



Cell-based therapies for limbal stem cell deficiency: a literature review

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Background and Objective: Limbal stem cell deficiency (LSCD) is characterized by the insufficiency of limbal stem cells to maintain the corneal epithelium. Severe cases of LSCD may be treated with limbal transplantation from healthy autologous or allogeneic limbal tissue. Multiple cell-based therapies have been studied as alternative treatments to improve success rates and minimize immunosuppressive regimens after allogeneic transplants. In this review, we describe the success rates, and complications of different cell-based therapies for LSCD. We also discuss each therapy's relative strengths and weaknesses, their history in animal and human studies, and their effectiveness compared to traditional transplants.

Methods: PubMed was searched for publications using the terms LSCD, cell-based therapy, cultivated limbal epithelial transplantation (CLET), cultivated oral mucosal epithelial transplantation (COMET), and mesenchymal stem cells from 1989 to August 2022. Inclusion criteria were English language articles. Exclusion criteria were non-English language articles.

Key Content and Findings: current cell-based therapies for LSCD are CLET and non-limbal epithelial cells. Non-limbal epithelial cell methods include COMET, conjunctival epithelial autografts, and mesenchymal stem/stromal cells (MSCs). Moreover, several alternative potential sources of non-limbal cells have described, including induced pluripotent stem cells (iPSCs), human embryonic stem cells (hESCs), human dental pulp stem cells, hair follicle bulge-derived epithelial stem cells, amniotic membrane epithelial cells, and human umbilical cord lining epithelial cells.

Conclusions: Cell-based therapies are a promising treatment modality for LSCD. While CLET is currently the only approved cell-based therapy and is only approved in the European Union, more novel methods have also been shown to be effective in human or animal studies thus far. Non-limbal epithelial cells such as COMET are also an alternative treatment to allogeneic transplants especially as a surface stabilizing procedure. iPSCs are currently being studied in early phase trials and have the potential to revolutionize the way LSCD is treated. Lastly, cell-based therapies for restoring the limbal niche such as mesenchymal stem cells have also shown promising results in the first human proof-of-concept study. Several potential sources of non-limbal cells are under investigation.

Keywords: Limbal stem cell deficiency (LSCD); cell-based therapy; cultivated limbal epithelial transplantation (CLET); cultivated oral mucosal epithelial transplantation (COMET)

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Introduction

Background

Limbal stem cell deficiency (LSCD) is characterized by damage to or loss of limbal epithelial stem cells, which maintain the continuous renewal of corneal epithelial cells (1). Limbal epithelial stem cells are located in the basal limbal epithelium, anatomically marked by the Palisades of Vogt located at the cornea-conjunctiva junction. Deficiency in these cells can result in persistent epitheliopathy, conjunctivalization of the cornea, and the loss of corneal transparency and visual function (2). Other LSCD consequences include persistent epithelial defects, corneal opacity, neovascularization, inflammation, ulceration, corneal thinning, and perforation. Several etiologies for LSCD have been introduced, which may be hereditary or acquired in origin. Inherited conditions such as aniridia are rare. Acquired conditions can be categorized as immune-related conditions such as Stevens-Johnson syndrome (SJS), mucus membrane pemphigoid (MMP), and non-immune pathologies like burns or previous multiple ocular surgeries (1,3).

The treatment of LSCD depends on the severity and extent of limbal stem cell loss. Conservative management with lubrication is suited for mild cases, while various surgical interventions can be utilized in severe cases. In unilateral cases, limbal stem cell transplantation is performed using limbal grafts taken from the patient's healthy second eye [conjunctival limbal autograft (CLAU)]. Autologous limbal stem cell transplants from a patient's unaffected eye in unilateral LSCD eliminate the need for immunosuppression, but it increases the risk of developing LSCD in the healthy eye. In allogeneic transplantations, limbal grafts are from healthy living-related donors or cadaver corneas. In all allogeneic transplants, systemic immunosuppression is required for a prolonged or indefinite period (1).

Rationale and knowledge gap

More recently, various cell-based therapies have been explored as alternative treatment options. Interestingly, ophthalmology is one of the first medical disciplines to

utilize stem cell application in regenerative medicine (4). The overall literature on LSCD and its treatment is growing rapidly and numerous papers are published annually on this topic. So, we aimed to provide and update on the cell-based therapies for LSCD to help clinicians and researchers alike.

Objective

This review discusses the mechanisms and utility of various cell-based therapies such as cultivated limbal epithelial transplantation (CLET) and other non-limbal and stem cell sources for transplantation (*Figure 1*). The key question to be explored is how cell-based therapies can play a role in LSCD treatment. We present this article in accordance with the Narrative Review reporting checklist (available at <https://aes.amegroups.com/article/view/10.21037/aes-22-55/rc>).

Methods

The literature search identification process included years starting from 1989, English language, published status, a database of coverage including PubMed, and study designs consisting of original articles, review articles, and case series (*Table 1*).

Limbal epithelial cells

CLET was first described in 1997 by Pellegrini *et al.* They showed that limbal epithelial cells could be cultured *ex vivo* and give rise to the stratified corneal epithelium. In this procedure, a 1 mm² biopsy is taken from the healthy eye, cultured *ex vivo* with various serums and growth factors, and subsequently transplanted on the deficient eye with grafts containing 2×10⁶ cells. It is of note that grafts may have an autologous or allogeneic origin (5).

More recently, CLET has been tested in more extensive studies which have shown reasonable success rates, defined as restoration of a stable, transparent, and avascular cornea. Rama *et al.* (6) reported a success rate of 68.2% at 1 year in 107 eyes after the first autologous CLET transplantation. Sangwan *et al.* (7) similarly found a success rate of 71% in a retrospective study of 200 eyes that underwent CLET

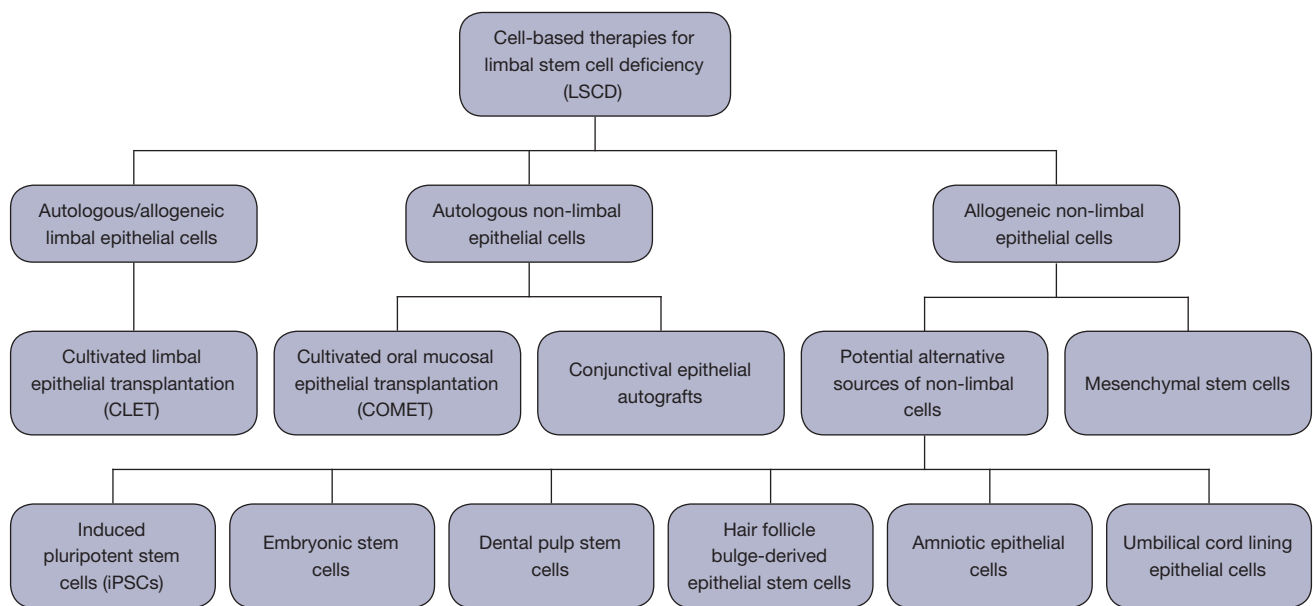


Figure 1 This diagram summarized updated treatment options for cell-based therapies in limbal stem cell deficiencies.

Table 1 The search strategy summary

Items	Specification
Date of search	August 10 th , 2022
Databases and other sources searched	PubMed
Search terms used	Limbal stem cell deficiency, cell-based therapy, cultivated limbal epithelial transplantation, cultivated oral mucosal epithelial transplantation, mesenchymal stem cells
Timeframe	1989 onwards
Inclusion and exclusion criteria	Inclusion: keywords in English literature from PubMed Exclusion: all other items not included in the inclusion criteria
Selection process	Conducted by Dr. Djallian, we searched the literature, and a consensus was reached according to author experience and source assessment

for LSCD from ocular surface burns. Fasolo *et al.* (8) also showed a lower success rate of 41% in 65 consecutive CLET transplantations of 59 eyes. However, an additional 39% of cases were classified as partially successful (relapsed neovascularization but not as extensive as admission). In the study by Rama *et al.*, it is found that 11 eyes that were partially successful or failed after primary CLET were re-grafted with 9 of the 11 (81.8%) classified as successful. This resulted in a final success rate of 76.6%. In this study, age, the underlying cause of LSCD, the severity of injury, culturing and postoperative complications, as well as inflammation were found to be associated with failure based

on univariate logistic-regression analysis. The severity of injury and culturing and postoperative complications were confirmed on multivariate logistic regression analysis. An interesting finding in this study was the role of p63-bright cell density (representing holoclones which are enriched in stem cells) in the success rate of grafts; cultures containing >3% of p63-bright cells had a success rate of 78% while cultures containing ≤3% of these cells population resulted in the success rate of 11%. This finding shows that >3% p63-bright cell density is necessary but not enough for successful treatment since over 20% of cultures with this property had failed (6).

Basu *et al.* (9) performed a retrospective study focusing on repeat CLET. These repeat transplants do not increase the risk of rejection or necessitate immunosuppressive treatment given the autologous nature of the transplant and have a success rate of 66% in the 50 eyes included, similar to the rates noted in primary CLET. Most of these eyes undergoing repeat autologous CLET also exhibit improvement in best corrected visual acuity (BCVA) of at least 2 lines.

Autologous CLET transplantation significantly reduces the risk of iatrogenic LSCD in the donor's eye compared to the larger traditional CLAU transplantations. However, as reported by the Holland group, if the donor's eyes are carefully screened and selected, the risk of iatrogenic LSCD in the donor's eye is minimal (10).

Although our knowledge regarding the long-term outcomes of CLET is limited, successful clinical results of up to 10 years (with a median of 2 years) were reported by Rama *et al.* (6). Regarding the comparison between long-term outcomes of CLET and CLAU, Sharma *et al.* (11) reported equal effectiveness in restoration of the ocular surface after chemical burns for both procedures. They performed CLET on 10 patients and CLAU in 51 eyes and all of the eyes experienced improvement in ocular surface condition. Although CLET provides more stem cells in the recipient's eye and avoids iatrogenic LSCD in the donor's eye, CLAU is cheaper and also can simultaneously address the pre-existing symblepharon (11).

Less data is available regarding clinical outcomes of CLET in children. One study looking at children under 15 years of age who underwent CLET for LSCD due to ocular burns showed a success rate was 37.4% in 107 eyes, with a significantly lower rate of success in children 6 years or younger (30%) compared to those over 6 years of age (70%). Multivariable analyses in this study also found that the survival rates of the grafts were significantly higher in eyes that underwent surgery more than 4 months after the injury. At 24 months, the survival rate was 12.8% in eyes operated at or earlier than 4 months after the initial injury, and 54% in eyes operated on more than 4 months after the injury. This lower success rate compared to the adult trials is likely multifactorial and may partially be attributed to an increased inflammatory response in children. In addition, children also have a much lower likelihood of visual improvement after surgery due to deprivation amblyopia (12).

CLET has also been performed with allografts, especially in cases of bilateral LSCD, although this is less commonly

performed than autologous CLET. A recent meta-analysis including 30 studies and 1,306 eyes reported a ratio of 75.2% to 24.7% of eyes had undergone autologous versus allogeneic CLET. No statistically significant difference was found in terms of graft survival and visual improvement. However, autologous CLET, whenever feasible, eliminates the need for long-term immunosuppression (13).

The precise process by which limbal stem cells are cultivated *ex vivo* and transplanted back onto the human cornea in CLET varies slightly between studies. There are differences in the use of feeder cells, culture media, serum, and scaffolds across these studies. Feeder cells help to create a suitable microenvironment and vary from irradiated or mitomycin-treated murine 3T3 (mitotically inactive) cells to human mesenchymal stem cells and limbal melanocytes. Similarly, animal and human-derived serums and growth products are used for the culture media. A few studies have also investigated serum-free culture protocols (1). The cultured cells are then transported to the diseased cornea using various scaffolds, including human amniotic membrane (HAM), collagen, fibrin, contact lenses, or other hydrogels (1).

In February 2015, CLET transplantation by the name of Holoclar[®] (Chiesi Farmaceutici SpA, Parma, Italy) was the first stem-cell containing advanced therapy medicinal product (ATMP) to be approved for use in the European Union by the European Medicines Agency and is approved to treat adult patients with moderate or severe LSCD due to burns, including chemical burns. It should be emphasized that at the time being, Holoclar has authorization only for application in cases due to burns with cultured grafts of autologous origin (7,14).

Non-limbal epithelial cells

In cases of total bilateral LSCD, allogeneic transplants are required either from living related donors or cadaveric donors, necessitating long-term chronic immunosuppression and increasing the risk of disease transmission. This has led researchers to seek other alternative non-limbal cells that may be used for autologous grafts. Studies have investigated a variety of non-limbal cells including oral mucosal epithelial cells (15-24), cultivated autologous conjunctival epithelial cells (25-28), induced pluripotent stem cells (iPSCs) (29-38), umbilical cord derived stem cells (39,40), hair follicle derived epithelial stem cells (41,42), dental pulp stem cells (43-46), and nasal mucosal cells (47,48).

Autologous non-limbal epithelial cell transplantation

Cultivated oral mucosal epithelial transplantation (COMET)

Of the non-limbal epithelial cell transplantations studied for the treatment of LSCD, the most well-studied is COMET. This procedure is also called *ex vivo* cultivated oral mucosal autograft (EVOMAU). COMET was first performed on humans in 2004 by Nakamura *et al.* (15). Six eyes of 4 patients with bilateral LSCD underwent COMET in this study, showing the survival of the transplanted oral epithelium at 2 days and improvement in visual acuity at follow-up of at least 11 months. Peripheral neovascularization, however, was found in all eyes. It has been reported that COMET is the most common autologous non-limbal epithelial cell transplantation in cases with bilateral LSCD (20). Lack of graft rejection and immunosuppressive regimen may justify this point.

Several subsequent studies evaluating COMET have since been performed (16-19) and COMET has proven to be promising in the treatment of LSCD with a variety of etiologies including ocular burns, aniridia, SJS, and ocular cicatricial pemphigoid (OCP). Cabral *et al.* performed a meta-analysis of 24 studies and 343 eyes, for which COMET was used to treat various etiologies of LSCD. This study showed a relative success rate defined as a stable ocular surface of 70.8% of 243 eyes (20). Dobrowolski *et al.* (21) and Venugopal *et al.* (22) also examined the outcomes of COMET in limbal stem cell deficient eyes exclusively due to aniridia and SJS, respectively. Dobrowolski *et al.* reported that 76.4% of eyes undergoing COMET for LSCD due to aniridia had a stable corneal epithelial surface after COMET transplantation, with 88% endorsing an improvement in the quality of vision (21). In 45 eyes that underwent COMET for severe SJS, COMET was also shown to be beneficial in 88.8% of eyes with a reduction in LSCD severity scores after the transplant (22). Phenotypic characterization of cells after COMET has also been performed and shows that cells retain markers for oral epithelial cells on impression cytology with immunofluorescence staining, even in successful cases (24,49). In addition, peripheral neovascularization, while not visually significant and reportedly stable at 6 months in the above studies, may continue to progress as oral mucosal cells have greater angiogenic potential compared to limbal epithelial cells (49,50).

While COMET has the main advantage of eliminating the need for chronic immunosuppression, its visual outcomes are inferior to other limbal transplant procedures.

This is partly because the oral mucosal epithelium is thicker and more opaque than normal corneal epithelium. A direct comparison study looking at allogeneic CLET and COMET by Wang *et al.* found a higher incidence of persistent epithelial defect, a worse ocular surface grade, a lower success rate, and a higher risk of graft failure after COMET compared to allogeneic CLET (23). Overall, while there is no consensus on the role of COMET in the management of bilateral LSCD, it may be considered a surface stabilization procedure especially when the patient is not a candidate for immunosuppression.

Conjunctival epithelial autografts

Human conjunctival epithelial cells are another type of non-limbal epithelial cells that have been studied for LSCD (25-28). Ang *et al.* compared cultivated human corneal epithelial cells and cultivated human conjunctival epithelial cells transplanted into rabbit eyes and found that immunohistochemical analysis of the conjunctival epithelial cells showed similar markers of corneal epithelium. Clinical outcomes were also comparable, with the majority of rabbit eyes demonstrating smooth, transparent corneas without epithelial defects (25). Jeon *et al.* demonstrated similar findings, showing that the cultivated conjunctival cells can acquire a corneal epithelial phenotype after transplantation (26). Although most studies thus far have been in animal models, Ricardo *et al.* demonstrate preliminary results of *ex vivo*-cultivated conjunctival epithelial cells in 12 human eyes with promising results. Re-epithelization with a transparent and regular epithelium without neovascularization was found in 8 of 12 eyes (66.6%) and they also demonstrated visual improvement. Overall, while the results are encouraging and avoid the need for immunosuppression, long-term restoration of the corneal phenotype is not likely with only conjunctival epithelial transplants (without any interventions to restore the limbal niche) and hence many of the problems associated with LSCD are likely to recur over time (28).

Allogeneic non-limbal stem cell-based transplantation

Mesenchymal stem/stromal cells (MSCs)

MSCs are a population of cells that can differentiate into a variety of mesodermal lineage cells including osteoblasts, adipocytes, and chondroblasts. In addition, MSCs secrete various cytokines and growth factors, creating an immunomodulatory and anti-inflammatory environment (51-53). Because of these features, MSCs have become

widely studied in a variety of conditions including severe corneal diseases. To date, more than 1,000 clinical trials have been registered using MSCs derived from bone marrow, fat, umbilical cord, and dental pulp.

Multiple animal models have shown promising results with MSCs in the regeneration of the corneal surface in different ocular surface diseases (54-63) as well as in LSCD (64). More recently, a proof-of-concept study was performed comparing allogeneic bone marrow MSC transplantation to allogeneic CLET (65). After 6–12 months, 72.7–77.8% of CLET cases and 76.5–85.7% of MSC cases were successful with no adverse events related to cell products. Central corneal epithelial phenotype was also examined in this study via *in vivo* confocal microscopy and showed improvement in the epithelial phenotype in both groups with no statistically significant difference. This report is the only clinical application of MSCs in LSCD (65).

Different routes of administration of MSCs have also been studied, including topical administration (56,66), sub-tenon injections, and intravenous injections (55,67,68). While the first human trial utilized HAMs as a carrier for the delivery of stem cells, Galindo *et al.* also showed promising results in utilizing subconjunctival injections to deliver MSCs (69). This study used an animal model, which may be a good alternative for clinical use in the future.

MSCs may have several benefits compared to limbal epithelial cells, including harvesting from multiple tissues, independency from cadaveric donors, as well as a faster and cheaper process. Moreover, 100% of the MSCs in a transplant are stem cells, whereas the population of stem cells in CLET may be extremely smaller (70). It seems that usage of MSCs in the treatment of LSCD is safe without subsequent adverse reactions, toxicity, and tumorigenicity (54,67,71-79). Currently, we are conducting a clinical trial to evaluate the safety and maximally tolerated dose of locally delivered allogeneic MSCs. In this study, different doses of bone marrow-derived MSCs were used in the route of subconjunctival injection to evaluate safety as well as anatomical and functional results in adult cases of neurotrophic keratitis (80). The results of the first three patients were reported in the annual ARVO 2022 meeting.

Alternative potential sources of non-limbal cells

In recent years, new promising options have been investigated and studied (81).

iPSCs

iPSCs have been at the forefront of regenerative medicine

and investigated as another possible treatment modality in LSCD (29,30). iPSCs can be induced from differentiated cells and reprogrammed into a variety of cells including corneal epithelial cells. Over the years, researchers have successfully transitioned from using mouse models (31,82) to human models (32,33). Mikhailova *et al.* have more recently studied iPSC-derived limbal epithelial stem cells and showed that they are able to retain their capacity for proliferation on bioengineered collagen matrices (34). Additional protocols and studies are also promising and suggest that human pluripotent stem cell-derived limbal stem cells may become a valuable cell source for treatments in the near future (35-38). Currently, more work needs to be done before this method can be implemented in large-scale clinical trials. Recently, a team of scientists from Osaka University reported the results of a world-1st trial on iPSC-based corneal transplantation (83). They performed this trial successfully on 4 patients without any rejection or tumorigenicity.

It should be mentioned that this technique carries a considerable expense, is time-consuming, and concerns about immune-related problems remain. HLA-typed iPSC banks can be a solution to these issues (84,85). Moreover, rigorous genetic analysis should be performed before transplantation to ascertain the lack of mutagenicity (86).

Embryonic stem cells

Human embryonic stem cells (hESCs) are pluripotent stem cells with the capability of differentiation into corneal and limbal epithelial cells (87). Hence, application of these cells may be beneficial in LSCD. Although challenging, several *in vitro* models have been successfully used to differentiate hESCs into corneal epithelial-like cells (88-92). Nevertheless, ethical challenges and potential immunogenicity and tumorigenicity may act as possible limiting factors (93).

Dental pulp stem cells

A similarity between marker expression of immature human dental pulp stem cells, mesenchymal stem cells, embryonic stem cells, and limbal epithelial stem cells has been shown. The success of transplantation of these stem cells in restoration of ocular surface structure and diminishing pathological features such as conjunctivalization and corneal neovascularization has been reported in animal LSCD models (43,44). Further studies are needed to investigate the safety and efficacy of this before clinical application.

Hair follicle bulge-derived epithelial stem cells

By providing a limbal-like microenvironment with a special

culture medium, stem cells harvested from hair follicles may differentiate into corneal epithelial-like cells (94). Although this source of stem cells has been used successfully in a mouse LSCD model (41), our knowledge regarding this technique is limited.

Amniotic membrane epithelial cells

It seems that expressed markers of amniotic membrane epithelial cells have a significant overlap with mesenchymal and embryonic stem cells. The other advantage of these cells is in showing immunomodulatory characteristics (95,96). In rabbit models, these cells have been successfully applied to treat LSCD (97-100).

Umbilical cord lining epithelial cells

Human umbilical cord lining epithelial cells can be considered another potential source for the management of LSCD. Animal models using this type of stem cells are available in the literature (39). Lack of mutagenicity and low immunogenicity are among the advantages of these cells (101).

The strengths of this review are describing the success rates, and complications of different cell-based therapies for LSCD and also discussing each therapy's relative strengths and weaknesses, their history in animal and human studies, and their effectiveness compared to traditional transplants.

Conclusions

Cell-based therapies are a promising treatment modality for both unilateral and bilateral limbal stem cell deficiency. While CLET is currently the only approved cell-based therapy and is only approved in the European Union, more novel methods have also been shown to be effective in human or animal studies thus far. Non-limbal epithelial cells such as cultivated oral mucosal cells (COMET) are also an alternative treatment to allogeneic transplants especially as a surface stabilizing procedure. Induced pluripotent stem cells are currently being studied in early phase trials and have the potential to revolutionize the way LSCD is treated. Lastly, cell-based therapies for restoring the limbal niche such as mesenchymal stem cells have also shown promising results in the first human proof-of-concept study. Several potential sources of non-limbal cells are under investigation.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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