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Concise Review: Stem Cells for Corneal Wound Healing

MEHRNOOSH SAGHIZADEH,[a,b] ANDREI A. KRAMEROV,[a] CLIVE N. SVENDSEN,[a,b] ALEXANDER V. LJUBIMOV [a,b]

Key Words. Corneal epithelium • Keratocyte • Corneal endothelium • Wound healing • Gene therapy • Stem cell • Pluripotent stem cell • Cell transplantation

[a]Cedars-Sinai Medical Center, Regenerative Medicine Institute, Los Angeles, California, USA; [b]David Geffen School of Medicine at UCLA, Los Angeles, California, USA

Correspondence: Alexander V. Ljubimov, Ph.D., D.Sc., FARVO, Cedars-Sinai Medical Center, Regenerative Medicine Institute, 8700 Beverly Boulevard AHSP, A-8319, Los Angeles, California 90048, USA. Telephone: 310-248-8583; Fax: 310-248-8066; e-mail: Ljubimov@csmc.edu

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ABSTRACT

Corneal wound healing is a complex process that occurs in response to various injuries and commonly used refractive surgery. It is a significant clinical problem, which may lead to serious complications due to either incomplete (epithelial) or excessive (stromal) healing. Epithelial stem cells clearly play a role in this process, whereas the contribution of stromal and endothelial progenitors is less well studied. The available evidence on stem cell participation in corneal wound healing is reviewed, together with the data on the use of corneal and non-corneal stem cells to facilitate this process in diseased or postsurgical conditions. Important aspects of corneal stem cell generation from alternative cell sources, including pluripotent stem cells, for possible transplantation upon corneal injuries or in disease conditions are also presented. STEM CELLS *2017;35:2105–2114*

SIGNIFICANCE STATEMENT

This is the first review directly addressing the role of various stem cells in corneal wound healing. The significance is that, in contrast with most other reviews, it covers all major corneal cell types in a comprehensive way, showing similarities and differences in the healing process and the usage of stem cells for therapy. Potential gaps in knowledge and future directions are specifically delineated.

INTRODUCTION

As the outermost part of the eye, cornea is directly exposed to the environment and is thus prone to potential injuries due to burns, abrasions, contact lens problems, insufficient tear production, infections and other disease conditions, as well as refractive surgeries. In many cases, such injuries cause wounds triggering the healing process in the tissue. Corneal wound healing is thus not only a basic science topic but is also a significant clinical concern. Cornea has three main cell types, the stratified surface epithelium, the stromal keratocytes, and the innermost single-layered endothelial cells, which are actually neuroepithelial in nature. These cells have similarities and differences in ways and mechanisms by which they heal wounds [1]. Similarities include cell migration and proliferation, growth factor and cytokine involvement, and reorganization of the extracellular matrix (ECM). Differences are related to specific behavior of healing cells. The epithelial cells migrate as a sheet and may proliferate in the process that involves peripheral stem cells, undergoing differentiation and stratification after closure of the defect. Epithelial wounds are also accompanied by apoptosis of stromal keratocytes under the wound caused by the epithelial interleukin-1. These keratocytes are gradually replaced by live cells usually without scarring. During healing of stromal wounds caused by injury or refractive surgery, quiescent keratocytes undergo transformation to activated fibroblasts and α-smooth muscle actin-containing myofibroblasts, with participation of both resident and circulating immune cells. This process involves transforming growth factor (TGF)-β and may be deregulated, leaving a stromal scar or haze due to excessive ECM deposition and hypercellularity. The corneal endothelium largely heals through migration and spreading, with documented TGF-β driven epithelial-mesenchymal transformation, whereas cell proliferation is less important. These cell type-dependent wound healing events are summarized in Figure 1. The corneal epithelial stem cells have been convincingly shown to participate in wound healing, but

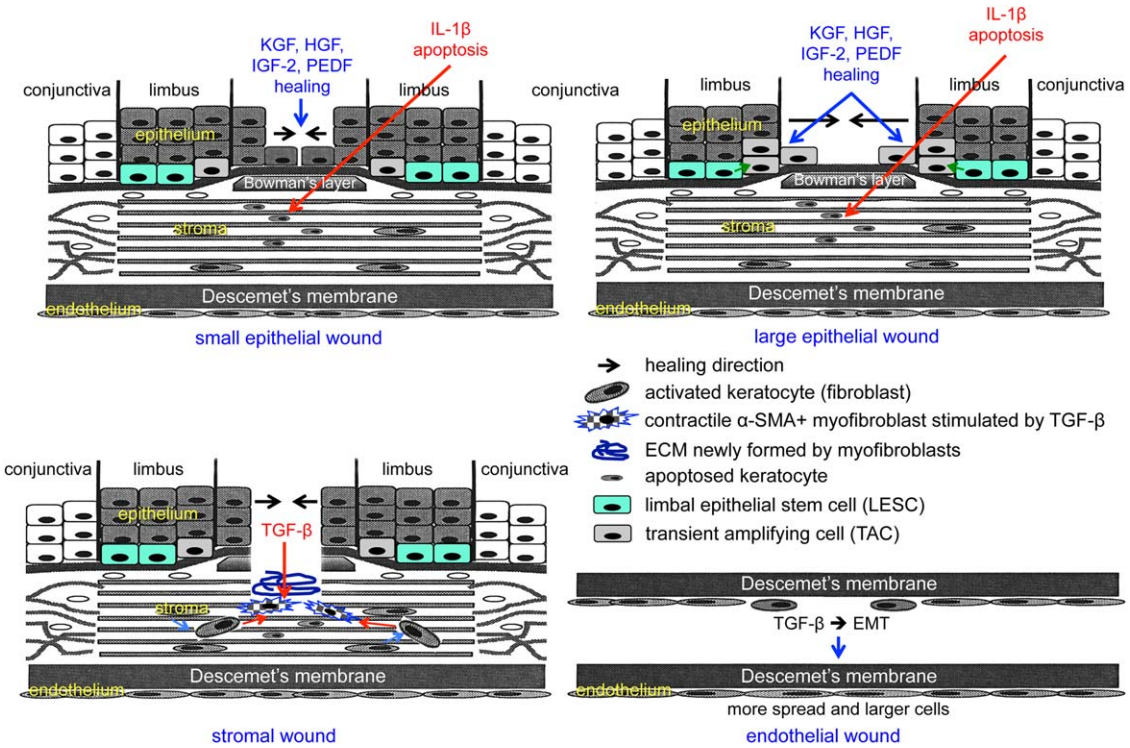

Figure 1. Schematic representation of main events during corneal epithelial, stromal, and endothelial wound healing. Top left, healing of small epithelial wound under the influence of several growth factors entails participation of central cells only. Keratocytes under the wound die by apoptosis mediated by epithelium-derived interleukin-1β. Top right, healing of large epithelial wound under the influence of several growth factors entails participation of both limbal epithelial stem cells and their progeny (transient amplifying cells), as well as of central cells. Bottom left, healing of a stromal wound entails activation of keratocytes to form fibroblasts that are transformed to motile myofibroblasts under the influence of transforming growth factor (TGF)-β. Myofibroblasts positive for α-smooth muscle actin contract the wound, and also produce and remodel the extracellular matrix in the wound bed. Burns are also associated with stromal neovascularization (not shown). Bottom right, healing of endothelial wound entails epithelial–mesenchymal transformation (EMT) and cell migration under the influence of TGF-β. Wound closure is accompanied by increased spreading and enlargement of endothelial cells that undergo the process opposite to EMT, that is, mesenchymal–epithelial transformation. Abbreviations: ECM, extracellular matrix; EMT, epithelial–mesenchymal transformation; HGF, hepatocyte growth factor; IGF-2, insulin-like growth factor-2; IL, interleukin; KGF, keratinocyte growth factor; PEDF, pigment epithelium-derived factor; TGF, transforming growth factor; α-SMA, α-smooth muscle actin.

the contribution of stromal and endothelial stem cells to this process is still debatable. In this review, we will analyze recent data on the identification of corneal stem cells, their possible roles in wound healing, and existing and future possibilities for using both autologous and allogeneic stem cell therapies.

Stem Cells for Epithelial Wound Healing

Limbal Epithelial Stem Cells in Wound Healing

Corneal epithelium comprises a single layer of basal cells and 4–6 layers of stratified squamous epithelial cells, which are continuously shed and replenished in corneal homeostasis. This cell turnover helps to maintain a uniform structure and thickness avoiding loss of corneal transparency. Corneal epithelial renewal depends on adult limbal epithelial stem cells (LESCs) located at the periphery of the corneoscleral junction, limbus (Fig. 1) [2, 3]. LESC are quiescent cells located in the basal layer of the limbal epithelium in a specific structured niche called palisades of Vogt, and/or in the deeper limbal epithelial crypts and focal stromal projections [4–6]. LESC have been localized and tentatively identified based on their colony-forming ability, proliferative potential, slow cycling nature (BrdU or EdU label-retaining cells), expression of specific antigens, and lack of terminal differentiation markers [1, 7, 8]. Loss of LESC leads to limbal stem cell deficiency (LSCD) that may be due to mechanical, chemical, and thermal injuries, genetic defects or chronic disease, leading to conjunctival ingrowth with neovascularization, corneal opacity, and vision loss [9]. LSCD is usually treated clinically by transplantation of an autologous or allogeneic limbal graft or cultured LESC [10–15].

During corneal homeostasis and wound healing, LESC proliferate and give rise to transient amplifying cells (TACs) that further divide and differentiate and migrate to the center of the cornea (Figs. 2; 3, left) to regenerate the epithelial layers [8, 12–16]. In vivo multicolor lineage tracing of keratin 14 (K14)-positive cells during closure of large wounds (Fig. 3, right) demonstrated centripetal miration of individual limbal cells as radial streaks to the center of the cornea [16–18]. Healing of small epithelial wounds may be achieved by central epithelial cells [19, 20]. After corneal injury with large wounds, limbal stem cell activation leading to healing occurs, which is mediated by environmental cues such as growth factors, cytokines, ECM, and integrin receptors [1, 17, 21–24]. Growth factor systems activating LESC upon corneal epithelial damage include among others keratinocyte growth factor in limbal fibroblasts and its receptor in the epithelial cells,

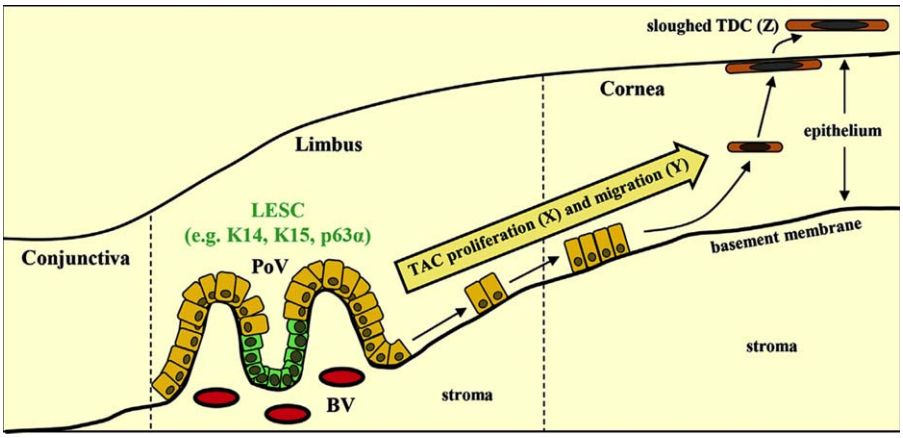

Figure 2. Corneal epithelial cell maintenance by limbal epithelial stem cell (LESC). LESC (expressing K14, K15, and p63α, and potentially other markers) residing in the basal epithelium of the palisades of Vogt, divide (X) and differentiate into transient amplifying cells while they migrate centripetally (Y), first horizontally along the basement membrane then diagonally through the epithelial tiers, before reaching the superficial epithelium in the central cornea as terminally differentiated cells that are sloughed from the ocular surface (Z). According to Thoft and Friend, X + Y = Z. Reproduced with permission from [8]. Abbreviations: BV, blood vessel; LESC, limbal epithelial stem cell; PoV, palisades of Vogt; TAC, transient amplifying cell; TDC, terminally differentiated cell.

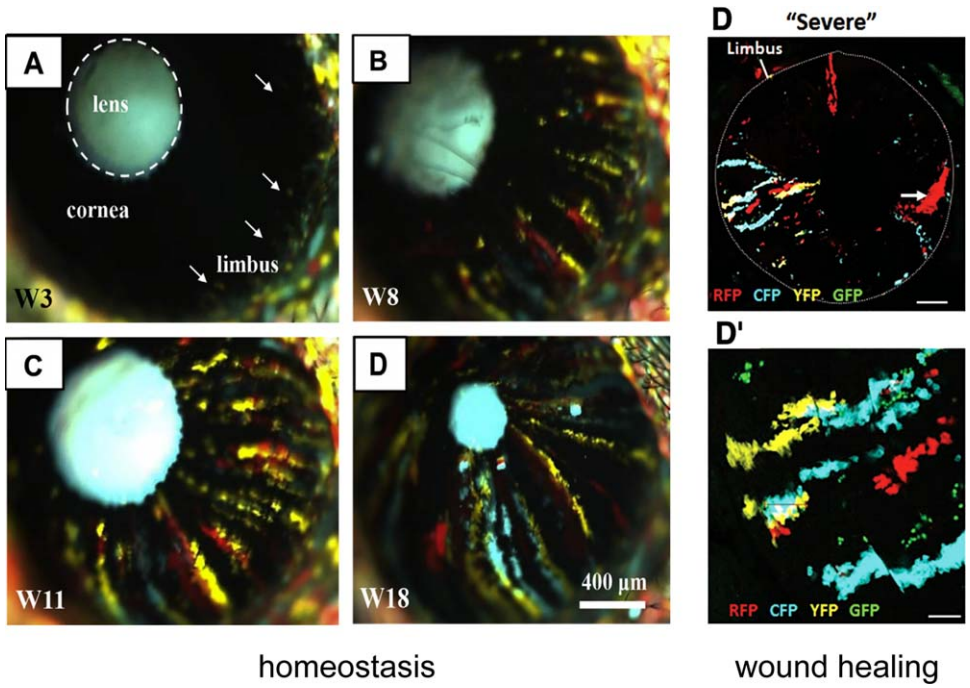

Figure 3. Clonal expansion and participation of limbal epithelial stem cell in corneal epithelial homeostasis and wound healing. Left, 6-week old transgenic mice were injected intraperetoneally with tamoxifen over 3 consecutive days. Mice were monitored long-term by intravital microscopy, as described previously. Colored patches were observed in the peripheral cornea at 3 weeks (W) post-tamoxifen (**A**, arrows), which developed into discrete stripes (**B, C**; 8 and 11 weeks post-tamoxifen, respectively) migrating toward the central cornea intersecting the apex by 18 weeks **(D)**. Notably, the intraocular lens autofluoresces and the eyelid skin recombined within 1 week of tamoxifen treatment. Scale bar = 400 μm. Reproduced with permission from [8]. Right, expansion and migration of K14+ fluorescent clones in Confetti transgenic mice during wound healing. Chemical burns to the corneal surface were achieved by topical application of dimethyl sulfoxide (DMSO) combined with tamoxifen induction. (D): Repeated DMSO application for 3 successive days caused a "severe" wound to the cornea. One week following the last DMSO treatment, multiple wide streaks of fluorescent cells were observed; (**D′**) is a magnification of (D). Limbal–corneal border is annotated by a dashed line. Scale bar = 500 μm (D); = 75 μm (D′). Reproduced with permission from [17]. Abbreviations: CFP, cyan fluorescent protein; GFP, green fluorescent protein; RFP, red fluorescent protein; YFP, yellow fluorescent protein.

insulin-like growth factor-1 and -2 and their receptors, and pigment epithelium-derived factor [23, 25–27]. A neuroprotective cytokine, ciliary neurotrophic factor, was shown to activate LESC in both normal and diabetic mouse corneal epithelial wound healing [28, 29]. A Rho-associated protein kinase (ROCK) inhibitor Y-27632 promotes limbal epithelial cell proliferation in vitro and wound healing in vivo [30]. The effects of Y-27632 may be due to the suppression of Smad2

expression [31], thus interfering with TGF-β signaling activation that delays re-epithelialization [32]. ROCK inhibitor can also block apoptosis by downregulating caspase-10 and -3 [33].

Our data show that in human diabetic corneas hepatocyte growth factor (HGF) receptor, c-met, plays a role in LESC activation and epithelial wound healing. In these corneas, HGF is upregulated, but c-met is downregulated. Corneas from long-term diabetics have dysfunctional LESC with significantly lower than normal expression of several putative stem cell markers [34]. Restoring c-met levels by gene therapy normalized delayed wound healing and significantly increased LESC marker expression in human diabetic organ-cultured corneas and primary LESC-enriched cultures [34–36]. Similar normalization was observed upon short hairpin RNA-mediated inhibition of proteinases matrix metalloproteinase-10 and cathepsin F that are upregulated in diabetic corneas [35, 36]. Corneal epithelial wound healing thus involves LESC and may be compromised in disease conditions such as diabetes. Therefore, gene and cell therapeutic approaches may help achieve faster wound closure and minimize complications.

Stem Cell Therapy

In various pathological conditions, such as hereditary disorders (e.g., aniridia or Stevens–Johnson syndrome), burns, diabetes, infections, and chronic inflammation, LESC damage or limbal niche disruption occurs leading to partial or total LSCD that seriously compromises epithelial regeneration and wound healing [1, 12, 14, 15, 35, 37, 38]. Therefore, limbal cell transplantation is now regarded as a promising therapeutic approach to restore the stem cell loss and function in LSCD of various etiologies [12, 14].

Thermal and especially chemical corneal burns represent the major clinical indication for transplanting LESC to improve compromised epithelial wound healing [12, 15, 39–41]. The most common alkaline burns cause necrosis of the corneal epithelium with partial or complete LSCD, dissolution of stromal collagen, significant inflammation that does not resolve until the epithelial defect is closed, and a later neovascularization brought about by invading conjunctiva [19]. Since the pioneering work of Barraquer [11], transplantation of limbal epithelium enriched in LESC is clinically used for corneal burns to achieve re-epithelialization, decrease inflammatory cell immigration, and suppress neovascularization. These impressive effects were very similar in a number of animal studies and clinical interventions involving hundreds of treated patients to date. The rates of clinical success are variable depending on the burn severity and are measured either as improved corneal conditions and visual acuity, or epithelialized, clear, and avascular cornea. After 1 year, complete or partial success in treating corneal burns with limbal cell transplantation was achieved in 75%–81% of adult patients; after 3 years, it was around 70% [12, 15, 37, 38, 40, 42]. The use of autologous and allogeneic LESC produced similar clinical results, although in the latter case patients received immunosuppressive drugs [12, 43].

LESC for LSCD are transplanted as autologous and allogeneic biopsies of the limbal tissue or as culture-expanded cells [10–12, 15, 38, 40, 41]. The prevailing methods used clinically differ depending on regulatory standards in a specific country. In the U.S., biopsy-based keratolimbal transplantation is still the only method allowed by the Food and Drug Administration, whereas in Europe, Japan, and especially in India, cell culture-based transplants are becoming very common.

The conjunctival limbal autografts (transplantation of a small biopsied piece of limbal tissue from a healthy eye to the region with LSCD in the fellow eye of the same patient) were first introduced for unilateral burn victims over 50 years ago by Barraquer [11] and remain the Food and Drug Administration-approved method of choice in the U.S. Allografts are also used, although they require long-term immunosuppression [10, 12, 14, 38]. For eyes with complete LESC failure, primarily due to burns, allogeneic penetrating limbokeratoplasty has been developed. The procedure consists of the transplantation of the central corneal button with LESC region by means of eccentric trephination of the donor cornea [41]. Autologous biopsy-based LESC grafting has a significant drawback related to the need of relatively large pieces of limbal tissue. For this reason, it should be used with caution because of a possibility of iatrogenic LSCD, as the LESC never regenerate at the biopsy site [15]. Bilateral LSCD calls for allogeneic tissue use requiring immunosuppression. The biopsy-based allogeneic grafts for corneal burns have a median survival of 3.4 years, requiring subsequent retransplantation [41].

Another way of managing LSCD, introduced in 1997 by Pellegrini's group, is the transplantation of LESC-enriched cell cultures expanded in vitro [10, 37, 38]. This procedure has now become a treatment of choice for LSCD in many countries [12, 15, 38, 42, 44, 45]. Recently, the first stem cell-based treatment Holoclar was granted license in the European Union for the management of moderate to severe LSCD in adults. The treatment consists of transplanting culture-expanded autologous limbal epithelial cells on a fibrin support [46]. Limbal cultures for clinical use are composed of stem cells and their progeny (TACs). Clonogenic assays in these cultures suggest a minor proportion of LESC, but they still can replace the damaged limbal epithelium and repopulate the corneal surface [37]. A comparison of transplantation results of cultured LESC versus conjunctival-limbal autografts for severe unilateral burns showed very similar success rates at 6 months post surgery. However, the amount of tissue required for cultured LESC expansion is minimal, making this procedure preferable [47]. The similarity of success rates upon transplantation of limbal grafts or cultured LESC was confirmed in other studies [12, 13]. Cultured LESC may be used autologously in "simple limbal epithelial transplantation" when small biopsies of limbal tissue are expanded, and the resultant cultures are used to treat burns to ensure epithelial wound healing [15, 42, 45, 47]. Allogeneic LESC cultures have also been used successfully. Interestingly, cryopreserved and expanded LESC cultures lose immunogenicity by downregulating major histocompatibility complex (MHC) protein expression, making immune rejection less of a problem [48]. Historically, LESC were expanded for transplantation using fetal bovine serum in the medium and mouse 3T3 cells as a feeder layer [10]. More recently, xenobiotic-free cultures were developed using a feeder-free system, and either serum-free or human serum-supplemented media to comply with rigorous regulatory requirements [49–52].

For easier handling of cells during transplantation, LESC are cultured on various biological supports. The cells on a

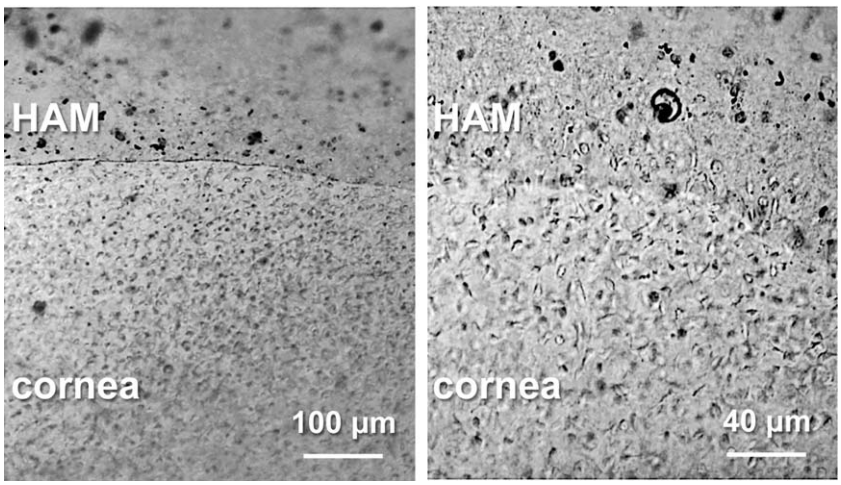

Figure 4. Re-epithelialization of the corneal surface by human amniotic membrane (HAM)-grown limbal epithelial stem cell-enriched limbal cultures. Left, low magnification showing HAM with attached limbal cells placed on top of human organ cultured cornea that has been de-epithelialized by mild NaOH treatment. HAM is secured to the corneal surface by derma + flex gel adhesive (formulated medical cyanoacrylate from Chemence Medical, Alpharetta, GA). Scale bar = 100 μm. Right, high magnification showing limbal cells that have migrated from HAM and repopulated the corneal surface. Scale bar = 40 μm. Abbreviation: HAM, human amniotic membrane.

support are sutured or glued to the limbal area of a damaged eye, and the cells migrate over time to the denuded area to heal the wound (Fig. 4; [15]). Some LESC supports are regularly used in the clinic, such as human amniotic membrane (HAM) or fibrin gel [15, 46]. A number of other supports are in preclinical development and may even offer better standardization and reproducibility than the clinically used ones [53]. Most often, cells are cultured on denuded HAM [12, 15, 42, 54], which is essentially a basement membrane with a composition similar to the limbal epithelial basement membrane [55]. For this reason, it may also provide the cells with the correct ECM that is an important part of the limbal stem cell niche [39, 56]. In addition to providing good support for cells, HAM supplies some growth factors and is non-immunogenic and anti-inflammatory. However, HAM needs to be thoroughly screened for infections and communicative diseases, and properly stored in sterile conditions [57]. Fibrin is used as LESC support in the Holoclar system approved for clinical use in Europe. Cells usually grow well on fibrin, but it may need to be stabilized against degradation [58]. It may also reduce cell migration, which should be taken into account when using it for LESC transplantation to heal corneal burn wounds [59]. It should be noted that growing cells on thermosensitive plates, from which they detach as sheets at room temperature, allows for transplanting them without a support/carrier and sutures. This was tried clinically on a small number of patients with LSCD using an alternative cell source from oral mucosa. Upon transplantation, all four patients retained clear corneas for the 14-month follow-up period [60].

A controversy recently emerged concerning the survival of transplanted LESC. In some studies, DNA analysis did not find transplanted allogeneic cells (either as cultures or as limbal grafts) after 3–9 months. At the same time, the corneal surface remained stable [38, 61]. In other clinical studies, however, grafts survived for 3–8 years before being rejected, attesting to the ability of transplanted cells to exist for a long time [15, 62]. It is unclear what cells assumed the limbal function in cases where donor cells disappeared early, and whether this may also happen with autologous transplants. In any event, if the LESC grafts eventually fail, repeat transplants can be made successfully, as a study in burn patients has shown [63].

Drawbacks of transplanting LESC-enriched cultures include a limit of cell passage number, increased risk of allograft rejection and disease transmission, and potential gene and/or cell contamination from mouse 3T3 cell feeder [64]. Many countries also face a shortage of donor corneas limiting allogeneic LESC transplantation in bilateral LSCD. For these reasons, alternative cell sources able to differentiate into the corneal epithelium have been tested for restoration of the corneal surface and wound healing. These include cultured oral mucosal epithelium, hair follicle, conjunctival and epidermal epithelium, amniotic epithelial cells, umbilical cord lining epithelial cells, as well as mesenchymal stem cells (MSCs) from adipose tissue, bone marrow, orbital fat, and immature dental pulp [13, 14, 22, 65–67]. Cultured autologous oral mucosal epithelium has been used clinically; again, most frequently, for chemical burns, although with somewhat lower success rates than with LESC transplantation (close to 70%) [13, 14, 65, 66]. Most other cell types have only been examined in preclinical models [22, 67].

Embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC) that are renewable, easily expandable and bankable are emerging as an attractive source for stem cell-based therapy in diseased or severely wounded cornea [68]. They can be directed to limbal differentiation with some degree of success, but this process is not yet fully optimized [68–71]. The use of combined soluble factors for specific time periods and proper extracellular support appears to be critical for achieving reliable limbal epithelial differentiation. Before the introduction of these cell sources into clinical practice certain critical issues need to be resolved, such as the risk of mutagenesis and tumorigenesis, high cost of the process, and reproducibility of differentiation in different ESC or iPSC clones [39].

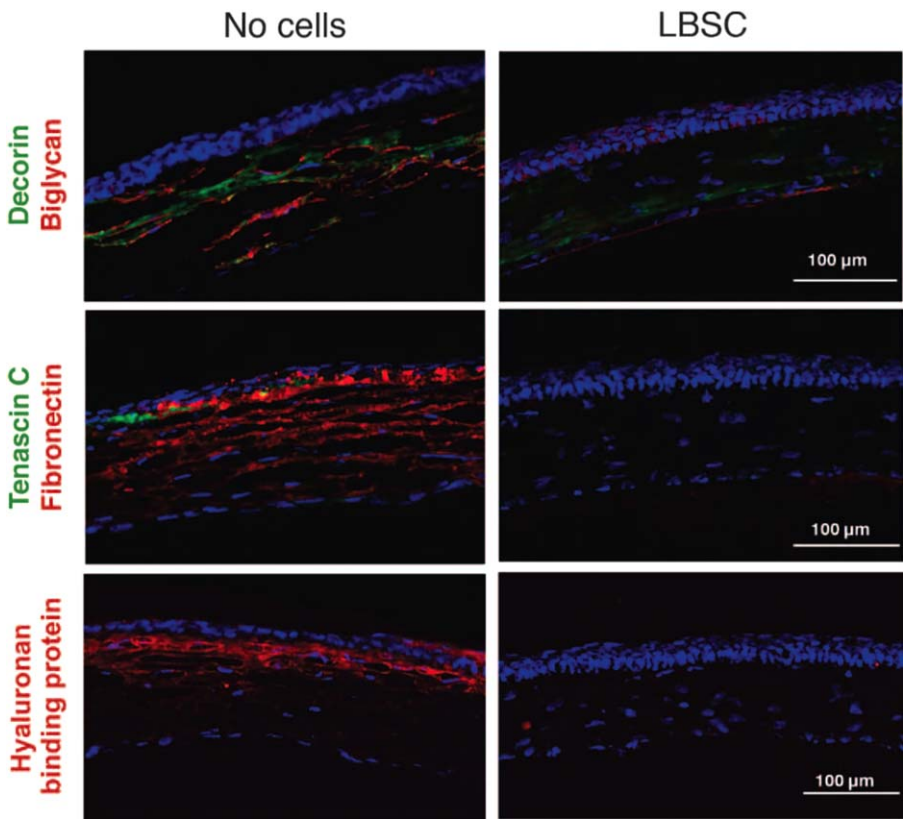

Figure 5. Debridement-wounded mouse corneas were treated with fibrin gel only (no cells) or with 50,000 limbal biopsy-derived stromal cells in fibrin gel. After 4 weeks of healing, histological sections (epithelium oriented up) were stained for fibrotic markers decorin, biglycan, tenascin C, fibronectin, and hyaluronan binding protein. Images are representative of sections from three corneas for each condition. Note lack of fibrotic proteins from the stem cell-treated corneas that are now similar to the untreated ones. Reproduced with permission from [76]. Abbreviation: LBSC, limbal biopsy-derived stromal cell.

In summary, the therapeutic potential of LESC biopsies or cultures is evident from ample clinical data on transplantation and wound healing, particularly in cases of severe LSCD caused by burns. Optimization of LESC cultures, and/or standardization of directed limbal differentiation of iPSC are important for the expansion of autologous stem cell-based therapy in corneal diseases associated with LESC damage. The use of various MSCs may also be advantageous due to autologous nature and ability to repair corneas after burns. However, potential problems related to cell support standardization and graft longevity need to be explored further.

Stem Cells for Stromal Wound Healing

Human limbal stroma contains cells that express Pax6 and ABCG2 progenitor markers and can be induced by fibroblast growth factor-2 to differentiate into corneal keratocytes in vitro. These cells also express markers of MSCs and can differentiate into non-keratocyte lineages in special culture media [72]. Hopkinson's group has found that such stromal stem cells from human limbus were multipotent and fulfilled the criteria for MSC developed by the International Society of Cellular Therapy [73]. Very similar MSCs have been recently isolated from human central corneal stroma as well [74]. Additionally, monocytic progenitor cells expressing CD133 marker and capable of keratocytic differentiation could be isolated as a side population from the stroma of donor human corneas and successfully differentiated into lumican-expressing keratocytes [75]. Corneal stromal stem cells have been already used for stromal engineering in vitro [72]. Importantly, injection of isolated human stromal stem cells restored stromal transparency in a lumican-null mouse model with corneal opacity [72].

In animal corneal wound models, the injection of human stromal stem cells from limbal biopsies prevented fibrotic scar formation (Fig. 5) and contributed to the normal regeneration of stromal ECM with the structure similar to uninjured corneas [76]. These limbal biopsy-derived stromal cells inhibited neutrophil migration into the wounded stroma, suppressing fibrotic tissue deposition [77]. Such cells also contributed to increased strength of adhesion of flaps created in the laser-assisted in situ keratomileusis procedure in organ-cultured corneas, which may be clinically important for flap integrity [78]. Additional to these endogenous cells, various MSCs in vivo significantly reduced stromal neovascularization in alkaline burn models and diminished corneal opacity and inflammation upon penetrating injury [79, 80].

In summary, corneal stromal stem cells and other MSCs appear to be a valuable tool for the treatment of burns and reduction of stromal fibrosis and opacity due to penetrating wounds in animal studies. These promising data should form the basis of the future clinical trials. The availability of iPSC-derived keratocytes [81] may eventually provide alternative stable sources for future stromal cell transplantation.

Stem Cells for Endothelial Repair and Wound Healing

Corneal endothelial cells (CEC) appear to close the wound mainly by migration and enhanced spreading, whereas cell proliferation plays a secondary role. Human CEC can hardly be expanded either in vivo or in vitro [82]. The existence of human corneal endothelial stem cells has not yet been conclusively established. Earlier studies used neurosphere-forming cell isolation and reported increased numbers of spheres from the endothelial periphery [83]. Expanded cells could be incorporated into the endothelial layer in a model of CEC deficiency [83]. Slow cycling endothelial cells and small cells with high nuclear-cytoplasmic ratio were also observed at the corneal periphery. They expressed stem cell markers Oct3/4, Wnt-1, Pax6, and Sox2 and had elevated telomerase activity. As such, they were postulated to be the endothelial progenitors capable of proliferation and expansion during wound healing [84, 85].

Recent studies addressed the possible application of other stem cells to enhance endothelial repair and wound healing. MSCs from umbilical cord blood were shown to engraft into wounded cultured endothelial sheets in vitro and acquire an endothelial phenotype [86]. As an alternative source for CEC transplantation, such cells can be obtained by in vitro differentiation of human pluripotent stem cells. CECs are derived from neural crest stem cells (NCSC), which may allow using NCSC to generate autologous CEC substitutes. The differentiation of rodent NCSC into functional CEC that covered the Descemet's membrane after transplantation was reported in the rat model of corneal endothelial deficiency [87]. Similar to the epithelial cells, ROCK inhibitor also enhanced CEC proliferation and migration in the in vitro and in vivo models of corneal endothelial wound healing [88], suggesting its potential therapeutic use. CEC were also generated from ESC and iPSC by a two-stage protocol [89] or directly by suppressing TGF-β and ROCK signaling [90]. Functional CEC-like cells derived from ESC restored transparency when transplanted into the eyes of rabbits with CEC dysfunction [91]. Such cells had high expression of CEC markers *AQP1*, Na^+-K^+-ATPase, type VIII collagen, and ZO-1, and a gene profile similar to CEC [92, 93].

In summary, a significant progress has been achieved in producing CEC from human ESC/iPSC-derived NCSC in an in vitro two-step process. This approach offers promise for the development of endothelial replacements suitable to treat corneal edema due to pathological CEC dysfunction (e.g., in Fuchs' endothelial corneal dystrophy) and wound-related complications of corneal surgeries.

Conclusion

Delayed, incomplete, or excessive corneal wound healing remains a significant clinical concern calling for the development of new efficient therapeutics. It is advantageous to accelerate epithelial wound healing, especially in conditions when it is slow or incomplete, such as in diabetes. During stromal healing, the treatment should counteract excessive tissue remodeling, which may lead to scarring and haze. During endothelial healing, a problem of low cell proliferative potential and possible complications from epithelial–mesenchymal transformation should be circumvented. In recent years, stem cell transplantation has emerged as an effective tool that may be able to fine tune wound healing and improve it for the patient's benefit. Clinical success in normal healing restoration has been achieved mainly for epithelial cells with the advent of cultured LESC transplantation for LSCD caused by various burns. This technique has proven to be as efficient as the previously introduced biopsy-based keratolimbal transplantation. At the same time, transplanted limbal cells need be further studied in regard to the factors that influence LESC-mediated healing, its molecular mechanisms, the duration of the transplant effect, and the longevity of the grafted cells [15, 38]. Organ-cultured human corneas could be used for this purpose, as animal data may not be fully relevant to the human conditions. There are some promising data using animal models on the reduction of scarring and haze by limbal stromal stem cells, but these results need to be confirmed with large animal models before their translation into clinical practice, in accordance with regulatory requirements. For the endothelial cells, there is clear need for more data on the identification of resident stem cells and their transplantability. In all three areas, alternative sources of stem cells for possible transplantation have been identified including various epithelial progenitors and MSCs. These important studies need to be expanded to streamline and standardize protocols for autologous non-corneal cell transplantations, especially in cases when the patient's respective corneal cells are not available.

This decade is seeing an exciting surge of studies using differentiation of ESC and iPSC into corneal cells, and some promising candidates may soon enter clinical trials. The resultant corneal epithelial cells may be closer to clinical use, whereas stromal and endothelial cells need to be generated from pluripotent stem cells with better reliability. ESC- or iPSC-derived differentiated cells offer the advantages of an autologous source, easy expansion and banking, but the safety issues including mutagenicity and tumorigenicity must be carefully addressed before clinical translation [39]. Another promising approach to consider and develop is the genetic manipulation of cultured stem cells in disease conditions, such as diabetes, where stem cell dysfunction may be reverted by a specific gain-of-function and/or loss-of-function gene therapy [36]. In the near future, viral gene therapy of stem cells may be complemented by nano vehicle-driven therapeutics. Finally, new gene editing techniques, such as CRISPR/Cas9, could also be applied to diseased/dysfunctional corneal stem cells to precisely and stably regulate gene expression. Such techniques may also be considered for targeting MHC proteins to decrease cell immunogenicity [94] to expand and facilitate the usage of allogeneic stem cell transplantation.

In conclusion, the available data show the importance of stem cells in corneal epithelial, stromal, and endothelial wound healing in disease, injury, or postsurgical conditions. Corneal epithelial stem cells transplantation has been successfully used in clinic to ensure healing upon serious injuries including burns, and preclinical data suggest similar benefits of stromal and endothelial stem cells. The field has expanded to include various non-corneal sources where the patient's corneal cells are not available, with ESC- and iPSC-derived limbal cells showing promise for future transplantation upon corneal injuries. The advances in transplant techniques and the range of available cell sources that can be used to optimize

the treatment of aberrant corneal wound healing can give reassurance to patients with corneal injuries that preserving vision may be possible in the near future.

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Author Contributions

M.S. and A.A.K.: manuscript writing, editing, and final approval; C.N.S.: manuscript editing, and final approval, financial support, and administrative support; A.V.L.: conception, manuscript writing, editing, and final approval.

Disclosure of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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