Oral Mucosal Cells in Treatment of Blindness

A Master Thesis by

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CONTENTS

INTRODUCTION

This master thesis is organized as follows.

Part I: This section gives a brief overview of oral mucosal epithelial cells (OMECs) used in treatment of Limbal Stem Cell Deficiency (LSCD) as well as future perspective of a novel surgical method drawn in the second section.

Part II: Section 2 is presented as a review article about this new technique: Simple Limbal Epithelial Transplantation (SLET). This paper was published in the journal "STEM CELLS Translational Medicine", December 2019. The review examines the question of SLET as a treatment option for LSCD. Section 2 is carried out by authors listed in the paper. My (Inger Thea Myklebust Ernø) contribution to this work, in addition to writing a paper, has mainly been to compare factors of high significance in reviewed papers. These include etiology, coverage of LSCD, number of patients, mean age, prior ocular surgery before SLET, simultaneous or follow-up surgery, percentage of stable corneal surface, percentage of visual improvement, complications and follow up time. With this in mind, I will call into question if SLET can be used with OMECs in the future.

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PART I

Limbal Stem Cell Deficiency

The ocular surface consist of corneal and conjunctival epithelium (1). The cornea is critical for normal vision by allowing light transmission to the retina. Corneal epithelium is useful because it keeps the surface transparent and avascular (2). A remarkable feature of the cornea together with other organs such as skin and gut, is its possibility to regenerate. If the tissue is injured in some way, stem cells will heal the tissue and ensure homeostasis (1).

The core problem in the disease called “Limbal Stem Cell Deficiency” (LSCD) is destroyed limbal stem cells (LSCs). LSCs can be destroyed by many factors. Some of them are chemical or thermal injuries, infections or surgeries involving the limbus (2). These stem cells are usually found in the periphery of the cornea, in limbal epithelial crypts, known as the limbus (3, 4). LSCs are critical for the homeostasis in cornea and usually protects it against ingrowth of conjunctival tissue (1). LSCD can reduce the number of LSCs or impair its function (5). If there is still some living stem cells that are not functioning properly, the patient can experience gradually increasing of symptoms from LSCD over time (5).

Pathologically when LSCs is dysfunctional, conjunctival tissue will find its way over the cornea. This migration of conjunctival tissue is also known as conjunctivalization (5). Conjunctivalization is the fundamental characteristic of LSCD and can be partial or total (5). This will eventually destroy the corneal epithelium and give rise to ulceration, scarring and may also lead to corneal perforation (5). LSCD is also recognized by loss of vision often companied by symptoms of heavy pain (5, 6).

Historical perspective of treatment of limbal stem cell deficiency

The treatment of LSCD has changed dramatically the last decades. In 1964, Jose Barraquer described ”Epithelial limbus, conjunctivocorneal taken from the other eye” for treatment of

burns on the surface of one eye. This was one of the first documentations for treatment of diseases on the surface of the eye (7). In 1977 Thoft described ”Conjunctival transplantation” as a technique for treatment of chemical injuries in one eye. This technique did not take the stem cells of the cornea into account, and could not regenerate the surface of the eye (7, 8).

The evidence from these studies points towards the idea of the autologous limbal transplantation from the healthy eye to the affected eye. Since this transferring happens in the same patient, it is a reduced risk for rejection due to an immunoreaction (9, 10). The most important limitation is due to the fact that the limbal transplant is large and can trigger an LSCD on the healthy cornea. In worst case, this can lead to bilateral LSCD (10, 11). With this follows another limitation. This treatment is not feasible for patients with bilateral LSCD (6, 10). A way to reduce the large transplant is to use conjunctival limbal autograft which is an addition of conjunctival tissue. If this tissue is used, there is no need for such large limbal tissue graft from the healthy eye. A consequence of this, is reduced chances for LSCD in the healthy eye (10, 12). A negative consequence is though that it increases chances for conjunctival growth over the cornea since the function to keep the invasion away has not been reestablished (10).

Later, in 1984, Thoft came with a new procedure where he used cornea from a deceased donor to treat bilateral LSCD (7, 13). This was the first allogenic transplantation technique to handle serious disease on the surface of the eye. This was modified in 1990 (7, 14). This technique uses the easiest way to harvest for transplantation: corneas from deceased people. The first corneal transplantation with some success was performed by Dr. Zirm already in 1905 (10, 15). The corneas from a deceased boy was transplanted to an adult patient. However, there was complications with one of the grafts. The success was due to the other cornea which maintained transparency. Allogenic transplantation is valuable for bilateral LSCD and is now used as an alternative treatment. As we will see further on, unilateral LSCD can be treated with autografts (6, 10, 12 , 16-19).

Despite major advances, it was not before the understanding of the location and function of LSCs, that they could develop successful protocols for treatment of LSCD. This knowledge was based on the work of Davanger and Evenson in 1970 (4), Schermer *et al.* (20), Kinoshita *et al.* (21) and Potten and Loeflller (22) in the 1980s (7). This research was the foundation for medical and surgical treatment that could preserve and replace destroyed or missing LSCs. The understanding of conjunctiva in eye diseases was also crucial for future treatment ways of LSCD (7).

Langer and Vacanti then explored tissue engineering. They combined biology and engineering to make artificial tissue that could reestablish or make tissue function better (10, 23). Tissue engineering uses stem cells isolated from any human organ (10). Kenyon and Tseng were the first who took the theory of LSCs out in practice. Their ”Limbal Autograft Transplantation” used tissue from conjunctiva and cornea from the healthy eye to a patient with unilateral LSCD. This is a treatment option today for serious unilateral eye disease (7, 24). In 1994, Tsai and Tseng described the first of many variations of today’s protocol for using limbal tissue from deceased donors (19). One year later, in 1995, the description of living relatives as a source for eye cells of Kwitko *et al*. was published. In their procedure, bites of the conjunctiva were taken from siblings or parents to treat LSCD (25). Kenyon and Repoza expanded this technique to include limbal region in periphery of cornea (7).

The evolution of treatment forms for injuries on the surface of the eye has led to development of many surgical techniques. It has also been researched on alternative sources to tissue, both allogenic and autologous. Progress in culturing techniques has led to the usage of cultured epithelium for transplantation. In 1957, Rheinwald and Green did many studies that strengthened the concept of cultured epithelium as stem cell mediated treatment (7, 26).

Pellegrini *et al.* were the first to describe this alternative technique. They used autologous cultured epithelium cells from the cornea to treat two patients with unilateral LSCD caused by chemical injuries. A small bite of limbal tissue from the healthy eye was used to grow corneal epithelium for transplantation. With this method cornea was restored and kept stabile in many months after transplantation (7, 27). Tsai *et al.* expanded this project with culturing the cells on amniotic membrane (7, 16). Schwab *et al.* did the same only with allogenic cells (7, 28). Tsai and Schwab also knew that limbal tissue from deceased donors could be used as an alternative source to allogenic cultured tissue (7).

Nakamura *et al.* then tried to use an alternative cell type for surface reconstruction of the eye with the first culturing and autologous transplantation of oral mucosal cells from rabbit on amniotic membrane (7, 29). Nishida *et al*. later used the same method with four humans with bilateral stem cell deficiency (7, 30). After these studies has a number of authors showed clinical results of cultured oral mucosal cell transplantation to the eye (7, 31-34).

In other words, it is used a lot of time and work to develop solid treatment of LSCD. To summarize, it is three different methodologies available to treat LSCD today. Either autogenous transplantation, allogenic transplantation or tissue engineering of auto- or allogenic cells with stem cell potential. It is a lot of research going on new treatment methods of LSCD. With an understanding of the history it is easier to predict future treatment alternatives for treatment of LSCD.

Oral mucosal epithelial cells as treatment for limbal stem cell deficiency

Bilateral LSCD is far more common than unilateral LSCD (2, 35). As mentioned earlier, bilateral LSCD can be treated with allogenic tissue transplantation. However, a major drawback of allogenic tissue is the need of immunosuppressive medications (5). The important advantage

of autologous tissue transplantation is therefore that it eliminates the risk of transmitting microorganism´s, rejection and thus the use of immunosuppressant´s (36, 37).

Bilateral LSCD is therefore one clinical indication to use autologous tissue like oral mucosal epithelial cells (OMECs) as the type of transplantation tissue. OMECs is increasingly becoming an attractive autologous alternative to corneal stem cells in these cases (35). A review from 2016 of the literature on this matter found a 72% success rate of cultured autologous oral mucosal epithelial cell sheets (CAOMECS) for treatment of LSCD. Follow up time was between 1-7.5 years and the number of tested patients was 242 (2, 30-35, 38-52).

Already in 1986, Gipson *et al*. developed the concept of transplanting OMECs. They transplanted oral mucosal epithelium to a destroyed rabbit cornea (53). This initiated the development of the use of oral mucosal epithelial cells to repair destructed corneal surfaces (10). The second study on a rabbit model wasn't performed before 2003. The group of this work transplanted OMECs on to rabbit eyes where corneal epithelium was removed first. The OMECs were grown on amniotic membranes (54). *Ex vivo* cultivated oral mucosal autograft (EVAMAU) was first described in humans in 2004 by Nishida *et al*. as earlier described in this thesis (10, 30).

Takahashi and Yamanaka discovered induced pluripotent stem cells (iPSCs) in 2006. They induced it by introduced stem cell factors in fibroblasts. iPSCs have the potential to differentiate into all types of cells under suitable cell culture environment (10, 55). Another type of stem cells that can be isolated from the organs is progenitor stem cells. Their capacity to differentiate is limited since these cells are already specialized to a type of cell (10). OMECs can differentiate into different types of epithelium. OMECs are progenitor stem cells and have a tendency to be pushed forward to differentiate to a specific type of cell depending on culture conditions. OMECs have been isolated to make cell sheets for esophagus treatment after

endoscopic submucosal dissection due to their biological closeness (10, 56-57). As we will see, OMECs has also been used to engineer cell sheets to reverse LSCD (10, 30).

Interestingly, there are more autologous reservoirs than the oral cavity that is considered for transplantation to cornea. Various approaches of autologous cell types have been put forward to solve this issue. In the literature embryonic stem cells, conjunctival epithelial cells, epidermal stem cells, dental pulp stem cells, bone marrow derived mesenchymal stem cells, hair follicle bulge derived stem cells and umbilical cord lining stem cells has been explored (35, 58-64).

However, only conjunctival and oral mucosal epithelial cells has been explored in clinical studies in human models of the non-limbal cell types (35). OMECs is interestingly the most documented autologous non limbal cell type for this use (5). As further mentioned by Utheim *et al*. it is a considerable amount of promising reports of transplantation treating LSCD in both animal and human models (5). OMECs are the most frequently used for *in vitro, in vivo,* and translational operations among the cells used for corneal regeneration (10).

Transplantation of OMECs has the advantage of easy isolation from biopsies. The wound heals quickly without residual scarring (2). When it comes to area of tissue harvesting, Islam *et al.* investigated this for *ex vivo* expanded transplants as the morphology, degree of keratinization and phenotype varies thorough the oral cavity. They investigated phenotype, attachment and growth and found that OMECs from lower lip and transition zone of lower lip cultured in Dulbecco's modified Eagle's medium/Ham's F12 (DMEM/F-12) are the most promising site for treatment as the cells from this site has the best growth capacity (65). However, the most common place of harvesting OMECs are inferior buccal mucosa (65).

The majority of LSCD causes do not affect OMECs (5). This cell source is also valuable in patients with Stevens Johnson Syndrome. This syndrome can give rise to an immunological form of LSCD. This condition can also appear in the oral cavity. Despite this finding,

transplantation of OMECs has proven successful (5, 39). Unfortunately, OMECs can give peripheral corneal neovascularization after transplantation (5, 31). In fact, they have greater angiogenetic potential than limbal cells (5, 66-68). This shortcoming has however been tried to solve by hypothesizing that angiogenetic therapy can regress this vascularization (5, 69).

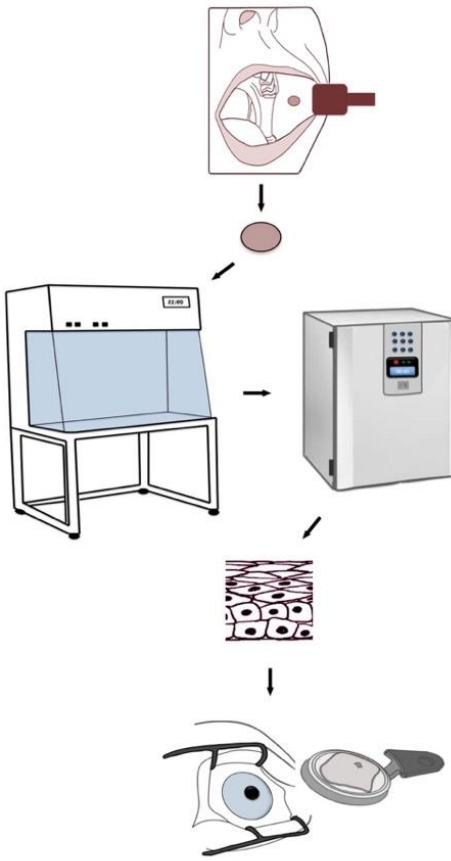

Fig. 1. Biopsy from oral mucosa, tissue engineering and autologous transplantation to cornea (from Utheim *et al*., 2016 (2)).

Simple limbal epithelial transplantation

Treatment of LSCD has received much attention the last years and new treatment options are rapidly developing. The next decade is likely to see that this field is orientated towards more

accessible treatments. Worldwide, blindness and marked visual impairment affect 160 million people (70). More than 80% live in developing countries. This imposes substantial costs on society, in particular to the patients and their families. In Japan, the costs of visual impairment are equivalent to 1.7% of the entire gross domestic product and almost one fifth of the national health budget (71). Due to demographic aging, the number of those with vision loss is likely to double over the next 20 years (72).

Many eye care interventions including research are cost saving. Even developing economies can afford to prevent or treat vision loss. Corneal diseases which can be caused by stem cell deficiency in the cornea are the second leading cause of blindness (73). The global need for treatment cannot be met since the culture from transplants which is needed for treatment must be performed in highly specialized centers (74).

As outlines previously, LSCD are often caused by infections, acid and chemical burns. In countries with poor sanitation, the disease is more often a fact. Therefore, most of the patients suffering from this eye disease live in developing countries. The necessary expertise is mainly found in the industrialized countries, including Norway. The problem is therefore that existing treatment for LSCD is not easily accessible for the patients needing it most. The goal for researchers in this field should therefore be to contribute in making the treatment accessible globally.

For that purpose, it is important to establish guidelines for minimal requirements and limitations. That's why Simple limbal epithelial transplantation (SLET) is so interesting as it eliminates the need of laboratory, storage and transport technology. With a transplantation of non-cultured cells, SLET is an elegant solution to reconstruct cornea than earlier methods described above. By transplanting in a one-step surgery, the cells do not need to be cultured in expensive laboratories and the treatment can be available worldwide in a completely new way. More details about SLET will be given in the next section of this master thesis.

Simple oral mucosal epithelial transplantation

Short term results so far for SLET have been very promising (75). If results in the long run are comparable, SLET can be favored over *ex vivo* cultivation of limbal cells since the SLET technique is less complicated. In our view, these results constitute excellent initial step toward simple oral mucosal epithelial transplantation (SOMET) where the only change is that uncultured oral mucosal epithelium are used instead of uncultured limbal epithelium.

As mentioned earlier there has been extensively work on *ex vivo* cultured oral mucosal cells. In fact there has also been done direct transplantation of OMECs to the eye with success (76). However, the wound left after biopsy surgery was larger than what is needed for EVAMAU or SOMET (35).

Clinical experiments were conducted on SOMET quite recently by a group of researchers from Japan which was published in Nature research (77). They did SOMET as treatment of LSCD in a rabbit model. This was done by creating LSCD in a SOMET group and a control group. The researchers harvested oral mucosa from the buccal region in the SOMET group and treated it with dispase. After that, they cut it into small pieces and placed it on to the cornea without graft sutures, amniotic membrane or glue. They placed a contact lens and did tarsorrhaphy in both groups. Then they used slit lamp microscopy to evaluate postoperative corneal neovascularization and fluorescein staining scores. After 2 weeks postoperatively, they excised the eyes and did immunohistochemically staining with different markers (CK3, CK13, CK15, p63). Their results showed complete recovery of LSCD in the SOMET group. That was defined by low neovascularization scores, low fluorescein staining scores, and detection of stratified K3/K13-positive cells on the stroma 2 weeks after surgery. In the control group, corneal epithelial defects persisted 2 weeks after surgery. This recent paper on this topic concluded that SOMET achieved re-epithelialization of the corneal surface in their rabbit LSCD

model. Moreover, the researchers highlights that the technique does not require culture and can be a promising option for ocular surface reconstruction in bilateral LSCD (77).

This paper from 2020 also report that their method was simpler than those reported previously and that dispase treatment resulted in the mucosal grafts becoming transparent more rapidly than with other techniques. Common points between this paper and techniques used in other studies about culture oral mucosal epithelium in animal and clinical studies is transplantation of oral mucosal epithelial stem cells, reconstruction of bilateral LSCD and expression of the same proteins as native oral mucosal epithelium by the grafted epithelium (77).

An advantage of oral mucosal epithelium that is cultured may be its anti-inflammatory effect. This is because the cultured sheet covers the entire cornea and protects it after surgery. Therefore, should transplantation of cultured oral mucosal epithelium be favored at hospital with tissue culture facilities in bilateral LSCD. However, where cell culture facilities and amniotic membrane are unavailable, the SOMET method technique can provide easy access for these patients (77).

As discussed for SLET, SOMET eliminate the chance of manipulation of the cells in the laboratory prior to transplantation. This is a disadvantage for both techniques (35, 78). The oral cavity house more pathogens that the eye and this can increase the risk of infections in the SOMET technique (35). However, the risk for long term complications after biopsies from the oral cavity are minimal compared to biopsies from the ocular surface that can induce LSCD in the healthy eye (35, 79).

SLET and SOMET has many beneficial applications. While transplantation of cultured cell sheets demands expensive facilities, careful monitoring when manufacturing epithelial cell sheets and legal restrictions, SLET and SOMET contribute in making the treatment more available. SLET and particularly SOMET is attractive because it circumvents the use of

immunosuppressive agents and the risk of transmitting diseases using foreign derived tissue and culture ingredients.

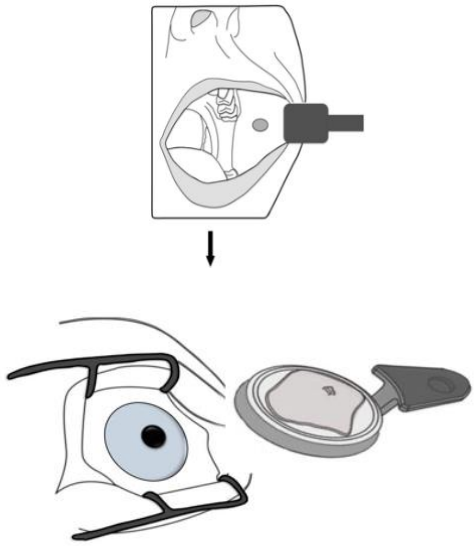

Fig. 2. One step surgery with biopsy and transplantation without culturing of OMECs (adapted from Utheim *et al*., 2016 (2)).

The design and development of SOMET will challenge us for years as the biopsy taking will be reserved for dentists. We suggest that further research should be undertaken in this area with basis in solid theoretical support in the literature of treatment of LSCD. This could eventually lead to better access to the treatment worldwide in the future. More research on this method that can avoid the use of culture facilities without weaken the clinical results is recommended (35). We hope that our review will serve as a base for future studies on SLET. The prospect of being able to do SLET, serves as a continuous incentive to future research of SOMET. Returning to the hypothesis, it is now possible to state that SOMET could be a promising treatment option for patients with bilateral LSCD in the future.

References

1. Land MF. The evolution of eyes. 1992;(1990):1–29.
2. Paaske Utheim T, Aass Utheim Ø, Khan Q-E-S, Sehic A. Culture of Oral Mucosal Epithelial Cells for the Purpose of Treating Limbal Stem Cell Deficiency. J Funct Biomater. 2016;
3. Cotsarelis G, Cheng SZ, Dong G, Sun TT, Lavker RM. Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: implications on epithelial stem cells. Cell. 1989 Apr;57(2):201–9.
4. Davanger M, Evensen A. Role of the pericorneal papillary structure in renewal of corneal epithelium. Nature. 1971 Feb;229(5286):560–1.
5. Utheim TP. Limbal Epithelial Cell Therapy: Past, Present, and Future. In 2013.
6. Espana EM, Grueterich M, Romano AC, Touhami A, Tseng SCG. Idiopathic limbal stem cell deficiency. Ophthalmology. 2002 Nov;109(11):2004–10.
7. Holland EJ. Management of Limbal Stem Cell Deficiency : A Historical Perspective , Past , Present , and Future. 2015;34(10):9–15.
8. Thoft RA. Conjunctival transplantation. Arch Ophthalmol (Chicago, Ill 1960). 1977 Aug;95(8):1425–7.
9. Keivyon KR, Tseng SCG. Limbal Autograft Transplantation for Ocular Surface Disorders. Ophthalmology [Internet]. 1989 May 1;96(5):709–23. Available from: https://doi.org/10.1016/S0161-6420(89)32833-8
10. Oliva J, Bardag-gorce F, Niihara Y. Clinical Trials of Limbal Stem Cell Deficiency Treated with Oral Mucosal Epithelial Cells. 2020;
11. Chen JJY, Tseng SCG. Corneal epithelial wound healing in partial limbal deficiency. Investig Ophthalmol Vis Sci. 1990;
12. Daya SM. Conjunctival-limbal autograft. Curr Opin Ophthalmol. 2017 Jul;28(4):370–6.
13. Thoft RA. Keratoepithelioplasty. Am J Ophthalmol. 1984 Jan;97(1):1–6.
14. Turgeon PW, Nauheim RC, Roat MI, Stopak SS, Thoft RA. Indications for Keratoepithelioplasty. Arch Ophthalmol [Internet]. 1990 Feb 1;108(2):233–6. Available from: https://doi.org/10.1001/archopht.1990.01070040085036
15. Armitage WJ, Tullo AB, Larkin DFP. The first successful full-thickness corneal transplant: a commentary on Eduard Zirm's landmark paper of 1906. Br J Ophthalmol

[Internet]. 2006 Oct 1;90(10):1222 LP-1223. Available from: http://bjo.bmj.com/content/90/10/1222.abstract

16. Tsai, Li, Chen. Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells(1). Am J Ophthalmol. 2000 Oct;130(4):543.
17. Theng JT, Tan DT. Combined penetrating keratoplasty and limbal allograft transplantation for severe corneal burns. Ophthalmic Surg Lasers. 1997 Sep;28(9):765–8.
18. Dua HS, Gomes JAP, Singh A. Corneal epithelial wound healing. BritishJournal ofOphthalmology. 1994;78:401–8.
19. Tsai RJ, Tseng SC. Human allograft limbal transplantation for corneal surface reconstruction. Cornea. 1994 Sep;13(5):389–400.
20. Schermer A, Galvin S, Sun TT. Differentiation-related expression of a major 64K corneal keratin in vivo and in culture suggests limbal location of corneal epithelial stem cells. J Cell Biol [Internet]. 1986 Jul 1;103(1):49–62. Available from: https://doi.org/10.1083/jcb.103.1.49
21. Kinoshiro S, Friend J, Thofr RA. Diphasic Cell Proliferation in Transdifferentiofion of Conjunciival to Corneol Epithelium in Rabbits. 1983;1008–14.
22. Potten CS, Loeffler M. Epidermal cell proliferation. I. Changes with time in the proportion of isolated, paired and clustered labelled cells in sheets of murine epidermis. Virchows Arch B Cell Pathol Incl Mol Pathol. 1987;53(5):279–85.
23. Langer R, Vacanti JP. Tissue engineering. Science. 1993 May;260(5110):920–6.
24. Kenyon KR, Tseng SC. Limbal autograft transplantation for ocular surface disorders. Ophthalmology. 1989 May;96(5):703–9.
25. Kwitko S, Marinho D, Barcaro S, Bocaccio F, Rymer S, Fernandes S, et al. Allograft conjunctival transplantation for bilateral ocular surface disorders. Ophthalmology. 1995 Jul;102(7):1020–5.
26. Rheinwald JG, Green H. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. Cell. 1975 Nov;6(3):331–43.
27. Pellegrini G, Traverso CE, Franzi AT, Zingirian M, Cancedda R, De Luca M. Long-term restoration of damaged corneal surfaces with autologous cultivated corneal epithelium. Lancet. 1997;
28. Schwab, Reyes, Isseroff. Successful transplantation of bioengineered tissue replacements in patients with ocular surface disease(1). Am J Ophthalmol. 2000 Oct;130(4):543–4.

29. Nakamura T, Endo K-I, Cooper LJ, Fullwood NJ, Tanifuji N, Tsuzuki M, et al. The successful culture and autologous transplantation of rabbit oral mucosal epithelial cells on amniotic membrane. Invest Ophthalmol Vis Sci. 2003 Jan;44(1):106–16.
30. Nishida K, Yamato M, Hayashida Y, Watanabe K, Yamamoto K, Adachi E, et al. Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium. N Engl J Med. 2004 Sep;351(12):1187–96.
31. Inatomi T, Nakamura T, Koizumi N, Sotozono C, Yokoi N, Kinoshita S. Midterm results on ocular surface reconstruction using cultivated autologous oral mucosal epithelial transplantation. Am J Ophthalmol. 2006 Feb;141(2):267–75.
32. Nakamura T, Takeda K, Inatomi T, Sotozono C, Kinoshita S. Long-term results of autologous cultivated oral mucosal epithelial transplantation in the scar phase of severe ocular surface disorders. Br J Ophthalmol. 2011 Jul;95(7):942–6.
33. Sotozono C, Inatomi T, Nakamura T, Koizumi N, Yokoi N, Ueta M, et al. Visual improvement after cultivated oral mucosal epithelial transplantation. Ophthalmology. 2013 Jan;120(1):193–200.
34. Satake Y, Higa K, Tsubota K, Shimazaki J. Long-term outcome of cultivated oral mucosal epithelial sheet transplantation in treatment of total limbal stem cell deficiency. Ophthalmology. 2011 Aug;118(8):1524–30.
35. Utheim TP. Concise Review: Transplantation of Cultured Oral Mucosal Epithelial Cells for Treating Limbal Stem Cell Deficiency-Current Status and Future Perspectives. Stem Cells. 2015;
36. Sehic A, Utheim ØA, Ommundsen K, Utheim TP. Pre-Clinical Cell-Based Therapy for Limbal Stem Cell Deficiency. 2015;6:863–88. Available from: www.mdpi.com/journal/jfb
37. Niethammer D, Kummerle-Deschner J, Dannecker GE. Side-effects of long-term immunosuppression versus morbidity in autologous stem cell rescue: striking the balance. Rheumatology (Oxford). 1999 Aug;38(8):747–50.
38. Ma DH-K, Kuo M-T, Tsai Y-J, Chen H-CJ, Chen X-L, Wang S-F, et al. Transplantation of cultivated oral mucosal epithelial cells for severe corneal burn. Eye (Lond). 2009 Jun;23(6):1442–50.
39. Nakamura T, Inatomi T, Sotozono C, Amemiya T, Kanamura N, Kinoshita S. Transplantation of cultivated autologous oral mucosal epithelial cells in patients with severe ocular surface disorders. Br J Ophthalmol. 2004 Oct;88(10):1280–4.
40. Chen H-CJ, Yeh L-K, Tsai Y-J, Lai C-H, Chen C-C, Lai J-Y, et al. Expression of

angiogenesis-related factors in human corneas after cultivated oral mucosal epithelial transplantation. Invest Ophthalmol Vis Sci. 2012 Aug;53(9):5615–23.

41. Ang LPK, Nakamura T, Inatomi T, Sotozono C, Koizumi N, Yokoi N, et al. Autologous serum-derived cultivated oral epithelial transplants for severe ocular surface disease. Arch Ophthalmol (Chicago, Ill 1960). 2006 Nov;124(11):1543–51.
42. Burillon C, Huot L, Justin V, Nataf S, Chapuis F, Decullier E, et al. Cultured autologous oral mucosal epithelial cell sheet (CAOMECS) transplantation for the treatment of corneal limbal epithelial stem cell deficiency. Invest Ophthalmol Vis Sci. 2012 Mar;53(3):1325–31.
43. Chen H-CJ, Chen H-L, Lai J-Y, Chen C-C, Tsai Y-J, Kuo M-T, et al. Persistence of transplanted oral mucosal epithelial cells in human cornea. Invest Ophthalmol Vis Sci. 2009 Oct;50(10):4660–8.
44. Gaddipati S, Muralidhar R, Sangwan VS, Mariappan I, Vemuganti GK, Balasubramanian D. Oral epithelial cells transplanted on to corneal surface tend to adapt to the ocular phenotype. Indian J Ophthalmol. 2014 May;62(5):644–8.
45. Hirayama M, Satake Y, Higa K, Yamaguchi T, Shimazaki J. Transplantation of cultivated oral mucosal epithelium prepared in fibrin-coated culture dishes. Invest Ophthalmol Vis Sci. 2012 Mar;53(3):1602–9.
46. Inatomi T, Nakamura T, Kojyo M, Koizumi N, Sotozono C, Kinoshita S. Ocular surface reconstruction with combination of cultivated autologous oral mucosal epithelial transplantation and penetrating keratoplasty. Am J Ophthalmol. 2006 Nov;142(5):757–64.
47. Kolli S, Ahmad S, Mudhar HS, Meeny A, Lako M, Figueiredo FC. Successful application of ex vivo expanded human autologous oral mucosal epithelium for the treatment of total bilateral limbal stem cell deficiency. Stem Cells. 2014 Aug;32(8):2135–46.
48. Nakamura T, Inatomi T, Cooper LJ, Rigby H, Fullwood NJ, Kinoshita S. Phenotypic investigation of human eyes with transplanted autologous cultivated oral mucosal epithelial sheets for severe ocular surface diseases. Ophthalmology. 2007 Jun;114(6):1080–8.
49. Priya CG, Arpitha P, Vaishali S, Prajna N V, Usha K, Sheetal K, et al. Adult human buccal epithelial stem cells: identification, ex-vivo expansion, and transplantation for corneal surface reconstruction. Eye (Lond). 2011 Dec;25(12):1641–9.
50. Satake Y, Dogru M, Yamane G-Y, Kinoshita S, Tsubota K, Shimazaki J. Barrier

function and cytologic features of the ocular surface epithelium after autologous cultivated oral mucosal epithelial transplantation. Arch Ophthalmol (Chicago, Ill 1960). 2008 Jan;126(1):23–8.

51. Sotozono C, Inatomi T, Nakamura T, Koizumi N, Yokoi N, Ueta M, et al. Cultivated oral mucosal epithelial transplantation for persistent epithelial defect in severe ocular surface diseases with acute inflammatory activity. Acta Ophthalmol. 2014 Sep;92(6):e447-53.
52. Takeda K, Nakamura T, Inatomi T, Sotozono C, Watanabe A, Kinoshita S. Ocular surface reconstruction using the combination of autologous cultivated oral mucosal epithelial transplantation and eyelid surgery for severe ocular surface disease. Am J Ophthalmol. 2011 Aug;152(2):195–201.e1.
53. Gipson IK, Geggel HS, Spurr-Michaud SJ. Transplant of oral mucosal epithelium to rabbit ocular surface wounds in vivo. Arch Ophthalmol (Chicago, Ill 1960). 1986 Oct;104(10):1529–33.
54. Nakamura T, Kinoshita S. Ocular surface reconstruction using cultivated mucosal epithelial stem cells. Cornea. 2003 Oct;22(7 Suppl):S75-80.
55. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006 Aug;126(4):663–76.
56. Takagi R, Murakami D, Kondo M, Ohki T, Sasaki R, Mizutani M, et al. Fabrication of human oral mucosal epithelial cell sheets for treatment of esophageal ulceration by endoscopic submucosal dissection. Gastrointest Endosc. 2010 Dec;72(6):1253–9.
57. Squier CA, Kremer MJ. Biology of Oral Mucosa and Esophagus. JNCI Monogr [Internet]. 2001 Oct 1;2001(29):7–15. Available from: https://doi.org/10.1093/oxfordjournals.jncimonographs.a003443
58. Homma R, Yoshikawa H, Takeno M, Kurokawa MS, Masuda C, Takada E, et al. Induction of epithelial progenitors in vitro from mouse embryonic stem cells and application for reconstruction of damaged cornea in mice. Invest Ophthalmol Vis Sci. 2004 Dec;45(12):4320–6.
59. Tanioka H, Kawasaki S, Yamasaki K, Ang LPK, Koizumi N, Nakamura T, et al. Establishment of a cultivated human conjunctival epithelium as an alternative tissue source for autologous corneal epithelial transplantation. Invest Ophthalmol Vis Sci. 2006 Sep;47(9):3820–7.
60. Yang X, Qu L, Wang X, Zhao M, Li W, Hua J, et al. Plasticity of epidermal adult stem cells derived from adult goat ear skin. Mol Reprod Dev. 2007 Mar;74(3):386–96.

61. Monteiro BG, Serafim RC, Melo GB, Silva MCP, Lizier NF, Maranduba CMC, et al. Human immature dental pulp stem cells share key characteristic features with limbal stem cells. Cell Prolif. 2009 Oct;42(5):587–94.
62. Ma Y, Xu Y, Xiao Z, Yang W, Zhang C, Song E, et al. Reconstruction of chemically burned rat corneal surface by bone marrow-derived human mesenchymal stem cells. Stem Cells. 2006 Feb;24(2):315–21.
63. Meyer-Blazejewska EA, Call MK, Yamanaka O, Liu H, Schlotzer-Schrehardt U, Kruse FE, et al. From hair to cornea: toward the therapeutic use of hair follicle-derived stem cells in the treatment of limbal stem cell deficiency. Stem Cells. 2011 Jan;29(1):57–66.
64. Reza HM, Ng B-Y, Gimeno FL, Phan TT, Ang LP-K. Umbilical cord lining stem cells as a novel and promising source for ocular surface regeneration. Stem cell Rev reports. 2011 Nov;7(4):935–47.
65. Islam R, Eidet JR, Badian RA, Messelt E. Tissue Harvesting Site and Culture Medium Affect Attachment , Growth , and Phenotype of Ex Vivo Expanded Oral Mucosal Epithelial. 2017;(August 2016):1–12.
66. Kanayama S, Nishida K, Yamato M, Hayashi R, Sugiyama H, Soma T, et al. Analysis of angiogenesis induced by cultured corneal and oral mucosal epithelial cell sheets in vitro. Exp Eye Res. 2007 Dec;85(6):772–81.
67. Kanayama S, Nishida K, Yamato M, Hayashi R, Maeda N, Okano T, et al. Analysis of soluble vascular endothelial growth factor receptor-1 secreted from cultured corneal and oral mucosal epithelial cell sheets in vitro. Br J Ophthalmol. 2009 Feb;93(2):263–7.
68. Sekiyama E, Nakamura T, Kawasaki S, Sogabe H, Kinoshita S. Different expression of angiogenesis-related factors between human cultivated corneal and oral epithelial sheets. Exp Eye Res. 2006 Oct;83(4):741–6.
69. Lim P, Fuchsluger TA, Jurkunas U V. Limbal Stem Cell Deficiency and Corneal Neovascularization. Semin Ophthalmol [Internet]. 2009 Jan 1;24(3):139–48. Available from: https://doi.org/10.1080/08820530902801478
70. Resnikoff S, Pascolini D, Etya'ale D, Kocur I, Pararajasegaram R, Pokharel GP, et al. Global data on visual impairment in the year 2002. Bull World Health Organ. 2004 Nov;82(11):844–51.
71. Roberts CB, Hiratsuka Y, Yamada M, Pezzullo ML, Yates K, Takano S, et al. Economic cost of visual impairment in Japan. Arch Ophthalmol (Chicago, Ill 1960). 2010 Jun;128(6):766–71.

72. Pararajasegaram R. VISION 2020-the right to sight: from strategies to action. Vol. 128, American journal of ophthalmology. United States; 1999. p. 359–60.
73. Whitcher JP, Srinivasan M, Upadhyay MP. Corneal blindness: a global perspective. Bull World Health Organ. 2001;79(3):214–21.
74. Ahmad S, Osei-Bempong C, Dana R, Jurkunas U. The culture and transplantation of human limbal stem cells. J Cell Physiol. 2010 Oct;225(1):15–9.
75. Jackson CJ, Myklebust Erno IT, Ringstad H, Tonseth KA, Dartt DA, Utheim TP. Simple limbal epithelial transplantation: Current status and future perspectives. Stem Cells Transl Med. 2020 Mar;9(3):316–27.
76. Promprasit D, Bumroongkit K, Tocharus C, Mevatee U, Tananuvat N. Cultivation and phenotypic characterization of rabbit epithelial cells expanded ex vivo from fresh and cryopreserved limbal and oral mucosal explants. Curr Eye Res. 2015 Mar;40(3):274–81.
77. Inamochi A, Tomioka A, Kitamoto K, Miyai T, Usui T. Simple oral mucosal epithelial transplantation in a rabbit model. Sci Rep [Internet]. 2019;1–11. Available from: http://dx.doi.org/10.1038/s41598-019-54571-7
78. Shimazaki J, Higa K, Kato N, Satake Y. Barrier function of cultivated limbal and oral mucosal epithelial cell sheets. Invest Ophthalmol Vis Sci. 2009 Dec;50(12):5672–80.
79. Mann I. A STUDY OF EPITHELIAL REGENERATION IN THE LIVING EYE. Br J Ophthalmol. 1944 Jan;28(1):26–40.

PART II

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CONCISE REVIEW

STEM CELLS TRANSLATIONAL MEDICINE

Simple limbal epithelial transplantation: Current status and future perspectives

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Abstract

Damage to limbal stem cells as a result of injury or disease can lead to limbal stem cell deficiency (LSCD). This disease is characterized by decreased vision that is often painful and may progress to blindness. Clinical features include inflammation, neovascularization, and persistent cornea epithelial defects. Successful strategies for treatment involve transplantation of grafts harvested from the limbus of the alternate healthy eye, called conjunctival-limbal autograft (CLAU) and transplantation of limbal cell sheets cultured from limbal biopsies, termed cultured limbal epithelial transplantation (CLET). In 2012, Sangwan and colleagues presented simple limbal epithelial transplantation (SLET), a novel transplantation technique that combines the benefits of CLAU and CLET and avoids the challenges associated with both. In SLET a small biopsy from the limbus of the healthy eye is divided and distributed over human amniotic membrane, which is placed on the affected cornea. Outgrowth occurs from each small explant and a complete corneal epithelium is typically formed within 2 weeks. Advantages of SLET include reduced risk of iatrogenic LSCD occurring in the healthy cornea at harvest; direct transfer circumventing the need for cell culture; and the opportunity to perform biopsy harvest and transplantation in one operation. Success so far using SLET is comparable with CLAU and CLET. Of note, 336 of 404 (83%) operations using SLET resulted in restoration of the corneal epithelium, whereas visual acuity improved in 258 of the 373 (69%) reported cases. This review summarizes the results of 31 studies published on SLET since 2012. Progress, advantages, challenges, and suggestions for future studies are presented.

KEYWORDS

cornea, limbus, limbal stem cell deficiency, simple limbal epithelial transplantation, stem cells

Abbreviations: alloSLET, allogenic SLET; AM, amniotic membrane; AMT, amniotic membrane transfer; CLAU, corneal limbal autograft; CLET, cultured limbal epithelial transplantation; COMET, cultured oral mucosal epithelial transplantation; lr-CLAL, living-related conjunctival limbal allograft; KLAL, keratolimbal allograft; LSCD, limbal stem cell deficiency; MMP, mucous membrane phemigoid; OSSN, ocular surface squamous neoplasia; PK, penetrating keratoplasty; SLET, simple limbal epithelial transplantation; SOMET, simple oral mucosal epithelial transplantation; VEGF, vascular endothelial growth factor; ΔNp63α+, delta p63 transcription factor alpha isoform positive.

1 | INTRODUCTION

The corneal epithelium is renewed by stem cells located in specialized niches in the limbus at the cornea-conjunctiva junction. Loss or damage to the limbal stem cell pool can lead to limbal stem cell deficiency (LSCD), where homeostatic maintenance of the corneal epithelium is

compromised, leading to ingrowth of the conjunctiva. Etiology includes autoimmune diseases (Steven-Johnson syndrome), infections (trachoma), contact lens wear, and thermal/alkali burns. LSCD may be partial or total depending on the extent of the damage.[1] Conjunctivalization is pathogenic for LSCD and is frequently accompanied by inflammation, neovascularization, persistent epithelial defects, and scarring resulting in decreased vision or blindness.

Several surgical and stem cell-based treatments for LSCD have been developed over the last decades.[1] Simple limbal epithelial transplantation (SLET) is a new treatment strategy introduced by Sangwan et al.[2] In this technique a small limbal biopsy is harvested from the healthy eye. The biopsy is divided into minute explant pieces that are distributed over human amniotic membrane (AM) and glued to the cornea (Figure 1). Outgrowth from individual explants merges with neighboring explant growth.[4] Reepithelialization is typically achieved within 2 weeks. This review summarizes 404 cases in 31 clinical studies using SLET to date.

Significance statement

The present review examines work reporting simple limbal epithelial transplantation (SLET), an innovative technique that uses minimal limbal tissue from the healthy eye to regenerate the cornea in the limbal deficient diseased eye. Results since the introduction of SLET in 2012 suggest that the success rate is comparable to established techniques, conjunctival-limbal autograft and cultured limbal epithelial transplantation. However, SLET has the advantages of requiring a smaller biopsy, achieving harvest and transplantation in a single operation, and the unnecessity of cell culture laboratories. AlloSLET, a novel modification of SLET using allogeneic tissue, promises to further improve outcome through promotion of early resolution of inflammation in the injured/diseased eye.

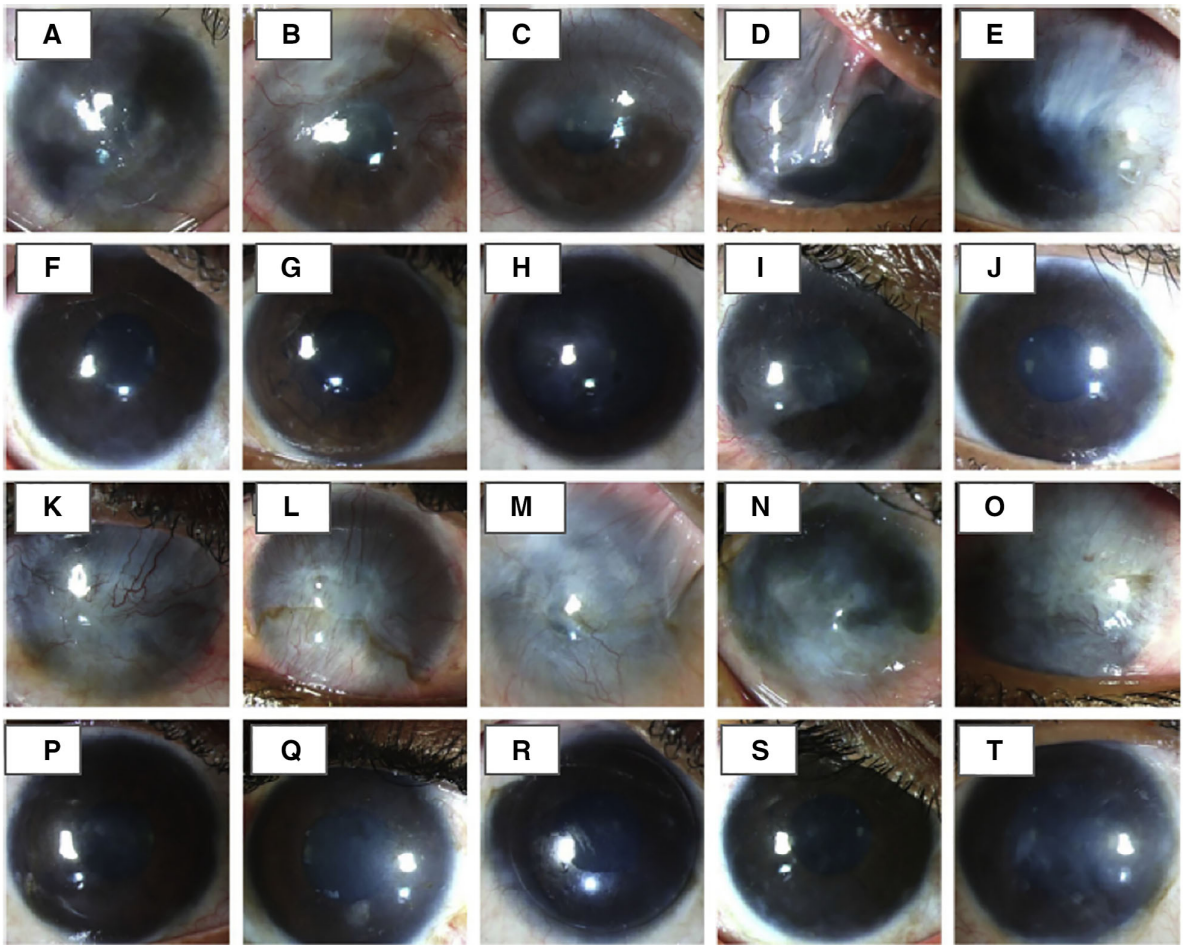

FIGURE 1 Illustration of 2-year outcomes following the use of simple limbal epithelial transplantation (SLET) for treatment of patients with partial and total limbal stem cell deficiency (LSCD). A-J, Patients with partial LSCD following ocular burns: A-F, Preoperative photographs and F-J, 2-year postoperative photographs showing a completely epithelized and stable corneal surface. K-U, Patients with total LSCD: K-O, Preoperative clinical photographs. P-T, 2-year postoperative photographs after SLET using Slit-lamp photography. Images reprinted from Basu et al[3]

2 | CURRENT OPTIONS FOR TREATMENT OF LSCD

Reepithelialization of the corneal surface and improved visual acuity are the primary and secondary aims in treating LSCD. Currently, there are two main surgical techniques available using autologous limbal tissue; conjunctival-limbal autograft (CLAU), and cultured limbal epithelial transplantation (CLET). In the CLAU technique two conjunctival-limbal biopsies are harvested (120° cornea circumference each as described in the original CLAU technique) and transferred directly to the affected limbal deficient eye.[5] Thus, an advantage of this procedure is that it does not require the use of a transplant substrate, saving the expense of using AM. Published reviews summarizing results of CLAU report a success rate of between 80% and 100% and improvement in visual acuity of 25% and 100%, with a survival rate of 62% at 6-year follow-up.[6,7]

The CLET technique depends on the culture of limbal biopsies to produce limbal cell sheets prior to transplantation.[8] The introduction of the CLET procedure by Pellegrini et al. in 1997 offered a significant advantage over CLAU by harvest of a smaller amount of limbal tissue, minimizing the risk of iatrogenic injury to the healthy eye.[8] Meta-analysis shows successful reepithelialization in 72% (n = 720) of cases and improved visual acuity in 63% (n = 539) of cases reporting the use of the standard CLET technique.[9] This technique has been criticized for use of mouse cells and other xenogeneic components in preparation of the cultured sheets, potentially resulting in infection and quality variation.[10] However, it is possible to substitute AM for mouse feeder cells.[11,12] As evidence of its safety, in 2015, the CLET technique advanced to become the first stem cell-based therapy to receive approval for application throughout the European Union (EU) under the trade name "Holoclar".[13]

Several non-limbal cell types have also shown promise in treating LSCD, offering options for treatment of bilateral LSCD using autologous cells and avoiding immunosuppression.[14] Among alternatives, the cultured oral mucosal epithelial transplantation (COMET) technique has been most widely reported.[15] Use of this tissue allows treatment of patients with Stevens-Johnson syndrome.[16] The success rate for COMET is comparable to CLET, resulting in reepithelialization in 63% (n = 230) of reported cases and improved visual acuity in 68% (n = 202).[17]

2.1 | Current challenges in treatment of LSCD

Though complications are rare and reepithelialization of the donor site usually occurs, the risk associated with taking two large limbal biopsies from the healthy donor eye is a concern associated with CLAU.[18–20] The CLET and COMET techniques address this challenge but require production of cultured sheets in a good manufacturing practice-regulated laboratory, which is expensive and limits accessibility. The COMET technique is promising, but peripheral neovascularization following surgery has been reported in many cases.[16] The use of anti-angiogenic agents in concert with COMET has shown benefit. However, inhibition of vascular endothelial growth factor has been shown to affect the overall wound healing response and induce corneal melt.[21]

SLET offers several advantages compared with the above options: (a) risk of iatrogenic damage to the donor eye is reduced; (b) a small biopsy means the procedure can be repeated if necessary; (c) SLET does not require expensive specialized culture facilities; and (d) The SLET procedure can be performed in one operation streamlining patient care, resource management, and reducing costs. Results of a recent study involving 125 patients show that SLET can be successfully used to treat partial and total LSCD (Figure 1).[3]

Treatment of bilateral LSCD remains a challenge. In addition to COMET, conjunctival-limbal allografts from a living-related relative (lr-CLAL) or cadaveric tissue (keratolimbal allograft [KLAL]) are options. There have been two reports of modified SLET using allogeneic limbal tissue (alloSLET) to treat bilateral LSCD. AlloSLET compared with lR-CLAL and KLAL procedures have so far not been directly compared. Regardless of the procedure, systemic immunosuppressants are critical for survival of allograft tissue.[22,23] A standard of care and recommended duration of immunosuppressants necessary to prevent allograft rejection has yet to be defined.[22] Large studies reporting KLAL and lr-CLAL procedures suggest an average duration of 42[24]-44[25] months, whereas the only large study reporting alloSLET for bilateral LSCD recommends gradual reduction over 2 years followed by the indefinite use of systemic and topical immunosuppressants.[23] Patients should be monitored for adverse systemic effects while taking immunosuppressants, which may include hypertension, diabetes mellitus, and biochemical abnormalities.[22]

3 | CHARACTERISTICS OF SLET STUDIES

The present review is based on a search of the National Library of Medicine (PubMed) database using the term "simple limbal epithelial transplantation" that gave a list of 31 publications reporting preclinical results of SLET (Table S1) and one publication optimizing the SLET technique.[26]

As of August 2019, 404 cases of SLET were reported. The eight largest case series' reported treatment of 125,[3] 68,[27] 30,[28] 30,[29] 30,[23] 18,[30] 15,[31] and 11[32] eyes. These included the largest prospective study to date, with 125 patients and a follow-up period of at least 1 year[3] and a multicenter international study of 68 patients.[27] The remaining 23 studies were noncomparative single case studies or case series of 10 eyes or less.[2,4,33–52] One study directly compared SLET with CLAU, with 10 patients randomly assigned to each group.[33] The Sangwan group in India published the most studies.[2,3,27,29,35,42–49] Other centers in India,[4,23,28,30–33,39–41,51,52] England,[38] Brazil,[36] Mexico,[34] Thailand,[50] and the United States[37] also contributed.

4 | ETIOLOGY OF CASES TREATED WITH SLET

Grading LSCD severity is important since some cases of partial LSCD may not require stem cell transplant.[53,54] SLET was mainly used in the

treatment of adults and children with uniocular total and partial LSCD resulting from burns and chemical injuries (Table S1). Patients with unilateral LSCD and a clinically non-inflamed wet ocular surface are ideal candidates for SLET[3,37] Preliminary reports also indicate that SLET has potential for use in non-LSCD ocular diseases; ocular surface squamous neoplasia (OSSN) (9 eyes),[35,41,51] laryngo-onycho-cutaneous syndrome (1 eye),[39] pterygium (9 eyes),[34] and recurrent pterygium (4 eyes) if results are confirmed in larger studies.[55] However, pterygium can be treated using pterygium extended removal followed by autologous extended conjunctival grafting, which has a high success rate and is safe, simple, and fast to perform.[56]

It has been shown that SLET can be used in patients with LSCD following failure of treatment with CLET.[29] At a mean follow-up of 2.3 years, 80% of the 30 eyes treated by SLET maintained a successful outcome without complications. Bilateral autoimmune diseases such as Steven-Johnsons syndrome and ocular cicatricial pemphigoid are contraindications for SLET using autologous tissue. A recent study also showed that scleral ischemia resulting from chemical injury is a poor prognostic indicator for success using SLET.[31]

SLET using biopsies of contralateral autologous tissue is most common. Although larger studies are necessary before recommendations can be made, recent case studies show the use of alloSLET for treatment of LSCD with a range of etiologies including extreme dry eye,[40] chemical injury,[47] and iatrogenic LSCD induced by mitomycin treatment for conjunctival melanoma.[38] Iyer et al. also suggested innovative use of alloSLET as an acute temporary biological bandage.[30] The goal of this treatment was to provide immediate stabilization of the wound environment, minimize more serious damage, and prepare the wound for future SLET using autologous tissue.

5 | THE SLET TECHNIQUE

Most studies used the original autologous SLET technique described by Sangwan et al. harvesting a small biopsy of limbal tissue from the healthy eye.[2] In summary, the injured eye is prepared with a 360° peritomy, and the vascular pannus covering the cornea is removed (Figure S1). The eye is covered by AM to the extent of the peritomy, secured with fibrin glue (Figure S1A). A small 2 × 2 mm biopsy (30° cornea circumference) is excised from the superior limbus of the healthy eye and placed in a balanced salt solution (Figure S1B). The limbal tissue biopsy is subsequently cut into tiny pieces that are fixed onto the AM epithelial side up in a circular arrangement (avoiding the visual axis) using fibrin glue (Figure S1C). A soft bandage contact lens is then applied along with topical antibiotics and corticosteroids for the first week or until healed (Figure S1D). A second layer of AM instead of the contact lens can also be used.[37,55] Use of cryopreserved AM instead of fresh AM has been shown to be equally effective and allows the use of this procedure in the United States.[37] Partial LSCD can be treated using a modified SLET technique, where superficial keratectomy is performed only in areas of fibrovascular pannus, thus avoiding the intact limbus areas.[3,28]

The SLET procedure has also been used as a preventative measure against development of LSCD. Wide excisional biopsies of ocular surface squamous neoplasia and SLET can be performed in the same procedure to prevent LSCD after resection.[35,41]

When severe stromal opacification is present, patients will additionally require penetrating keratoplasty (PK). It is sometimes necessary to perform PK simultaneously with SLET if patients are unwilling to undergo a second operation.[27] Results from the three largest studies point to a correlation of failure with simultaneous performance of PK with SLET.[3,27,28] Furthermore, SLET improves the corneal environment, which may promote self-clearing of the stroma.[3,28] Therefore, delaying PK for at least a year post-SLET is recommended. In support of this, a large multicenter study reported an overall success rate of 84% (n = 68), but this dropped to 20% in the eight eyes receiving simultaneous PK and SLET.[27] However, the authors cautioned that the unsuccessful cases may have presented with more serious etiology. Singh et al. described performance of deep anterior lamellar keratoplasty in pediatric patients 9–15 months post-SLET giving visual improvement of 64% (n = 11).[32]

Failure of SLET is correlated with regeneration of the cornea by migrating conjunctival cells.[50] Thus, in vivo confocal microscopy and impression cytology can be useful in determining the phenotype of regenerated epithelium on the cornea, allowing early diagnostic assessment of failure and management before clinical symptoms appear.[50]

6 | MECHANISM OF REGENERATION

The success of SLET is in large part attributed to in vivo expansion of transplanted explants on the corneal surface. However, the exact mechanism in terms of the individual contribution of fibrin glue, AM, limbal biopsy size, distribution of the biopsies, preexisting stem cells, and migration pattern of transplanted cells is as yet unknown. Detailed discussion of the role of these factors in reestablishing an epithelialized cornea is beyond the scope of the present review. The proposed benefits of the major components, fibrin glue and AM, and the source of proliferating stem cells will be briefly discussed here.

Fibrin glue was first reported as a replacement for sutures in an AM transplantation (AMT) procedure in 11 patients with partial LSCD.[57] Here, reepithelialization was achieved through growth from residual limbal and corneal tissue, without the need to transplant limbal cells. Kheirkhah and colleagues suggested that the glue forms a full contact seal between the transplanted AM and the corneal surface, ensuring reepithelialization occurs on the surface of the AM rather than underneath, taking full advantage of the AM microenvironment.[57] in vitro work has shown that fibrin glue inhibits cell migration, which in SLET may prevent ingrowth of conjunctival tissue at a critical phase and promote expansion of epithelial cells from explants on the AM surface.[58] Growth rates vary between explants from the same donor placed in the same eye, which may be attributed to the amount of fibrin glue used for their individual attachment or to differences in handling during transplant.[4] Fibrin glue has also been shown to extend the beneficial effects of AM by delaying its breakdown compared with sutures.[7,57]

The main benefit of AM is in its early application to control inflammation. It provides a substrate to promote the formation of a well-differentiated stratified corneal epithelium.[59] The advantage of the addition of limbal tissue in SLET (compared with AM alone as used in the AMT procedure) is highlighted where limbal explants have been lost postoperatively, resulting in failure of SLET despite the presence of AM.[3] Amescua et al. used ultra-high-resolution optical coherence tomography to reveal that the transplanted AM persisted at least 4 months post-SLET (in one patient).[37]

The relative contribution of transplanted cells and residual surviving stem cells to the regenerated epithelium is unknown. A stable source of proliferating stem cells is necessary to restore long-term homeostasis of the corneal epithelium. These may be established through transplanted stem cells becoming embedded or by dormant residual stem cells becoming reactivated. It is possible that in some cases removal of fibrotic tissue and paracrine signals from transplanted explants is sufficient to stimulate residual stem cells to resume their homeostatic function. Preliminary investigation to resolve these questions has revealed the presence of focal points of basal layer cells expressing putative markers for stem cells (ΔNp63α and ABCG2) post-SLET[3]; the presence of a mix of patient and donor cells on the cornea several months post-alloSLET[30]; and patches of outgrowth emerging from individual explants growing in a centripetal pattern that eventually merge with outgrowth from neighboring explants.[37] Although it is clear that the role of transplanted cells needs further investigation in larger studies, these initial analyses suggest that the rapid reepithelialization seen post-SLET can be attributed, at least in part, to transplanted proliferating cells from limbal explants.

7 | RESULTS

Most studies used reversal of the main features of LSCD as the primary measure of success. This is defined as complete reepithelialization, a clinically stable corneal epithelium and reversal of vascularization.[60] Improved visual acuity was used as a secondary definition of success. Reported follow-up periods ranged from 6 to 59 months. Failure usually occurred within 6 months of surgery (Table 1). Combined results show that 83% (n = 336) of SLET operations were successful by the primary criterion and visual acuity improved in 69% of cases (n = 258) (Table 1). Summarizing results by severity, the success rates were 74.2% (n = 35) for partial LSCD and 76.8% (n = 151) for total LSCD (Table 1).

The overall success rate compares well with other procedures that use autologous limbal tissue.[7,9,17] A direct comparison between SLET and CLAU performed on patients with the same etiology and in the same clinical setting supports SLET as an equally safe and effective treatment for LSCD.[33] A primary success rate of 62.5% (n = 30) was also reported where SLET was performed in cases of failed CLET.[29]

The success rate declined in treatment of pediatric patients with LSCD, with a rate of 71% compared with 85.5% in adults.[3,28,52] Successful treatment of pediatric LSCD using CLET has also been disappointing (46.7%),[61] suggesting pediatric LSCD is especially challenging. This could be linked to the pressure for young patients to undergo surgery earlier (before inflammation is fully controlled) in order to reduce the risk of developing amblyopia (lazy eye),[28] which often accompanies sensory vision loss. Furthermore, ocular inflammation is correlated with failure[62] and children generally experience more inflammation.[63]

Overall, SLET compares well with other procedures (CLAU, CLET, COMET) that use autologous tissue for treatment of LSCD, resulting in similar average primary and secondary criteria success rates. Importantly, SLET has now been validated in several larger studies and in several international centers since the first published report in 2012.[3,27–29] Many studies have reported long-term success, with follow-up periods of 12 to 59 months (Tables 1 and 2).[3,27,28,30,35,38,39]

8 | RISK FACTORS FOR FAILURE AND COMPLICATIONS

8.1 | Preoperative

The presenting features of the patient eye should be considered when deciding treatment. Absolute contraindications include a dry ocular surface, blind eye with no visual potential, disorganized anterior segment, and the continued presence of adnexal pathologies.[23] Presentation with LSCD resulting from acid injury is also correlated with failure.[28] The association of presenting features with prognosis post-SLET has been summarized in a review by Shanbhag et al. (see their table 1[23]).

Preexisting symblepharon is correlated with failure.[3,27,28] According to Basu et al., the presence of symblepharon extending toward the cornea pre-SLET could indicate conjunctival deficiency, and outcomes may improve if symblepharon is addressed before or at the time of SLET.[3] A retrospective case series of four children where only one patient had a completely successful outcome also noted that recurrence of LSCD coincided with areas of severe preoperative symblepharon.[52] The three partial success patients had initially presented with more severe injury and extensive LSCD. Thus, authors suggested that damage to conjunctival stem cells may have contributed to failure. Repeat SLET combined with conjunctival autograft transplant resulted in reepithelialization and an avascular surface.

Optimization of the ocular surface including fast resolution of inflammation prior to SLET is important to give the best chance for successful outcome, especially in pediatric cases.[3,27,52] Glucocorticoids and AM transfer are often used to reduce inflammation in the acute phase and induce epithelization. Iyer and colleagues have shown that alloSLET can also be successfully used for this purpose.[30]

8.2 | Intraoperative

Based on poor results when combining PK with CLET, Basu and colleagues recommend identifying patients with thin corneas by optical coherence tomography or ultrasound bio-microscopy before surgery so that lamellar corneal graft can be performed simultaneously with SLET if required.[3,64]

TABLE 1 Outcomes of studies using simple limbal epithelial transplantation (SLET)

	References	Surgery	No. of patients	Stable corneal epithelium %	Improvement in visual acuity %	Complications in recipient eye	Follow-up period
1	Shanbhag et al.[23]	AlloSLET: Living-related alloSLET ×16 Cadaveric ×14	30	83.3% (25/30) 14/16 (87.5%) in Living-related 11/14 (78.6%) in cadaveric	60% (18/30)		Median 28 months (range, 13-66 months)
2	Prabhasawat et al.[50]	SLET ×5 Living-related alloSLET ×5	10 eyes in 9 patients	70% (7/10)	70% (7/10) Ranging within improvement to 6/60 or better from HM	Failure: Central/peripheral neovascularization correlated with regrowth by conjunctival cells ×3	3-18 months
3	Gupta et al.[31]	SLET 6 months later ×15	7 x non-isch. 8 x isch.	75% (6/8) in non-isch. 29% (2/7) in isch.			1 year
4	Narang et al.[51]	SLET ×1	1	100% (1/1)	Unchanged ×1	Cataract	31 months
5	Mednick et al.[55]	Pterygium surgery + adjunct mitomycin C treatment	4	100% (4/4)	100% (4/4)	Symblepharon ×1 Pterygium in new area ×1 Neovascularization resolved with anti-VEGF injections ×1	8-30 months
6	Gupta et al.[28]	Repeated SLET ×1 resulted in failure PK ×3	30	Partial 66.6% (10/15) Total 73.3% (11/15)	71.4 % of patients BCVA at presentation: 20/200 or worse (blindness) ×24 20/70-20/160 (low vision) ×2 20/60 or better ×4 Final BCVA: 20/200 or worse (blindness) ×19 20/70-20/160 (low vision) ×2 20/60 or better ×9	Progressive conjunctivalization (30%) Foreign body sensation in donor eye (×3; 10%)	1.1 years (range, 6 months-3.5 years)
7	Basu et al.[29]	PK ×1 Corneal transplantation ×4 Repeated SLET ×2	30	80% (24/30)	63% of successful outcomes (15/24) Improved to 20/200 or better	Recurrence of LSCD ×6 (20%) Hemorrhage beneath the AM ×2 (7%) Persistent epithelial defect with corneal graft infiltrate ×1 (3%) Donor eye: Subconjunctival hemorrhage ×6 (20 %)	2.3 ± 1.5 years
8	Singh et al.[32]	DALK 9-15 months after SLET ×11	11	82% (9/11)	64% (7/11) Improvement by ≥2 lines	Recurrence of keratolimbal vascularization or conjunctivalization with graft opacification ×2 Focal recurrences of mild keratolimbal conjunctivalization/ vascularization ×9 Mid-to-deep stromal involvement with visual axis opacification ×9 Deeper stromal involvement of varying degrees which did not involve the visual axis ×2	11.63 ± 2.21 months

(Continues)

TABLE 1 (Continued)

	References	Surgery	No. of patients	Stable corneal epithelium %	Improvement in visual acuity %	Complications in recipient eye	Follow-up period
9	Vasquez-Perez[38]	PK	1	100% (1/1)	100% (1/1) From hand movements to 6/12	Recurrence of herpes simplex keratitis ×1	>20 months
10	Mohamed et al.[39]		1	100% (1/1)	100% (1/1) 20/160 improved to 20/40 vision	Focal LSCD and recurrence of symblepharon ×1 Recurrence of granulation tissue ×1	18 months
11	Kaliki et al.[35]	Wide excisional biopsy of OSSN + Primary SLET ×7 Plaque radiotherapy×3	7	Clear cornea: 71.4% (5/7)		Peripheral corneal opacity ×2 (29%)	12 months
12	Arora et al.[33]		10 SLET 10 CLAU	100% (20/20)	100% (20/20) Preoperative in SLET group was 2.13 ± 1.0, which improved to 1.53 ± 0.72 and 1.62 ± 0.86 at 3 and 6 months, respectively	SLET group: Hemorrhage under AM ×1 (Spontaneous resolution) No complications at donor site in either group	6 months
13	Iyer et al.[30]	AlloSLET Subsequent limbal autograft (SLET technique) ×5	17 (18 eyes)	94.11% (17/18)	72.2% (13/18) Better than 20/120	Gradual failure of allograft ×7 Symblepharon formation ×3 (16.7 %)	10.28 ± 6.7 (3-23) months
14	Basu et al.[3]	Standard SLET + modified for partial LSCD ×125 PK ×10 Cataract surgery ×5	125	76% (95/125) overall Adults—Tot. 80% (44/55) Adults—Part. 80% (8/10) Children—Tot. 71.2% (37/52) Children—Part. 76% (6/8)	75.2% (94/125) overall Two-line improvement	Donor eye: Subconjunctival hemorrhage ×35 (28%) Pyogenic granuloma ×2 (1.6%) LSCD ×0 Recipient eye: Conjunctivalization ×23 (18.4 %) Symblepharon ×21 (16.8%) Hemorrhage under hAM ×10 (8%) Loss of transplants ×7 (5.6%) Detached hAM ×4 (3.2 %) Keratitis ×8 (6.4 %) Corneal melting with perforation ×2 (1.6 %) Lignocaine allergy ×1 (0.8 %)	1.5 years (range, 1-4 years)
15	Arya et al.[40]	Standard + cadaveric AlloSLET	2	100% (2/2)	100% (2/2) Case 1: From HM to 20/20 by six weeks Case 2: From light perception to hand movements close to the face	Case 2: underlying optic atrophy	3 months

(Continues)

TABLE 1 (Continued)

	References	Surgery	No. of patients	Stable corneal epithelium %	Improvement in visual acuity %	Complications in recipient eye	Follow-up period
16	Queiroz et al.[36]		4	50% (2/4)	25% (1/4) From hand motion to 20/80 vision	No adhesion of limbal grafts to cornea ×1 Recurrence of corneal neovascularization and persistent epithelial defect ×1	6 months
17	Vazirani et al.[27]		68	83.8% (57/68)	64.7% (44/68) 20/200 or better	Focal recurrences of pannus not progressing to the center of the cornea ×21 (30.9%) Microbial keratitis ×5 Ocular hypertension secondary to steroid use ×1 Pyogenic granuloma ×1 Focal iatrogenic LSCD at the site of the donor limbus ×1	>6 months. Median, 12 months. Range, 6-59 months
18	Mittal et al.[41]	Excisional biopsy of cornea/limbus + SLET in same setting Radiotherapy	1	100 % (1/1)	100% (1/1) From 20/50 to 20/40	None	2 years
19	Mittal et al.[52]	Repeated SLET with conjunctival autograft ×3	4	100% (4/4)	100% (4/4) From PLPR to counting fingers close to face ×1 From PR to 6/36 x 2 From HM to 6/18 x 1	LSCD focal recurrence with symblepharon ×3	12-60 months after first SLET and 13-36 months after repeat SLET
20	Vazirani et al.[42]	Customized SLET for treating focal recurrent conjunctivalization after SLET	1	100% (1/1)	100% (1/1) From light perception to 20/50	Focal recurrences of conjunctivalization on the cornea and recurrence of symblepharon after first SLET ×1	5 months
21	Nair et al.[43]	Cataract surgery	1	100% (1/1)	100% (1/1) From light perception to 20/60	Recurrence LSCD ×1	7 months
22	Bogantes[34]	Pterygium surgery + SLET	10 eyes in 9 patients	100% (10/10)		Pyogenic granuloma at the junction of AM and conjunctiva ×1	8 months
23	Das et al.[44]		1	100% (1/1)	100% (1/1) From counting fingers at 1m to 20/50		27 months
24	Mittal et al.[4]		5	100% (5/5)	80% (3/5) 2 line improvement	SPK ×2 Resolved after increasing lubricant	10.8 months (range, 8-36 months)
25	Amescua et al.[37]		4	100% (4/4)	100% (4/4) From worse than 20/200 to 20/50 or better		7.5 ± 1.3 months
26	Vazirani et al.[45]	Conjunctival autografting with supplemental SLET ×1	1	100% (1/1)	100% (1/1) From light perception to 20/40	Recurrence of LSCD with symblepharon and forniceal shortening ×1	6 months

(Continues)

TABLE 1 (Continued)

	References	Surgery	No. of patients	Stable corneal epithelium %	Improvement in visual acuity %	Complications in recipient eye	Follow-up period
27	Lal et al.[46]		1	100% (1/1)	100% (1/1) From 20/50 to 20/25		2 years
28	Bhalekar et al.[47]		1	100% (1/1)	100% (1/1) From hand motions to 20/100	Allograft rejection managed with aggressive immune suppression	6 months
29	Bhalekar et al.[48]		1	100% (1/1)	100% (1/1) Improved to 20/80		13 months
30	Bhalekar et al.[49]		1		100% (1/1) From light perception with accurate projection to 20/200	Whitish plaque and corneal epithelial hyperplasia ×1	3 weeks
31	Sangwan et al.[2]	Original SLET protocol	6	100% (6/6)	66.6% (4/6) From worse than 20/200 in all recipient eyes to 20/40 or better in four eyes		9.2 ± 1.9 months

Abbreviations: AMT, amniotic membrane transplantation; BCVA, best corrected visual acuity; CLET, cultivated limbal epithelial transplantation; DALK, deep anterior lamellar keratoplasty; HM, hand movements; ISCH, ischemia; LSCD, limbal stem cell deficiency; LSCT, limbal stem cell transplantation; MMC, mitomycin C; OSSN, ocular surface squamous neoplasia; Part., partial LSCD; PK, penetrating keratoplasty; PLPR, hand motions, accurate projection of rays; SLET, simple limbal epithelial transplantation; SPK, superficial punctuate keratitis; Tot., total LSCD.

8.3 | Postoperative

The most common complications following SLET reported in the three largest follow-up studies (involving 125,[3] 68,[27] and 30[28] patients) were focal recurrence of LSCD,[27] progressive conjunctivalization,[3,28] progressive symblepharon,[3] and keratitis (Table 1).[3,27] More unusual complications were loss of transplants following surgery,[3] epithelial defects that persisted for more than 6 months,[29,36] and pyogenic granuloma.[3,27,34]

One study reported corneal epithelial hyperplasia following SLET in an 11-year-old boy.[49] The authors suggested that in young patients the contact lens should be removed as soon as possible after corneal epithelialization is complete due to the high rate of cell proliferation that is typically seen.

A trial of 30 patients who underwent SLET after failed CLET reported zero cases of iatrogenic LSCD despite harvest of multiple biopsies from donor eyes.[29] Harmless subconjunctival hemorrhage after biopsy harvest, which resolved within 1 month was noted in 28% of donor eyes in the largest study involving 125 patients.[3] Iatrogenic LSCD at the site of the donor limbus was also noted in one patient.

In summary, preexisting symblepharon and simultaneous performance of PK with SLET are the main features correlated with SLET failure. Complications following SLET are relatively benign and manageable. The risk of iatrogenic LSCD at the donor site is also low even after harvest of multiple biopsies for repeat SLET.

9 | ALLOGENIC SLET

Very little has been published on the use of alloSLET for permanent restoration of the cornea for treatment of bilateral LSCD. Bilateral LSCD often occurs secondary to Stevens-Johnson syndrome, mucous membrane phemigoid (MMP), and severe chemical burns, which produce extensive cicatrization or dryness making patients unsuitable candidates for treatment with SLET.[23] A total of 56 eyes in six separate studies have used alloSLET.[23,30,38,40,47,50] Immunosuppressant steroids were prescribed topically (19 eyes[30,38]), systemically (1 eye[47]), or in combination (30 eyes[23]). Transplant rejection can be managed by increasing the dose of systemic and topical immunosuppressants.[23,47] A total of 30 eyes were treated in the largest alloSLET study reported so far; 16 eyes received living-related donor tissue and 14 eyes of 13 patients received cadaveric donor tissue.[23] At the final follow-up (median 28 months), the overall improvement in visual acuity was from hand-motion to 20/60 in more than 60% of eyes. Achievement of a stable corneal surface indicating successful outcome varied slightly between the two groups with success noted in 14 of 16 (87.5%) eyes receiving living-related SLET and in 11 of 14 (78.6%) eyes in the cadaveric group at the final follow-up (average 28 months). No serious systemic complications were noted. These results compare well with typical results using lr-CLAL and KLAL techniques, for example, in a large retrospective case series 105 of 136 patients (77.2%) achieved ocular surface stability.[24]

Iyer et al. investigated the effectiveness of alloSLET in management of acute inflammation in 17 patients (18 eyes) with severe grade 4 or worse chemical injury (Dua's classification).[30] Ten of the patients were children with an age range of 3 months to 10 years. Systemic immunosuppressants were not used since later rejection of allogenic transplants was expected. Follow-up ranged from 3 to 23 months. The authors performed alloSLET with the intention of aiding fast epithelialization of the denuded cornea and to promote early reconstruction of the corneal surface and not with an aim toward long-term survival of the allogenic cells (Figure S2). They speculated that the small size of the allogenic explants may have reduced the antigenic load leading to slow rejection. Complete reepithelialization was achieved within 10-40 days in 17 of 18 (94%) eyes. Improved visual acuity was seen in 13 of 17 (76%) patients. Symblepharon involving one or two quadrants was noted in three eyes.

Iyer and colleagues hypothesize that early resolution of inflammation facilitated by the use of AM and topical steroids may have been influential in preventing further damage to residual stem cells.[30] Furthermore, early reepithelialization by allogenic explants may have also reduced ocular surface inflammation allowing residual stem cells to repopulate the cornea.

Though studies are so far limited, reports suggest that use of AM in the alloSLET procedure and regeneration of an epithelial layer using allogenic explants quietens inflammation on the ocular surface. Therefore, in addition to offering an alternative treatment for bilateral LSCD, alloSLET may be especially applicable for fast temporary treatment of pediatric patients, where inflammation has been reported as a key factor hindering successful outcome. AlloSLET offers the advantage of quickly restoring a clear epithelial layer, albeit of a temporary nature, which aids in improvement in visual acuity as early as a month following injury.[30] Thus, the risk of amblyopia can be reduced or addressed earlier in pediatric patients. Importantly, the use of allogeneic tissue as a temporary application maintains an undisturbed healthy alternate eye. Valuable autologous limbal tissue can then later be harvested for use in SLET once inflammation in the injured eye has subsided, giving a higher chance of success.

10 | FUTURE STUDIES

The AM carrier could be a critical factor to the success of SLET. It contains anti-inflammatory cytokines, growth factors, and provides a substrate that may allow stem cells in SLET explants to embed. SLET results may be further improved with the use of cross-linked AM.[65] Comparison of the effect of using denuded vs. intact AM would also be useful. Consideration of a standardized synthetic replacement for AM could also be evaluated to eliminate the inherent variability found in AM, a natural tissue.

Cumulative results show that although regeneration of the corneal epithelium occurs in 83.5% of SLET operations, visual acuity is improved in only 68.7% of patients (Table 1). Avenues for improvement include the pursuit of work indicating that inflammation plays a key role in SLET operations with poor outcomes.[3] Inflammatory state may be influenced by the time between injury and operation, as reported in several studies.[3,28,62] To advance the treatment of LSCD in children, it may be necessary to focus on faster resolution of inflammation before SLET.[28] Temporary application of alloSLET may accomplish this, and larger studies are needed to confirm.[30]

Mittal et al. showed that individual explants from the same donor often vary in outgrowth.[4] Follow-up studies could optimize the amount of fibrin glue used for mounting explants, as well as limbal explant size, orientation, harvest site, and handling techniques.

Although SLET minimizes the amount of biopsy harvested from the donor eye, the same technique using an alternative source of autologous tissue may have the additional benefit of offering treatment of bilateral LSCD. Oral mucosal tissue has proved effective in treating LSCD transplanted as cultured sheet transplants (COMET).[15,66] Transfer of small oral mucosal biopsies in a simple oral mucosal epithelial transplantation (SOMET) technique would avoid the need to harvest ocular limbal material altogether.

Direct comparison of the effectiveness of CLAU, CLET, and SLET in a large randomized prospective study would be useful.

11 | CONCLUSION

In conclusion, results so far indicate that SLET offers a comparable alternative to CLAU and CLET using the two main criteria for success: corneal re-epithelialization and improvement in visual acuity. In addition, there are advantages to harvesting a smaller biopsy for transplant, such as lowered risk of iatrogenic LSCD and the option for repeat operations. Importantly, harvest and transplantation are accomplished in a single operation, which increases efficiency, promotes accessibility, and reduces cost. Latest work shows limbal allografts can be used successfully in treatment of bilateral LSCD.

Direct transfer of limbal explants may support superior maintenance of stem cell phenotype and function following transplant. On the other hand, analysis of biopsies used for CLET transplants has shown a correlation between clinical success and stem cell content suggesting stem cells are maintained during culture.[67] The opportunity for gene editing prior to transplantation may also be an important advantage of the CLET technique.

Long-term follow-up studies equivalent to CLAU and CLET are now becoming available, and results using SLET are promising. AlloSLET used as a temporary treatment to resolve initial inflammation and quickly recover an intact epithelial layer also holds great potential. This may be especially important in treating pediatric cases of LSCD. Avenues for improvement should be further explored, including the feasibility of using non-limbal autologous tissue from the oral cavity for treatment of bilateral LSCD (SOMET).

CONFLICT OF INTEREST

The authors indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

C.J.J.: conception and design, collection of studies, analysis and interpretation of studies, manuscript writing, final approval of manuscript; I.T.M.E., H.R.: collection of studies, manuscript writing, analysis and interpretation of studies, final approval of manuscript; K.A.T., D.A.D.: conception and design, analysis and interpretation of studies, final approval of manuscript; T.P.U.: conception and design, analysis and interpretation of studies, manuscript writing, final approval of manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

1. Utheim TP. Limbal epithelial cell therapy: past, present, and future. *Methods Mol Biol*. 2013;1014:3–43.
2. Sangwan VS, Basu S, MacNeil S, et al. Simple limbal epithelial transplantation (SLET): a novel surgical technique for the treatment of unilateral limbal stem cell deficiency. *Br J Ophthalmol*. 2012;96:931–934.
3. Basu S, Sureka SP, Shanbhag SS, et al. Simple limbal epithelial transplantation: long-term clinical outcomes in 125 cases of unilateral chronic ocular surface burns. *Ophthalmology*. 2016;123:1000–1010.
4. Mittal V, Jain R, Mittal R. Ocular surface epithelialization pattern after simple limbal epithelial transplantation: an in vivo observational study. *Cornea*. 2015;34:1227–1232.
5. Kenyon KR, Tseng SC. Limabl autograft transplantation for ocular surface disorders. *Ophthalmology*. 1989;96:709–722.
6. Yin J, Jurkunas U. Limbal stem cell transplantation and complications. *Semin Ophthalmol*. 2018;33:134–141.
7. Liang L, Sheha H, Li J, et al. Limbal stem cell transplantation: new progresses and challenges. *Eye*. 2009;23:1946–1953.
8. Pellegrini G, Traverso CE, Franzi AT, et al. Long-term restoration of damaged corneal surfaces with autologous cultivated corneal epithelium. *Lancet*. 1997;349:990–993.
9. Holland EJ. Management of limbal stem cell deficiency: a historical perspective, past, present, and future. *Cornea*. 2015;34:S9–S15.
10. Schwab IR, Johnson NT, Harkin DG. Inherent risks associated with manufacture of bioengineered ocular surface tissue. *Arch Ophthalmol*. 2006;124:1734–1740.
11. Utheim TP, Lyberg T, Raeder S. The culture of limbal epithelial cells. *Methods Mol Biol*. 2013;1014:103–129.
12. Tsai RJ, Li LM, Chen JK. Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. *N Engl J Med*. 2000;343:86–93.
13. Pellegrini G, Ardigo D, Milazzo G, et al. Navigating market authorization: the path holoclar took to become the first stem cell product approved in the European Union. *Stem Cells Translational Medicine*. 2018;7:146–154.
14. Sehic A, Utheim OA, Ommundsen K, et al. Pre-clinical cell-based therapy for limbal stem cell deficiency. *J Funct Biomater*. 2015;6:863–888.
15. Nakamura T, Inatomi T, Sotozono C, et al. Transplantation of cultivated autologous oral mucosal epithelial cells in patients with severe ocular surface disorders. *Br J Ophthalmol*. 2004;88:1280–1284.
16. Prabhasawat P, Ekpo P, Uiprasertkul M, et al. Long-term result of autologous cultivated oral mucosal epithelial transplantation for severe ocular surface disease. *Cell Tissue Bank*. 2016;17:491–503.
17. Utheim TP. Concise review: Transplantation of cultured oral mucosal epithelial cells for treating limbal stem cell deficiency-current status and future perspectives. *Stem Cells*. 2015;33:1685–1695.
18. Jenkins C, Tuft S, Liu C, et al. Limbal transplantation in the management of chronic contact-lens-associated epitheliopathy. *Eye*. 1993;7:629–633.
19. Tan DT, Ficker LA, Buckley RJ. Limbal transplantation. *Ophthalmology*. 1996;103:29–36.
20. Miri A, Said DG, Dua HS. Donor site complications in autolimbal and living-related allolimbal transplantation. *Ophthalmology*. 2011;118:1265–1271.
21. Baradaran-Rafii A, Eslani M, Haq Z, et al. Current and upcoming therapies for ocular surface chemical injuries. *Ocul Surf*. 2017;15:48–64.
22. Ballios BG, Weisbrod M, Chan CC, et al. Systemic immunosuppression in limbal stem cell transplantation: best practices and future challenges. *Can J Ophthalmol*. 2018;53:314–323.
23. Shanbhag SS, Patel CN, Goyal R, et al. Simple limbal epithelial transplantation (SLET): review of indications, surgical technique, mechanism, outcomes, limitations, and impact. *Indian J Ophthalmol*. 2019;67:1265–1277.
24. Holland EJ, Mogilishetty G, Skeens HM, et al. Systemic immunosuppression in ocular surface stem cell transplantation: results of a 10-year experience. *Cornea*. 2012;31:655–661.
25. Ang AY, Chan CC, Biber JM, et al. Ocular surface stem cell transplantation rejection: incidence, characteristics, and outcomes. *Cornea*. 2013;32:229–236.
26. Kethiri AR, Basu S, Shukla S, et al. Optimizing the role of limbal explant size and source in determining the outcomes of limbal transplantation: An in vitro study. *PLoS One*. 2017;12:e0185623.
27. Vazirani J, Ali MH, Sharma N, et al. Autologous simple limbal epithelial transplantation for unilateral limbal stem cell deficiency: multicentre results. *Br J Ophthalmol*. 2016;100:1416–1420.
28. Gupta N, Joshi J, Farooqui JH, et al. Results of simple limbal epithelial transplantation in unilateral ocular surface burn. *Indian J Ophthalmol*. 2018;66:45–52.
29. Basu S, Mohan S, Bhalekar S, et al. Simple limbal epithelial transplantation (SLET) in failed cultivated limbal epithelial transplantation (CLET) for unilateral chronic ocular burns. *Br J Ophthalmol*. 2018;102:1640–1645.
30. Iyer G, Srinivasan B, Agarwal S, et al. Outcome of allo simple limbal epithelial transplantation (alloSLET) in the early stage of ocular chemical injury. *Br J Ophthalmol*. 2017;101:828–833.
31. Gupta N, Singh A, Mathur U. Scleral ischemia in acute ocular chemical injury: long-term impact on rehabilitation with limbal stem cell therapy. *Cornea*. 2019;38:198–202.
32. Singh D, Vanathi M, Gupta C, et al. Outcomes of deep anterior lamellar keratoplasty following autologous simple limbal epithelial transplant in pediatric unilateral severe chemical injury. *Indian J Ophthalmol*. 2017;65:217–222.
33. Arora R, Dokania P, Manudhane A, et al. Preliminary results from the comparison of simple limbal epithelial transplantation with conjunctival limbal autologous transplantation in severe unilateral chronic ocular burns. *Indian J Ophthalmol*. 2017;65:35–40.
34. Hernandez-Bogantes E, Amescua G, Navas A, et al. Minor ipsilateral simple limbal epithelial transplantation (mini-SLET) for pterygium treatment. *Br J Ophthalmol*. 2015;99:1598–1600.
35. Kaliki S, Mohammad FA, Tahiliani P, et al. Concomitant simple limbal epithelial transplantation after surgical excision of ocular surface squamous neoplasia. *Am J Ophthalmol*. 2017;174:68–75.

36. Queiroz AG, Barbosa MM, Santos MS, et al. Assessment of surgical outcomes of limbal transplantation using simple limbal epithelial transplantation technique in patients with total unilateral limbal deficiency. *Arq Bras Oftalmol*. 2016;79:116–118.
37. Amescua G, Atallah M, Nikpoor N, et al. Modified simple limbal epithelial transplantation using cryopreserved amniotic membrane for unilateral limbal stem cell deficiency. *Am J Ophthalmol*. 2014;158:469–475.e462.
38. Vasquez-Perez A, Nanavaty MA. Modified allogenic simple limbal epithelial transplantation followed by keratoplasty as treatment for total limbal stem cell deficiency. *Ocul Immunol Inflamm*. 2017;26:1–3.
39. Mohamed A, Sangwan VS. Ocular surface reconstruction in laryngo-onycho-cutaneous syndrome. *Ocul Immunol Inflamm*. 2017;25:460–462.
40. Arya SK, Bhatti A, Raj A, et al. Simple limbal epithelial transplantation in acid injury and severe dry eye. *J Clin Diagn Res*. 2016;10: ND06–ND07.
41. Mittal V, Narang P, Menon V, et al. Primary simple limbal epithelial transplantation along with excisional biopsy in the management of extensive ocular surface squamous neoplasia. *Cornea*. 2016;35: 1650–1652.
42. Vazirani J, Lal I, Sangwan V. Customised simple limbal epithelial transplantation for recurrent limbal stem cell deficiency. *BMJ Case Rep*. 2015;2015.
43. Nair D, Mohamed A, Sangwan VS. Outcome of cataract surgery following simple limbal epithelial transplantation for lime injury-induced limbal stem cell deficiency. *BMJ Case Rep*. 2015;2015.
44. Das S, Basu S, Sangwan V. Molten metal ocular burn: long-term outcome using simple limbal epithelial transplantation. *BMJ Case Rep*. 2015;2015.
45. Vazirani J, Basu S, Sangwan V. Successful simple limbal epithelial transplantation (SLET) in lime injury-induced limbal stem cell deficiency with ocular surface granuloma. *BMJ Case Rep*. 2013;2013.
46. Lal I, Panchal BU, Basu S, et al. In-vivo expansion of autologous limbal stem cell using simple limbal epithelial transplantation for treatment of limbal stem cell deficiency. *BMJ Case Rep*. 2013;2013.
47. Bhalekar S, Basu S, Sangwan VS. Successful management of immunological rejection following allogeneic simple limbal epithelial transplantation (SLET) for bilateral ocular burns. *BMJ Case Rep*. 2013;2013.
48. Bhalekar S, Basu S, Lal I, et al. Successful autologous simple limbal epithelial transplantation (SLET) in previously failed paediatric limbal transplantation for ocular surface burns. *BMJ Case Rep*. 2013;2013.
49. Bhalekar S, Sangwan VS, Basu S. Growth of corneal epithelial cells over in situ therapeutic contact lens after simple limbal epithelial transplantation (SLET). *BMJ Case Rep*. 2013;2013.
50. Prabhasawat P, Luangaram A, Ekpo P, et al. Epithelial analysis of simple limbal epithelial transplantation in limbal stem cell deficiency by in vivo confocal microscopy and impression cytology. *Cell Tissue Bank*. 2019;20:95–108.
51. Narang P, Mittal V, Menon V, et al. Primary limbal stem cell transplantation in the surgical management of extensive ocular surface squamous neoplasia involving the limbus. *Indian J Ophthalmol*. 2018;66: 1569–1573.
52. Mittal V, Jain R, Mittal R, et al. Successful management of severe unilateral chemical burns in children using simple limbal epithelial transplantation (SLET). *Br J Ophthalmol*. 2016;100:1102–1108.
53. Sangwan VS, Matalia HP, Vemuganti GK, et al. Amniotic membrane transplantation for reconstruction of corneal epithelial surface in cases of partial limbal stem cell deficiency. *Indian J Ophthalmol*. 2004; 52:281–285.
54. Westekemper H, Figueiredo FC, Siah WF, et al. Clinical outcomes of amniotic membrane transplantation in the management of acute ocular chemical injury. *Br J Ophthalmol*. 2017;101:103–107.
55. Mednick Z, Boutin T, Einan-Lifshitz A, et al. Simple limbal epithelial transplantation for recurrent pterygium: A case series. *Am J Ophthalmol Case Rep*. 2018;12:5–8.
56. Hirst LW. Recurrence and complications after 1,000 surgeries using pterygium extended removal followed by extended conjunctival transplant. *Ophthalmology*. 2012;119:2205–2210.
57. Kheirkhah A, Casas V, Raju VK, et al. Sutureless amniotic membrane transplantation for partial limbal stem cell deficiency. *Am J Ophthalmol*. 2008;145:787–794.
58. Yeung AM, Faraj LA, McIntosh OD, et al. Fibrin glue inhibits migration of ocular surface epithelial cells. *Eye*. 2016;30:1389–1394.
59. Dietrich-Ntoukas T, Hofmann-Rummelt C, Kruse FE, et al. Comparative analysis of the basement membrane composition of the human limbus epithelium and amniotic membrane epithelium. *Cornea*. 2012; 31:564–569.
60. Sangwan VS, Basu S, Vemuganti GK, et al. Clinical outcomes of xeno-free autologous cultivated limbal epithelial transplantation: a 10-year study. *Br J Ophthalmol*. 2011;95:1525–1529.
61. Sejpal K, Ali MH, Maddileti S, et al. Cultivated limbal epithelial transplantation in children with ocular surface burns. *JAMA Ophthalmol*. 2013;131:731–736.
62. Dogru M, Tsubota K. Current concepts in ocular surface reconstruction. *Semin Ophthalmol*. 2005;20:75–93.
63. Ganger A, Vanathi M, Mohanty S, et al. Long-term outcomes of cultivated limbal epithelial transplantation: evaluation and comparison of results in children and adults. *Biomed Res Int*. 2015;2015: 6 pages.
64. Basu S, Mohamed A, Chaurasia S, et al. Clinical outcomes of penetrating keratoplasty after autologous cultivated limbal epithelial transplantation for ocular surface burns. *Am J Ophthalmol*. 2011;152: 917–924.e911.
65. Utheim TP, Aass Utheim O, Salvanos P, et al. Concise review: altered versus unaltered amniotic membrane as a substrate for limbal epithelial cells. *Stem Cells Translational Medicine*. 2018;7:415–427.
66. Nishida K, Yamato M, Hayashida Y, et al. Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium. *N Engl J Med*. 2004;351:1187–1196.
67. Rama P, Matuska S, Paganoni G, et al. Limbal stem-cell therapy and long-term corneal regeneration. *N Engl J Med*. 2010;363:147–155.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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