called conjunctival limbal autograft (CLAU) and the use of amniotic membrane (AM). We present the results of CLAU in three patients with total unilateral LSCD due to chemical injury.

Methods: Autologous limbal transplantation CLAU begins with the removal of the fibrovascular pannus from the diseased corneal surface and the harvesting of two conjunctival-limbal grafts from the healthy eye. The grafts are then transplanted onto the limbal area of the recipient eye. AM is used as a patch to cover the denuded cornea and limbal grafts, and serves as a barrier preventing the conjunctival epithelium from encroaching onto the temporal and nasal sides of the corneal surface. In the donor eye, AM is used to cover the donor sites. The combination of CLAU and AM transplanation was used in three patients with unilateral LSCD due to chemical eye injury. In one patient, limbal transplantation was combined with symblepharon lysis for entropium repair. In all cases AM was removed three to six days postoperatively to assess the growth of new epithelium from the limbal grafts. In all patients the ocular surface was covered with another AM that was left in place until the cornea was completely epithelialized and the new epithelium stabilized. One patient required subsequent corneal regrafting and cataract removal.

Results: During the follow up period CLAU proved successful in two patients and partially successful in one patient. In all cases the growth of new epithelium from the limbal grafts was noted on days three to six after CLAU. The cornea completely epithelialized within two weeks in two patients and within 35 days in one patient. In two patients the corneal epithelium remained clear, smooth and stable during the follow up of three and a half years and four months, respectively. In one patient, uneven epithelium, probably representing a mosaic pattern of corneal and conjunctival cells, was noted in the central corneal region, where a small corneal ulcer developed five months after CLAU. In the donor eyes no postoperative complications were noted, the donor sites epithelialized within a few days.

Conclusions: Autologous limbal transplantation using the combination of CLAU and the use of AM is a successful and safe therapy for restoring corneal surface in total unilateral LSCD after chemical injury. It enables surgeons to perform further surgical procedures for restoring the vision, such as corneal transplantation and cataract surgery.

Cite as: Zdrav Vestn. 2017; 86:266–75.

1. Introduction

A healthy cornea is covered with smooth and transparent epithelium that is crucial for its protection and transparency (1). The corneal epithelium is renewed by a population of stem cells (SC) that reside in the limbus, the anatomical border between the cornea and conjunctiva. SCs are concentrated in the limbus and maintained in special supporting microenvironments, known as the SC niches. The limbus serves as a barrier between the conjunctiva and the cornea preventing the growth of conjunctiva onto the cornea. In the case of absolute absence or dysfunction of limbal SCs or their niche, ocular surface failure may result from insufficient corneal epithelium renewal;it is clinically referred to as limbal SC deficiency (LSCD) (2). It is characterized by conjunctivalization and vascularization of the cornea, persistent epithelial defects, chronic inflammation, corneal haze, pain and vision deterioration (3). The diagnosis is based on the patient's history and clinical presentation (4,5). Although LSCD can be congenital (aniridia), in the majority of cases it is acquired (chemical and thermal injures, Stevens-Johnson syndrome, ocular cicatricial pemphigoid, contact lens misuse).LSCD can either be unilateral or bilateral, partial or total.

Figure 1: Photograph of the eye with total unilateral LSCD 4 years prior to CLAU (Case 1): chronic inflammation, vascularization and conjunctivalization of the cornea and corneal graft.

In partial LSCD the conjunctival epithelium that grows over the cornea can be removed by sequential sector conjunctival epitheliectomy. It is the main procedure that prevents the conjunctivalization of the cornea in the acute phase after njury (6). To accelerate epithelialization with the healthy corneal epithelium covering the deepithelialized cornea with AM was proposed by Tsang and co-workers in 1998. It markedly improves the healing process and visual acuity outcome (7).

In patients with total LSCD, LESC transplantation is required to reconstruct the ocular surface. Classical surgical approaches with direct transplantation of the limbal tissue can be used as well as the treatment using *ex vivo* expanded limbal epithelium that involves LESC harvested from a small limbal biopsy (cultivated limbal epithelial transplantation, CLET) (8,9). In both cases, the transplantation can either be autologous or allogenic, depending on whether involvement is unilateral or bilateral. The treatment of total LESC deficiency by direct limbal transplantation was introduced by Kenyon and Tseng in 1989 (10). They described the procedure suitable for treating unilateral ocular involvement, known today as conjunctival-limbal autograft (CLAU) (11). Unlike in unilateral LSCD, in total bilateral LSCD no autologous source of healthy donor limbal tissue is available, therefore limbal allografts are harvested from a living related donor(living related conjunctival-limbal allograft -lr-CLAL) or from a cadaver donor (keratolimbal allograft KLAL). The same surgical technique is used for CLAU and lr-CLAL. Contrary to autologous limbal grafts, limbal allografts have a low survival rate (12-15). Despite systemic immunosuppressive

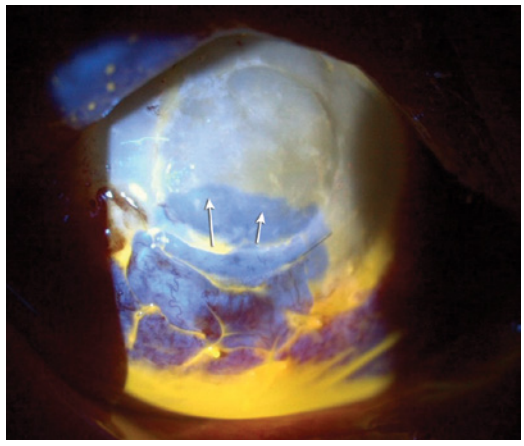

Figure 2: Fluorescein staining image of the ocular surface after CLAU (Case 1):the growth of new epithelium (arrows) from the inferior limbal graft on day 3 after surgery.

therapy, rejection occurs in more that 50 % of cases by five years after transplantation (12-15).

2. Methods and patients

Three consecutive patients with unilateral total LESC deficiency were included in the study. The limbus was transplanted from the healthy eye using CLAU as described by Dua (16) with slight modifications. Only in one patient keratectomy of the diseased cornea at 12 o'clock was performed to make appropriate recipient bed for the transplanted graft. In addition to limbal transplantation we used AM in both eyes in all cases. Prior to the procedure ocular surface inflammation and dry eye were properly addressed so that all affected eyes were inflammation free for at least 18 months. All patients were operated under general anesthesia. After the preparation of the recipient eye surface 360° peritomy was performed and all the conjunctival epithelium and subepithelial fibrovascular tissues were removed from the cornea. The cornea was covered with AM and then the limbal tissue was harvested from the healthy eye. We obtained two conjunctival-limbal grafts with two hours of circumference at the 12 and 6 o'clock positions and transferred them onto the diseased eye. The grafts comprised 1 to-2 mm of the peripheral cornea, the limbus and 3 mm of the conjunctiva. They were held in place each by two single 10–0 nylon sutures on the limbus of the affected eye and two single 8–0 vicryl sutures on the conjunctiva at the 12 and 6 o'clock positions. Both, corneal and scleral grafts were covered with AM that was sutured onto the conjunctiva. Next, AM was sutured by 8–0 vicryl sutures onto the donor sites of the healthy eyes in order to accelerate their epithelialization. From the very first day the patients were treated with preservative free eye drops: (0.5 % moxifloxacin eye drops four times daily (Vigamox®, Alcon Pharmaceuticals, Fort Worth, TX, USA), 0.1 % dexamethasone eight times daily (Dexamono®, Laboratoires Thea, Clermond-Ferrand, FR). Every hour they received artificial tears and autologous serum drops in an alternating fashion. The benefits of this therapy in limbal transplantation have been described by Dua and co-workers (17). Antibiotic eye drops were applied until the ocular surface became fully epithelialized. Corticosteroid drops were used for six months in decreasing doses, or until inflammation subsided Dexamethasone was then replaced by 0.5 % loteprednol (Lotemax®; Bausch and Lomb Incorporated, Rochester, NY, USA) given two times daily in the first month and afterwards once daily as a long-lasting treatment until the scheduled corneal transplantation. In all patients AM in the recipient eye was removed a few days after the transplantation to allow accurate assessment of epithelial growth from the limbal grafts. The exposed cornea of the recipient eye and both limbal grafts were then recovered with a new AM. It was sutured under the nasal and temporal conjunctiva in order to restrain the migration of conjunctival epithelium onto the cornea. Corneal epithelialization was monitored using fluorescein dye. If conjunctival ep-

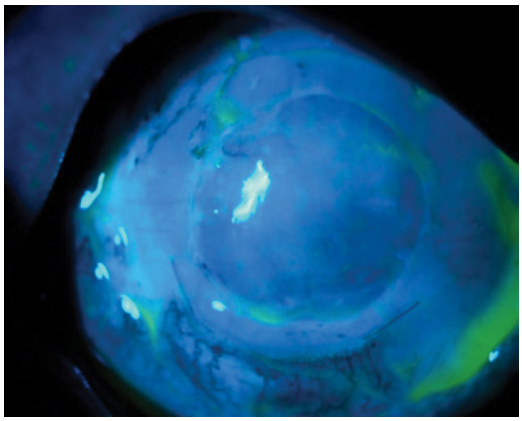

Figure 3: Fluorescein staining image of ocular surface after CLAU (Case 1): 14 days after the procedure: the cornea in fully epithelialized with smooth epithelium from limbal grafts.

ithelium was seen to have grown on the cornea, it was removed by mechanical abrasion using a slit lamp, as previously described (18). AM was finally removed once the cornea of the recipient eye was fully epithelialized and the epithelium was stable enough.

3. Case 1

A 64 year-old lady suffered a chemical injury to her right eye in 2003. As a consequence, total limbal stem cell insufficiency developed. Four years after the injury she underwent corneal transplantation. After surgery, conjunctivalization due to total LSCD. Occurred in her own and in the transplanted corneas. In addition, the cornea became hazy because of graft rejection (Figure 1). By the time she visited our clinic her right eye surface had become heavily inflamed with conjunctivalization and vascularization of the graft with a persistent, centrally located epithelial ulcer. The anterior chamber was of appropriate depth with a circular, centrally lying pupil; deeper ocular parts could not be seen. Ultrasound examination of her right eye did not reveal any pathological changes in her optic disc or chorioretina. The left eye showed no signs of LSCD; the cornea was clear and the deeper ocular parts appeared normal. Visual acuity was counting fingers at 1 m in the right eye and at 1.0 m without correction in the left eye. Intraocular pressure was normal. Preoperative diagnostic procedures revealed marked hyposecretion of the lacrimal gland with Schirmer test being 2mm/5min in the right eye and 3mm/5min in the left. The ocular surface of the patient's healthy left eye was smooth and without any signs of inflammation. In July 2012, after the inflammation and dry eye had been properly addressed, CLAU using AM was performed. On day three post-surgery, after the removal of AM, growth of the epithelium from the limbal grafts was noted; the growth being faster from the lower graft (Figure 2). The epithelium was smooth, transparent and stable and from a clinical point of view it exhibited all characteristics of the corneal epithelium. After AM re-transplantation the cornea became fully epithelialized within two weeks of limbal transplantation (Figures 3 and 4). Six months later the cornea was re-transplanted, and in June 2013 cataract surgery was performed. The patient was followed up for three and a half years during which time the cornea and the corneal transplant of the recipient eye remained inflammation-free and epithelialized with smooth, clear and stable corneal epithelium(Figure 5). There were no complications in the donor eye (Figure 6). At the end of the follow up period the patient's visual acuity

Figure 4: Photograph of the eye 14 days after CLAU (Case 1): newly formed epithelium is transparent, smooth, stable and without vascularization, which improves postoperative visual acuity.

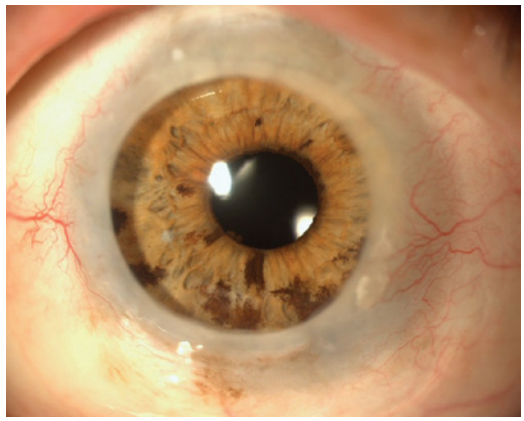

Figure 5: Photograph of the recipient eye 3.5 years after CLAU and subsequent corneal transplantation and cataract surgery (Case 1): the epithelium of the cornea and corneal graft is still transparent, smooth and stablel.

with eye glasses was 0.6 in the right eye and 0.8 in the left eye.

4. Case 2

In 2012, a 47 year-old man sustained an injury to his left eye caused by metal cleaning acid. On clinical examination there was extensive 360-degree limbal ischemia, ischemia of the whole bulbar and tarsal conjunctiva with superficial necrosis of the nasal part of the upper tarsal and bulbar conjunctiva. Almost entire ocular surface was devoid of epithelium, the cornea was cloudy; there were precipitates and marked hyperemia of the iris. Because of poor epithelialization and subsequent onset of keratomalacia with persistent epithelial ulcer, the patient underwent three AM transplantations over the next three months. After AM placement ocular surface became covered with conjunctival epithelium due to total LSCD. In the next year the patient was treated continuously for persistent ocular surface inflammation. Eighteen months after the injury the inflammation finally calmed down, yet visual acuity was restricted to hand motion perception. There was a symblepharon, superiorly nasally, with entropion of the upper eyelid; the conjunctiva was heavily thickened with marked scarring, especially in the nasal half.The cornea was completely overgrown by the conjunctiva, the corneal stroma was hazy; the temporal part of the anterior chamber was visible, whereas deeper structures could not be inspected (Figure 7). Intraocular pressure by digital palpation was normal under the therapy . Ultrasound examination revealed a slightly excavated optic disc, Schirmer test was normal. The left eye was healthy with visual acuity of 1.0 without correction. In August 2015 the patient underwent CLAU with the use of AM and symblepharon lysis. On removing AM six days after CLAU, we noted that the lower half and the superior temporal parts of the cornea next to the grafts were covered with smooth and transparent epithelium. At the temporal border of the upper graft we noticed growth of the conjunctival epithelium towards the limbus. The upper fornix was deep, and the eyelids fitted snugly against the globe. On day 14 after surgery the cornea was largely epithelialized with only a small 3×3 mm defect nasally, that healed rather slowly. Total epithelializationoccurred as late as 35 days after CLAU surgery. At three months after surgery we noticed irregular epithelium in the central part, showing a characteristic mosaic pattern of the epithelium of the cornea and conjunctiva, and the onset of vascularization over the limbus, nasally. In January 2016, the patient was admitted to the hospital with a small shallow central corneal ul-

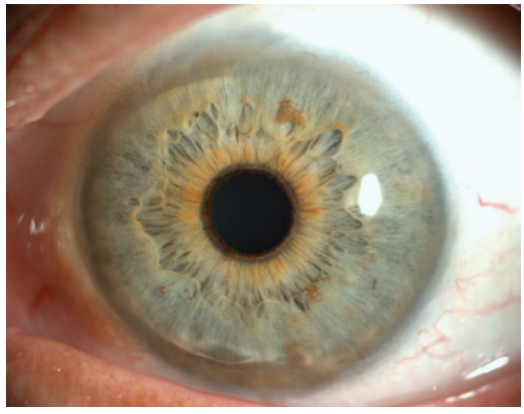

Figure 6: Photograph of the donor eye 3.5 years after CLAU surgery (Case 1).

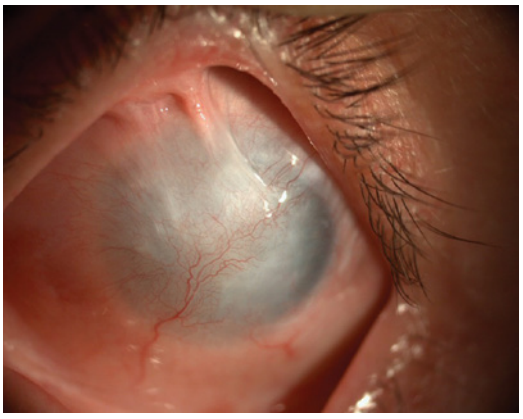

Figure 7: Photograph of the eye with total unilateral LSCD before surgery (Case 2): total conjunctivalization, vascularization of the cornea, symblepharon.

cer from which alpha-hemolytic Streptococcus was isolated. The ulcer healed on treatment with penicillin eye drops 20.000 IU/ml (magistral drug) (Figure 8). At the end of the six-month follow up period visual acuity was the same as before CLAU (hand motion). The globe was still injected and the cornea was epithelialized; the central epithelium, however, showed a mosaic pattern and was smooth only inthe 2–3-mm-wide zone on the borders of both grafts. Once the inflammation calms down, sequential sector conjunctival epitheliectomy (SSCE) should be done to promote epithelialization of the cornea from the limbal grafts only. A subsequentl histological examination of the epithelium should be considered. In the donor eye there were no complications and the patient's visual acuity at the end of the follow up was the same as before surgery.

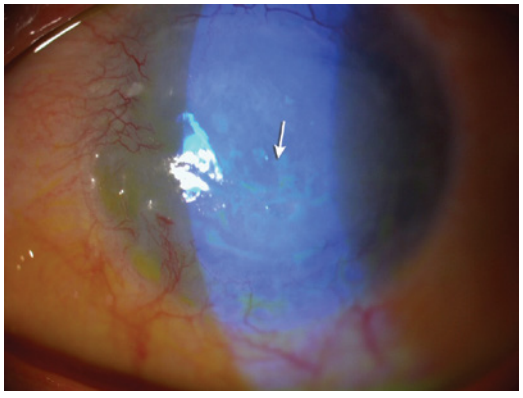

Figure 8: Fluorescein staining image of ocular surface 6 months after CLAU (Case 2): mosaic corneal and conjunctival epithelium in the central region (arrow), next to the grafts the epithelium is smooth.

5. Case 3

In 1991, a 44-year-old man suffered chemical injury to his right eye while handling a grout gun. He underwent three corneal transplantations. With each transplant the graft became hazy and conjunctivalized and there was recurrent eye inflammation. At presentation his visual acuity in the affected eye was counting fingers at 10 cm; the ocular surface was free of inflammation, Total conjunctivalization of the cornea and deep neovascularization of the corneal grafts were seen (Figure 9). Multiple paracentral anterior synechiae with a notably atrophic iris and dense cataract were present. Deeper ocular parts were unvisualizable. Ultrasound examination of the right eye did not reveal any abnormalities of the optic disc or chorioretina. Visual acuity of the healthy eye was 1.0 without correction.There were no clinical signs of dry eye in either eye. In September 2015, we performed CLAU surgery with the use of AM. Within the next few days the nasal and temporal parts of AM became overgrown with the epithelium that originated from the nasal and temporal conjunctiva. It was removed six days after surgery; the underneath cornea was epithelialized with the epithelium growing from both limbal grafts (Figure 10). The cornea was re-covered with AM; it was left in place until full epithelialization

two weeks after CLAU. Within the follow up period of four months, the cornea remained epithelialized with smooth, transparent and stable epithelium.Visual acuity improved to counting fingers at 0.5 m (Figure 11). On day six after the procedure the donor sites were epithelialized and at the end of the follow up period the patient's visual acuity was the same as before surgery. There were no complications recorded.

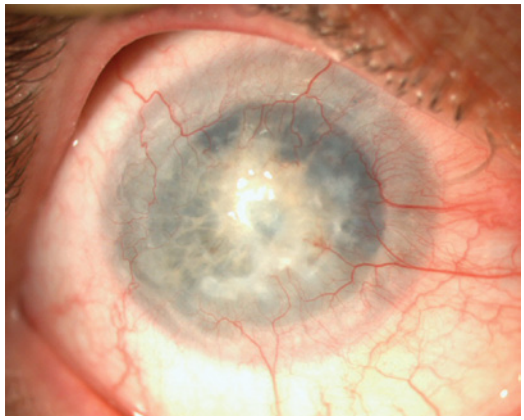

Figure 9: Photograph of the eye before surgery with total unilateral LSCD and 2 hazy corneal grafts (Case 3): vascularization and conjunctivalization of cornea and corneal grafts.

6. Results

We have used the combination of autologous transplantation of the limbus (CLAU) and AM transplantation in three patients, one woman and two men, with an average age of 52 years at the time of surgery. In all patients the indication for CLAU was unilateral total insufficiency of LESC due to chemical injury to the eye. There were no intraoperative complications. One patient was followed up for 42 months, and the other two patients, for six and four months, respectively. Three to six days after surgery we removed AM and noticed growth of the epithelium from limbal grafts in all patients. In two cases the cornea became fully epithelialized within 14 days of the procedure whereas in one case it took as long as 35 days to epithelialize. In two patients the epithelium retained the properties of corneal epithelium, it remained smooth, transparent, stable and with no signs of neovascularization for the duration of follow up.. Furthermore, in both patients visual acuity was improved after CLAU. In the patient who underwent symblepharolysis and CLAU, performed in the same session, the central region of the cornea was covered with unstable, mosaic corneal and conjunctival epithelium. At the end of the follow up period visual acuity was the same as it was before surgery. The donor sites became epithelialized within a few days in all cases and no complications were recorded in the donor eyes, either intra- or postoperatively. In one patient visual acuity in the donor eye decreased because of progressing age-related cataract In the other two patients it remained the same as before surgery. Dry eye did not influence the final outcome in our series of patients.

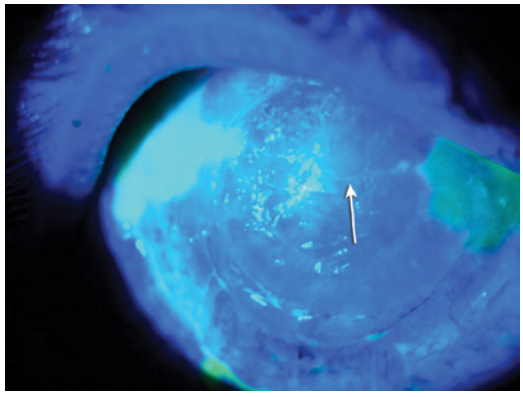

Figure 10: Fluorescein staining image of ocular surface of the recipient eye 1 week after CLAU (Case 3): the cornea is largely epithelialized with the limbal graft epithelium. Horizontal contact line (arrow) between the epithelium from the inferior and superior grafts is visible paracentrally superiorly. There are also some smaller areas without epithelium, nasally and temporally next to the limbus.

7. Discussion

CLAU is an effective method for the treatment of total unilateral LSCD (10,14,19–21). In a very few cases, however, it may cause complications in the donor eyes, such as filament keratitis and even microperforation (16,22–25). In order to reduce the risk for complications, transplantation of limbal epithelium cultivated from a small limbal biopsy of the healthy eye (CLET) (8) has been used with increasing popularity. In contrast to CLAU where 6x1 mm of cornea and 6x3 mm of conjunctiva (2 hours of corneal circumference) have to be harvested, for CLET only a small corneal biopsy of 1x2 mm is obtained Given that CLET is performed in few European centres and is not used in Slovenia, and taking into consideration the high costs of preparation of cultivated epithelium (14), CLAU remains the method of choice for treatment of total unilateral

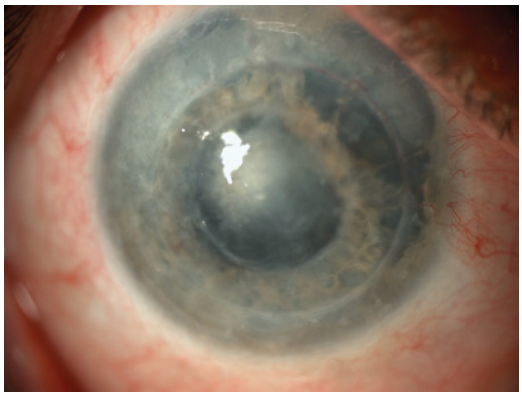

Figure 11: Photograph of the recipient eye 4 months after CLAU (Case 3): the epithelium of the cornea and corneal grafts is still transparent, smooth and stable. Repeat corneal transplantation and cataract surgery are proposed.

LSCD in this centre. In our series of cases no intra- or postoperative complications were recorded in the heathy eye, despite marked dry eye in Case 1. Therefore we believe that CLAU is a safe treatment option provided that appropriate topical treatment is used and the patient has frequent follow up examinations.

For CLAU to be successful it is crucial to monitor closely the growth of conjunctival epithelium towards the limbus, and to prevent the growth onto the cornea, as first pointed out by Dua (16). This is in agreement with the results of our study: in two cases the cornea was epithelialized from the grafts whereas in one patient (Case 2) the conjunctival epithelium probably "overtook" the epithelialization from the limbal grafts. In the cases described we used no other methods (e.g. impression cytology) for the identification of the conjunctival epithelium. However, the technique may be of great value in patients with clinical features of both corneal and conjunctival epithelium, because it could confirm the presence of goblet cells and, therefore, of conjunctival epithelium. In order to prevent growth of the conjunctival epithelium over the cornea we used both, bmechanical removal, and AM suturing under the conjunctiva in the nasal and temporal parts, as proposed by Dua and co-workers (26). AM served as a patch placed over the limbal grafts and the deepithelialized cornea, thus protecting the transplanted limbal tissue and creating favorable environment for survival of LESC and growth of new epithelial cells over the cornea. In Case 3 we noticed extensive growth of conjunctival epithelium over AM. The epithelium was removed together with AM. It can be speculated that in Case 2 this technique was not adequate to fully prevent the growth of conjunctival epithelial cells onto the cornea. As a consequence, the cornea became epithelialized with corneal epithelium from the grafts, as well as with conjunctival epithelium.

Furthermore, for CLAU to be successful the eye should be free of inflammation for at least six months before surgery (27). Careful and precise postoperative treatment is mandatory to establish conditions for the survival of LESC and growth of new corneal epithelium. Thus the cornea became epithelialized despite the presence of marked dry eye, although dry eye was described as a major risk factor affecting the survival of LESC (28).

According to previous reports treatment by symblepharon lysis was successful regardless of whether it was performed prior to or at the same time as CLAU (29). As shown by the results in Case 2, who had simultaneous symblepharon lysis and CLAU, limbus transplantation should be the final procedure in ocular surface reconstruction, e.g.eyelid correction or symblepharon lysis. It can be speculated that simultaneous symblepharon lysis in our case was the reason for the poorer epithelialization of the cornea from the limbal grafts. In view of the fact that every additional conjunctival procedure induces inflammation and leads to uneven ocular surface, symblepharon lysis per se might decrease the rate of LESC survival.

8. Conclusion

Autologous transplantation of limbal tissue using CLAU technique in combination with AM transplantation, is a successful and safe method for the treatment of total unilateral LSCD due to chemical injury. The success rate of corneal epithelialization from limbal grafts' depends on the frequency of follow up examinations, as well as on recognition and prevention of growth of conjunctival epithelium onto the cornea and provision of the appropriate microenvironment for the growth of new epithelium.

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