Review paper

DOI: https://doi.org/10.5114/ceji.2023.132033

The immunology of corneal limbal stem cells: the up-to-date approach to stem cell transplantation

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Abstract

Limbal epithelial stem cells (LSC, LESC) are multipotent cells used as regenerative treatment of the cornea in patients with limbal epithelial stem cell deficiency (LSCD, LESCD).

There are different types of stem cell grafting including cultivated limbal epithelial transplantation (CET) and simple limbal epithelial transplantation (SLET). The outcomes of the techniques have been assessed as similar, with differences in the sample size required during the procedures.

The most important culture components for stem cell cultivation include 3T3 murine fibroblasts, human amniotic membrane (HAM), fibrin gel, and culture medium. The culture medium may be enriched with serum or not; however, xenobiotic-free materials are preferred because of the low risk of pathogen transmission.

Multiple studies have defined molecules important for maintaining the function of LSC including C/EBPδ, Bmi-1, p63α, interleukins (IL-6), epithelial structural proteins – keratins, and antibodies against epidermal growth factor receptor (EGFR). The cell phenotype of LSC has been described with factors of transplantation success rate such as a high percentage of p63 positive cells.

The article emphasizes the role of recipient tissue preparation, modern cultivation techniques and pathophysiological processes in LSC transplantation effectiveness.

Key words: *CLAU, CLET, limbal epithelial stem cells, limbal epithelial transplantation, SLET, stem cell deficiency, stem cell cultivation.*

(Cent Eur J Immunol 2023; 48 (3): 245-250)

Introduction

The cornea is a part of the eye present in most vertebrates, including mammals, birds, reptiles, amphibians, and fish. It is a part of the eye surface and it maintains the integrity of the eyeball. However, its histological structure varies among animals, even within the same genus [1, 2]. The cornea plays the key role in the visual process: it is (apart from the tear film) the first refractive layer of the eye. In order to maintain its refractive function, it has to be transparent and of regular shape, allowing the maintenance of the refractive power. The transparency of the cornea is the result of the collagen fibrils in the stroma, which are devoid of blood vessels. Any immunological process of the cornea may lead to corneal scar formation and pathological angiogenesis.

The human cornea, histologically, consists of five main layers: epithelium, Bowman layer, stroma, Descemet's membrane and endothelium. The most anterior layer – the epithelium – consists of around 5-6 layers of non-keratinized epithelial tissue cells. The deepest cells (called the basal cells) undergo mitosis and form the more superficial layers of the epithelium. The integrity of the epithelium provides a barrier against potentially invasive microorganisms. Moreover, the tight junctions present between the superficial layers of the corneal epithelium provide their polarity, enable their shedding [3, 4]. The corneal epithelium remains an immunologically active structure with high activity of cytokines, such as IL-1, IL-6, IL-8 and granulocyte monocyte colony stimulating factor (GM-CSF) [3, 5].

The average human corneal central thickness is within 450-550 mm. The average diameter is 10-11.5 mm.

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Submitted: 27.06.2023, Accepted: 05.09.2023

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The limbus is the peripheral part of the cornea, on the edge of the sclera within the structure palisades of Vogt. Its function remains vital for the cornea to maintain its function due to the presence of the limbal epithelial stem cells [6].

Limbal epithelial stem cells (LSC, LESC) are multipotent cells responsible for restoration of the corneal epithelium. They are smaller in size than regular basal epithelial cells of the cornea, densely packed and characterized by a prominent nucleus [7]. Davanger and Evensen, cited by Notara, described the corneal limbus as a 'generative organ for corneal epithelial cells' and identified the cause of pterygium formation as a failure in limbal structure [4, 8]. The presence of the limbal epithelium forms a barrier which prevents conjunctivalization of the cornea [9].

Indications for LSC transplantation

Limbal stem cells transplants have been successfully used as regenerative treatment of the cornea. They became a possible therapeutic tool in patients with limbal epithelial stem cell deficiency (LSCD, LESCD). LSCD may be a result of multiple hereditary or acquired conditions such as chemical or thermal corneal injuries, contact lens-induced keratopathy, ectrodactyly-ectodermal dysplasia-clefting (EEC) syndrome, or Stevens-Johnson syndrome [10-12]. All of the above may lead to conjunctivalization of the corneal epithelium with subsequent opacification, inflammation, neovascularization and final scarring of the cornea [13].

Limbal stem cells transplantation methods include direct autologous transplantation (the graft is harvested from the patient's healthy eye), direct allogenic transplantation (the graft is harvested from a healthy donor) and cultivated autologous or allogenic transplantations (the graft is harvested and expanded from LESC samples obtained from the patient's or donor's eye) [14].

Immunological response and neovascularization

The LSC, anatomically, lie within the limbus in the microenvironment called a 'niche', which is similar in function to other epithelial niches, such as hair follicles or intestinal crypts [7]. The cell phenotype of the LSC has been broadly described, indicating the primary expression of proteins p63, ABCG2 and α9 integrin. The antibodies found at the limbal cells are those against integrin β1, epidermal growth factor receptor (EGFR), K19, enolase α, and CD71 [15, 16].

The ATP-dependent transporters ABCG2 and ABCB5 are the mainly studied transporters found in the LSC. The transporter ABCB5 is a transporter protein proposed as a marker of mammalian LSC [17]. Other molecules important for maintaining the function of the LSCS are C/EBPδ, Bmi-1, p63α. Their role is to maintain the cell cycle [7]. Apart from their physiological function, they may be used as a predictor of positive LSC transplantation results. Rama *et al.* found the high percentage of p63-positive cells in the LSC graft to be a predictive factor for success of LSC transplantation [18]. The corneal limbus niche cells also have their proper integrins identified, such as α9 integrin, which was found to be absent in the central corneal basal epithelial cells in a mouse model [19].

Interleukins, such as IL-6, also play a key role in the function and structure of the limbus. IL-6 was found to be present in the stem cells, as well as in the underlying stroma keratocytes. This finding suggests that IL-6 is vital for the limbal-stromal interaction. Additionally, IL-6 and STAT3 interaction has been described. The STAT3-mediated involvement of IL-6 in the maintenance of LSC keeps them in a progenitor-like state [20].

The role of epithelial structural proteins – keratins – is to provide the stability and integrity of the tissue, as well as cell differentiation and intracellular signaling. The keratins K3/K12, K5/K14, K5/K12, K8/K18, and K8/K19 are considered reliable markers for epithelial stem cells, whereas K5/K14 is also present in the basal epithelial cells, which makes them a bad candidate for a marker [16].

Antibodies against EGFR, together with the function ADAM10-dependent sheddase of EGFR ligands, are important for maintaining the homeostasis of the limbus in the normal state. In the case of injury, however, EGFR becomes active and destabilizes E-cadherin-dependent junctions in order to promote corneal epithelial wound healing [21].

A recent study by Sasamoto *et al.* discovered ten-eleven translocation (TET) dioxygenase's role in corneal epithelial cells' gene expression. Its action is exerted by downregulation of differentiation markers such as MUC4, MUC16 and keratin 12 (KRT12) There is a potential in therapy inducing TET in diseases with abnormal epithelial maturation [22].

The lack of stromal vascularization is a condition for maintaining clear media. Vascular epithelial growth factors (VEGFs) secreted by keratocytes promote not only neovascularization, but also proliferation and metaplasia of epithelial progenitor cells at the central cornea. VEGF inhibition in the pathway involving the proteins Notch1 and Hif1a maintains the clear structure of the corneal stroma and remains a possible treatment option in some disorders related to corneal neovascularization [23].

The history of limbal epithelial stem cell transplantation

Initially, autologous grafts were in use, as they do not require immunosuppressive therapy and do not pose a risk of immunological rejection. Donor tissue collection in autologous transplantation is not always possible in bilateral LSCD such as in post-chemical bilateral ocular burns with complete corneal destruction. However, multiple researchers have proven that even a tiny part of uninjured cornea

may be a source of limbal cells to be cultured on fibrin or human amniotic membrane [18, 24].

Kenyon and Tseng introduced conjunctival limbal autografting (CLAU) in which the donor lenticules consisting of conjunctiva and limbus obtained from healthy eyes are passaged to the ocular surface of the LSCD eye [25]. A possible complication of the treatment is the development of LSCD in the 'donor eye', which was reported as uncommon. More recent studies have focused on the way of transplanting the limbal cells without creating a risk for the donor eye upon tissue collection.

Direct autologous transplants require the harvesting of the arc of limbal tissue from a healthy eye. It was proven that a sufficient transplantation necessitates limbal tissue grafts acquired from more than a 90° arc, whereas grafts derived from more than 240° may result in iatrogenic LSCD [7, 26].

The cultivation techniques make it possible to depend on minimal-size limbal biopsies (from the contralateral healthy eye, healthy donors or cadaveric corneal tissue), isolation of the LSC and their culture. The common LSC harvesting and cultivating conditions include using the amniotic membrane, collagen shields, and fibrin gels. However, these xenogenic reagents increase the risk of non-human pathogen transmission [27]. Hence there is a search for potential new non-animal derived reagents. Nakatsu *et al*. managed to reduce the xenobiotic burden and proved the supportive role of mesenchymal cells in LSC culture [28].

The first report of autologous corneal epithelial stem cell cultivation was reported by Pellegrini *et al.* in 1997 [29]. Pellegrini introduced a new technique: cultivated limbal epithelial cultivation (CLET). The authors took a 1 mm^2 cell sample of the limbus of the healthy eye, plated it on irradiated 3T3-J2 cells and grafted the cultivated autologous corneal sheets on the eye surface damaged due to alkali burn of two patients. The two-year follow-up showed promising results.

Donor tissue location in the limbus

The site of obtaining the stem cells seems to be important for ensuring the good quality of the graft. The study of Ekpo *et al.* was based on five human cadaveric donor corneoscleral tissues. The culture details were described by Prabhasawat *et al.* [30, 31]. After preparation, the explant culture was washed in phosphate-buffered saline (PBS), incubated in dispase for 20 minutes at 37°C, washed in PBS again and then placed on a culture plate. The medium used was CELLnTEC-Prime (CELLnTEC, Bern, Switzerland) enriched in ROCK inhibitor (Y27632). The medium was replaced every two days. The ROCK inhibitor was needed so the explants grew at an adequate rate. The incubation time was until the explants reached 70-80% confluence: usually 7-14 days. As the result, the explant site source ('limbal middle' and 'limbal adjacent to conjunctiva') was proven to provide more successful growth than the explants from sites adjacent to the cornea. The mRNA expression coding Δ*Np63* and *ABCG2* was checked. The Δ*Np63* expression was higher in the culture from the "limbal middle" than from the "limbal conjunctival" site, which was significant. The difference in expression of *ABCG2* between the explants from different locations was not significant.

Stem cell cultivation: cultivated limbal epithelial transplantation (CLET) and simple limbal epithelial transplantation (SLET)

Limbal stem cell deficiency is the indication for stem cell grafting. Donor tissue removal has its limitations as removing a significant amount of the tissue may lead to damage of the donor eye, as mentioned above. Limbal stem cells may be cultivated and autografted from limbal epithelium of another eye (CLET). CLET, however, is only possible when the second eye of the patient has not been injured and the limbus is efficient [33]. If there is no such possibility, the source of the cells may be autologous oral mucosa (using protocols such as CAOMECS and COMET) [32, 33], which will be described below.

Simple limbal epithelial transplantation (SLET) is a newer technique described by Sangwan *et al.* in 2012 [34]. It may be used in patients with unilateral limbal stem cell deficiency and, oppositely to CLET, does not require cultivation of the cells – as in CLAU. In SLET the donor tissue is obtained from a 2 mm × 2 mm limbal area and divided into ten or more grafts smaller in size. The recipient eye surface is prepared by pannus removal and cauterization of the bleeding points. The graft is then placed under the human amniotic membrane (HAM), which is previously secured using fibrin glue.

There are more innovations proposed for the SLET technique, such as modified SLET [35], where the stem cell graft is placed between the 2 layers of amniotic membranes, the second of which is sutured to the limbal area. A possible indication for SLET in an eye with LSCD due to chemotherapy for ocular surface tumor has been reported with a good outcome [36] as well as mini-SLET implantation in the eye after pterygium removal [37].

Overview of cultivation protocols

Culture components most commonly used for stem cell cultivation are: 3T3 murine fibroblasts, human amniotic membrane (HAM), fibrin gel, and culture medium. The culture medium may be enriched with serum or not. Bovine serum may be in use, but the proposed solution nowadays is the xenobiotic-free culture systems using autologous serum. It is mostly due to the fact that animal-derived materials may cause a risk of pathogen transmission, immune reaction and graft rejection [38, 39].

Cultured autologous oral mucosal epithelial cell sheet (CAOMECS) is one of the techniques of epithelial cell culturing. It includes the use of amniotic membrane, temperature-responsive culture plates, fibrin gel, fibrin-coated culture plates, collagen IV-coated culture plates and culture plates with no substrate [33]. The donor tissue in CAOMECS is derived from the buccal mucosa (oral cavity), then it is cultured for 1-4 weeks and then, upon producing 2-12 layers, it is transplanted. Amniotic membrane is one of the most frequently used substrates in stem cell culture. HAM includes the xenogeneic components FBS and 3T3 fibroblasts. It has been proved that HAM should be previously denuded (deepithelialized) so that it contains a higher amount of growth factors [40].

Fibrin gel is used on the eye surface *in vivo* in cases of ocular surface reconstruction. In 2001 there was published the first report of a culture system using fibrin substrate in order to perform LSC transplantations from the contralateral eye in limbal stem cell deficiency following ocular burns. The grafts were successful in 14/18 patients in which re-epithelialization occurred within the first week [41]. CAOMECS has a success rate for treating LSCD of 72%.

There are reports proposing modifications to the aforementioned technique. A study by Ilmarinen *et al.* [33] reported the serum-free cultivation of epithelium cells – they used collagen IV-coated culture plates. Other studies used non-coated substrate-free culture plates. Kolli *et al.* suggested that the use of autologous serum is more effective than fetal calf serum (FCS) [42]. Substrate-free cell sheets are more challenging in transplantation in the technical aspect: they lack mechanical strength. The technique used is an air-lifting technique, which promotes migration, proliferation, epithelial stratification, and increases the barrier function of limbal epithelial cells [43].

Another protocol, cultivated oral mucosal epithelial transplantation (COMET), promotes re-epithelialization and stabilization of the corneal surface [33]. Grafts cultured with COMET reportedly remain successful for 90 months. COMET protocols usually use serum and murine 3T3 feeder cells. Serum-free media are proposed as the solution for improving COMET efficacy. In serum-free cultivation there are additional factors needed, such as BPE (bovine pituitary extract). Also serum-free, feeder-free and BPE-free systems have been developed [42].

Outcomes of LSC transplantation

Stem cell therapy has applications in several branches of medicine, including ophthalmology [6, 27]. Outcomes of different types of LSC transplantations have been compared in several clinical studies. They revealed superiority of autologous methods with higher rates of improved visual acuity and significantly fewer side effects compared to allogenic technique [45]. Le *et al.* analyzed the results of 40 LSC transplantations and concluded that there was ocular surface improvement in 85.7% vs. 84.7% of cases in autologous and allogenic methods, respectively [46]. Limbal tissues sourced from cadavers, stored at the appropriate temperature, may be used for complete corneal healing; however, the postoperative results of visual acuity improvement are temporary. Short *et al.* reported that clinical outcomes of allografts using cultured limbal cells gradually declined over 3 years after the procedure [47]. The results presented by Vazirani *et al.* indicated more than 80% autologous SLET graft survival in 12-month follow-up. The risk factors for graft rejection were identified as the presence of symblepharon and simultaneous keratoplasty [48].

The effectiveness of the direct and cultivated autologous techniques has also been compared. Although the success rates of visual improvement in both methods are similar, limbal cell cultivation requires larger samples of LSC, which increases the procedure costs [13]. The clinical trial HOLOCORE (NCT02577861) introducing autologous cultivated LSC transplantation in eyes with LSCD was conducted in European countries between 2015 and 2022. The results have not been published yet.

The first step that should be taken in LSCD treatment is optimization of the ocular surface conditions, which consists of controlling causative factors, and controlling comorbid conditions. Eyelid position assessment (e.g. in lagophthalmos), as well as lubrication agents, lacrimal punctal occlusion and autologous serum tears, should be introduced if necessary [49].

Restoration of LSC function usually is not enough to restore the transparency of the cicatricial tissue of the corneal stroma. Eyes with LSCD in some cases demand corneal transplantation to improve the vision. Two-step procedures consisting of LSC transplantation and, as another step, keratoplasty are found to have a better outcome in terms of corneal tissue allograft survival than one-step procedures [48, 49].

The most common adverse effect of LSC transplant is recurrent or persistent erosion [14]. Intraocular pressure (IOP) elevation may commonly occur after the procedures. Both complications were proved to be more frequent in allogenic than autologous LCS transplantations [14].

Conclusions

The pathway of learning the accurate treatment for corneal pathologies has led to LSC transplantations that differ greatly from the original procedures. The modern cultivation techniques, as well as the recipient tissue preparations, are relatively easy and have good outcomes. Understanding the pathophysiological processes present in the corneal limbal tissue upon injury remains the basis for the research and for development of new, even more accurate therapies.

The authors declare no conflict of interest.

References

1. Nautscher N, Bauer A, Steffl M, Amselgruber WM (2016): Comparative morphological evaluation of domestic animal cornea. Vet Ophthalmol 19: 297-304.
2. Klećkowska-Nawrot J, Goździewska-Harłajczuk K, Barszcz K (2022): Comparative histology of the cornea and palisades of Vogt in the different wild ruminants (Bovidae, Camelidae, Cervidae, Giraffidae, Tragulidae). Animals (Basel) 12: 3188.
3. Skopinski P, Radomska-Leśniewska DM, Izdebska J, et al. (2021): New perspectives of immunomodulation and neuroprotection in glaucoma. Cent Eur J Immunol 46: 105-110.
4. Notara M, Lentzsch A, Coroneo M, Cursiefen C (2018): The role of limbal epithelial stem cells in regulating corneal (lymph)angiogenic privilege and the micromilieu of the limbal niche following UV exposure. Stem Cells Int 86: 201-272.
5. Ambroziak AM, Szaflik J, Szaflik JP, et al. (2016): Immunomodulation on the ocular surface: a review. Cent Eur J Immunol 41: 195-208.
6. Dąbrowska AM, Skopiński P (2017): Stem cells in regenerative medicine – from laboratory to clinical application – the eye. Cent Eur J Immunol 42: 173-180.
7. Dziasko MA, Daniels JT (2016): Anatomical features and cell-cell interactions in the human limbal epithelial stem cell niche. Ocul Surf 14: 322-330.
8. Davanger M, Evensen A (1971): Role of the pericorneal papillary structure in renewal of corneal epithelium. Nature 229: 560-561.
9. Dua HS (1998): The conjunctiva in corneal epithelial wound healing. Br J Ophthalmol 82: 1407-1411.
10. Daniels JT, Dart JK, Tuft SJ, et al. (2001): Corneal stem cells in review. Wound RepairRegen 9: 483-494.
11. Di Iorio E, Kaye SB, Ponzin D, et al. (2011): Limbal stem cell deficiency and ocular phenotype in ectrodactyly-ectodermal dysplasia-clefting syndrome caused by p63 mutations. Ophthalmology 119: 74-83.
12. Barbaro V, Bonelli F, Ferrari S, et al. (2023): Innovative therapeutic approaches for the treatment of the ocular morbidities in patients with EEC syndrome. Cells 12: 495.
13. Masood F, Chang JH, Akbar A, et al. (2022): Therapeutic strategies for restoring perturbed corneal epithelial homeostasis in limbal stem cell deficiency: current trends and future directions. Cells 11: 3247.
14. Le Q, Chauhan T, Yung M, et al. (2020): Outcomes of limbal stem cell transplant. JAMA Ophthalmol 138: 660.
15. Chen Z, de Paiva CS, Luo L, et al. (2004): Characterization of putative stem cell phenotype in human limbal epithelia. Stem Cells 22: 355-366.
16. Ruan Y, Jiang S, Musayeva A, et al. (2021): Corneal epithelial stem cells-physiology, pathophysiology and therapeutic options. Cells 10: 2302.
17. Ksander B, Kolovou P, Wilson B, et al. (2014): ABCB5 is a limbal stem cell gene required for corneal development and repair. Nature 511: 353-357.
18. Rama P, Matuska S, Paganoni G, et al. (2010): Limbal stem-cell therapy and long-term corneal regeneration. N Engl J Med 363: 147-151.
19. Pajoohesh-Ganji A, Pal-Ghosh S, Simmens SJ, Stepp MA (2006): Integrins in slow-cycling corneal epithelial cells at the limbus in the mouse. Stem Cells 24: 1075-1086.
20. Notara M, Shortt AJ, Galatowicz G, et al. (2010): IL6 and the human limbal stem cell niche: A mediator of epithelial-stromal interaction. Stem Cell Res 5: 188-200.
21. Kaplan N, Ventrella R, Peng H, et al. (2018): EphA2/Ephrin-A1 mediate corneal epithelial cell compartmentalization via ADAM10 regulation of EGFR signaling. Invest Ophthalmol Vis Sci 59: 393-406.
22. Sasamoto Y, Wu S, Lee CAA, et al. (2023): Epigenetic regulation of corneal epithelial differentiation by TET2. Int J Mol Sci 24: 2841.
23. Biswas S, Shafiquzzaman M, Yu G, et al. (2022): Notch1 signaling in keratocytes maintains corneal transparency by suppressing VEGF expression. Stem Cell Reports 17: 1442-1457.
24. Olli S, Ahmad S, Lako M, Figueiredo F (2010): Successful clinical implementation of corneal epithelial stem cell therapy for treatment of unilateral limbal stem cell deficiency. Stem Cells 28: 597-610.
25. Kenyon KR, Tseng SC (1989): Limbal autograft transplantation for ocular surface disorders. Ophthalmology 96: 709-722.
26. Amescua G, Atallah M, Palioura S, Perez V (2016): Limbal stem cell transplantation: Current perspectives. Clin Ophthalmol 10: 593-602.
27. Woronkiewicz M, Skopiński P (2010): Clinical applications of limbal epithelial stem cells. Centr Eur J Immunol 35: 179-182.
28. Nakatsu MN, González S, Mei H, Deng SX (2014): Human limbal mesenchymal cells support the growth of human corneal epithelial stem/progenitor cells. Investig Ophthalmol Vis Sci 55: 6953-6959.
29. Pellegrini G, Traverso CE, Franzi AT, et al. (1997): Long-term restoration of damaged corneal surfaces with autologous cultivated corneal epithelium. Lancet 349: 990-993.
30. Prabhasawat P, Ekpo P, Uiprasertkul M, et al. (2012): Efficacy of cultivated corneal epithelial stem cells for ocular surface reconstruction. Clin Ophthalmol 6: 1483-1492.
31. Ekpo P, Inthasin N, Matamnan S, et al. (2020): Characterization of limbal explant sites: Optimization of stem cell outgrowth in in vitro culture. PLoS One 15: e0233075.
32. Utheim TP, Utheim A, Khan QE, Sehic A (2016): Culture of oral mucosal epithelial cells for the purpose of treating limbal stem cell deficiency. J Funct Biomater 7: 5.
33. Ilmarinen T, Laine J, Juuti-Uusitalo K, et al. (2013): Towards a defined, serum- and feeder-free culture of stratified human oral mucosal epithelium for ocular surface reconstruction. Acta Ophthalmol 91: 744-750.
34. Sangwan VS, Basu S, MacNeil S, Balasubramanian D (2012): Simple limbal epithelial transplantation (SLET): a novel surgical technique for the treatment of unilateral limbal stem cell deficiency. Br J Ophthalmol 96: 931-934.
35. Amescua G, Atallah M, Nikpoor N, et al. (2014): Modified simple limbal epithelial transplantation using cryopreserved amniotic membrane for unilateral limbal stem cell deficiency. Am J Ophthalmol 158: 469-475.
36. Polania-Baron EJ, Graue-Hernandez EO, Ramirez-Miranda A, et al. (2021): Visual rehabilitation with limbal autologous stem cells transplant and cataract surgery in a patient with ocular surface squamous neoplasia. Am J Ophthalmol Case Rep 23: 101167.
37. Hernandez-Bogantes E, Amescua G, Navas A, et al. (2015): Minor ipsilateral simple limbal epithelial transplantation (mini-SLET) for pterygium treatment. Br J Ophthalmol 99: 1598-1600.
38. Barut Selver Ö, Yağcı A, Eğrilmez S, et al. (2017): Limbal stem cell deficiency and treatment with stem cell transplantation. Turk J Ophthalmol 47: 285-291.

39. Kolli S, Ahmad S, Lako M, Figueiredo F (2010): Successful clinical implementation of corneal epithelial stem cell therapy for treatment of unilateral limbal stem cell deficiency. Stem Cells 28: 597-610.
40. Koizumi N, Fullwood NJ, Bairaktaris G, et al. (2000): Cultivation of corneal epithelial cells on intact and denuded human amniotic membrane. Invest Ophthalmol Vis Sci 41: 2506-2513.
41. Rama P, Bonini S, Lambiase A, et al. (2001): Autologous fibrin-cultured limbal stem cells permanently restore the corneal surface of patients with total limbal stem cell deficiency. Transplantation 72: 1478-1485.
42. Kolli S, Ahmad S, Mudhar HS, et al. (2014): Successful application of ex vivo expanded human autologous oral mucosal epithelium for the treatment of total bilateral limbal stem cell deficiency. Stem Cells 32: 2135-2146.
43. Dua HS, Miri A, Alomar T, et al. (2009): The role of limbal stem cells in corneal epithelial maintenance: Testing the dogma. Ophthalmology 116: 856-863.
44. Yokoo S, Yamagami S, Usui T, et al. (2008): Human corneal epithelial equivalents for ocular surface reconstruction in a complete serum-free culture system without unknown factors. Invest Ophthalmol Vis Sci 49: 2438-2443.
45. Borderie VM, Ghoubay D, Georgeon C, et al. (2019): Long-term results of cultured limbal stem cell versus limbal tissue transplantation in stage III limbal deficiency. Stem Cells Transl 8: 1230-1241.
46. Le Q, Chauhan T, Yung M, et al. (2020): Outcomes of limbal stem cell transplant. JAMA Ophthalmol 138: 660.
47. Shortt AJ, Bunce C, Levis HJ, et al. (2014): Three-year outcomes of cultured limbal epithelial allografts in aniridia and Stevens-Johnson syndrome evaluated using the Clinical Outcome Assessment in Surgical Trials assessment tool. Stem Cells Transl Med 3: 265-275.
48. Vazirani J, Ali MH, Sharma N, et al. (2016): Autologous simple limbal epithelial transplantation for unilateral limbal stem cell deficiency: multicentre results. Br J Ophthalmol 100: 1416-1420.
49. Atallah MR, Palioura S, Perez VL, Amescua G (2016): Limbal stem cell transplantation: current perspectives. Clin Ophthalmol 10: 593-602.