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The Limbal Niche and Its Role in Maintaining Corneal Regeneration

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Abstract

In recent years, stem cells have been a focal point in research designed to evaluate the efficacy of ophthalmologic therapies, specifically those for corneal conditions. The corneal epithelium is one of the few regions of the body that maintains itself using a residual stem cell population within the adjacent limbus. Stem cell movement has additionally captivated the minds of researchers due to its potential application in different body regions. The cornea is a viable model for varying methods to track stem cell migratory patterns, such as lineage tracing and live imaging from the limbus. These developments have the potential to pave the way for future therapies designed to ensure the continuous regeneration of the corneal epithelium following injury via the limbal stem cell niche. This literature review aims to analyze the various methods of imaging used to understand the limbal stem cell niche and possible future directions that might be useful to consider for the better treatment and prevention of disorders of the cornea and corneal epithelium.

Keywords

Cornea, Limbus, Molecular Biology, Stem Cells, Lineage Tracing, Live Imaging

1. Introduction

The cornea, a transparent dome-shaped tissue positioned at the front of the eye, plays a pivotal role in vision and eye protection [1] [2]. Structurally, the cornea has several layers, including the epithelium, Bowman's layer, the stroma, Descemet's membrane, and the endothelium (**Figure 1**) [3] [4]. The cornea is also an avascular tissue, meaning it lacks the presence of blood vessels. This characteristic allows for the successful movement of light to the retina, thus enabling clear vision. Additionally, a layer of tear film, a lubricant that plays a role in

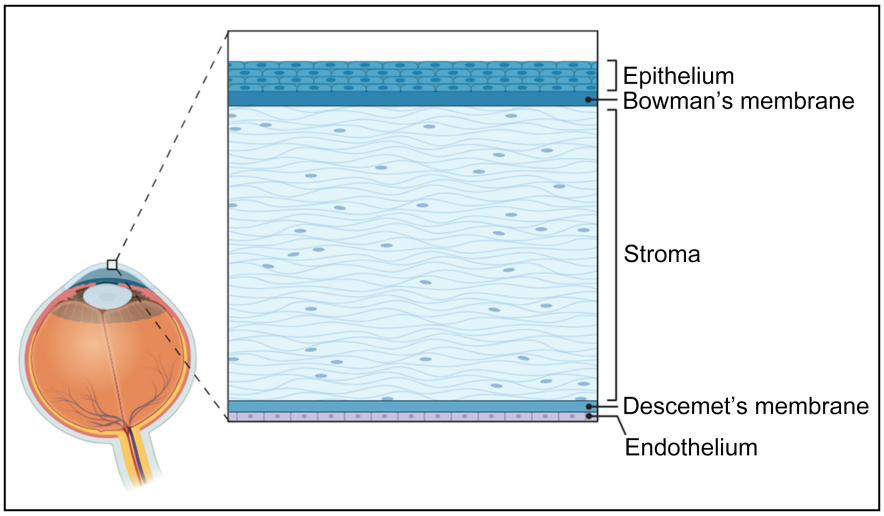

Figure 1. Schematic of the cornea. The layer identified as the pre-descemet's membrane, or Dua's layer, is not included. Only the corneal epithelium is fully known to undergo complete regeneration and self-maintenance (Created with BioRender.com).

maintaining a smooth surface needed for light refraction and ensuring the cornea and conjunctiva's health, coats the cornea [1] [5]. The cornea is, therefore, responsible for around 2/3 of the refractive capabilities of the eye. In terms of composition, corneal tissue is composed of both cellular and acellular elements. Its cellular components include epithelial cells, endothelial cells, and keratocytes-dormant cells that play a role in post-injury repair [1] [6]. Additionally, the cornea contains acellular components like collagen and glycosaminoglycans, which are crucial for cell proliferation [1].

The corneal epithelium stretches across 5 - 7 layers of cells in terms of overall thickness, making it relatively uniform. It participates in a symbiotic relationship with the tear film layer above it that maintains comfort and wound healing following injury (**Figure 1**). As the first layer of the cornea, the epithelium protects the lower layers of the cornea from the environment and infectious agents while maintaining corneal transparency [1] [5] Corneal epithelial cells typically live for 7 - 10 days before being shed through the tear film due to the rapid turnover rate. The corneal epithelium is formed from three kinds of cells from most anterior to least: superficial, wing, and basal. The second layer of the corneal epithelium, comprised of wing cells, restricts the movement of white blood cells through the cornea, contributing to its isolated immune environment [7].

The stroma comprises the main body of the different layers in the cornea, comprising anywhere between 80% - 85% of its depth (**Figure 1**). Due to stromal fibers consisting of different types of collagen, the stroma is transparent. Stromal transparency is necessary, particularly when considering the percentage of the actual cornea it constitutes. The primary cell types inhabiting the stromal layer are keratocytes, which play a prominent role in maintaining the stroma's extracellular matrix. These cells can create collagen and glycosaminoglycans, contributing to the stroma's role of maintaining corneal strength and structural capa-

bilities. Its functioning heavily relies on the strict assembly of collagen into fibrils, parallel bundles of fiber [1] [8].

Finally, the corneal endothelium comprises metabolically active cells and pumps regulating water content. This portion of the cornea primarily regulates water flow from the stromal layer to a region known as the aqueous humor, a fluid located anterior to the lens [9] [10]. In addition, the endothelium's density decreases as age increases, and the tissue cannot regenerate in adults [1].

Stem cells reserve the ability to differentiate into various specialized tissues and self-renew [11]. Researching their modes of collective repair, replication, and signaling pathways is vital. Therapeutically, stem cells might be used for organ replacement or regrowth, cancer research, studies on the causes of genetic defects, and measuring the safety or efficiency of different drugs [12].

The cornea is an ideal model to observe stem cell movement and replication following injury and repair. Due to its transparency, the corneal model benefits from live-imaging processes that directly monitor the movement of stem cell populations. In addition, the cornea is a highly accessible form of tissue, as it is relatively simple to remove and dissect [13]. Monitoring the movements of stem cell populations vertically and horizontally transforms into a visually straightforward task within the cornea's relatively simple, organized structure. Lastly, it is widely accepted in the scientific community that the cornea acts as an isolated environment, anatomically and functionally distinct from the surrounding conjunctiva [14] [15]. The cornea's immune response system is separate from other parts of the eye and body, making it an ideal model for studying stem cell interactions without concerns about host compatibility or immune cell interference.

Overall, further experimentation on both the cornea and the capabilities of its self-repair is vital for developing new procedures designed to reduce invasive surgeries to combat different corneal diseases. Current findings report that over half of the world's population lacks access to corneal donors and that for every cornea available for transplant, around 70 are in a complete deficit [16]. An alternative to corneal transplants could involve generating new corneas from stem cell populations, potentially reducing the demand for donor corneas. Similarly, avenues of personalized self-maintenance could be explored through continued research on the role of stem cells in corneal maintenance, as this solution would remove the danger of non-compatibility between donors and recipients undergoing transplant surgeries. Using stem cells from a patient's own body could minimize the risk of compatibility issues.

This literature review will focus on initially laying out the existing anatomical corneal model by presenting its various functions in terms of positioning relative to other eye regions and the different layers that compose it. Chemical compositions of layers will be included, as well as functions that are pertinent to the topic of observing stem cell movement. Next, stem cells will be defined and explained in the cornea's context, leading to their ultimate role within the limbal niche. A

description of live imaging and the uses of a corneal-based model to observe stem cell movement will be provided to characterize usability and the importance of possible stem cell contributions to the cornea. The rest of the literature will detail how studies referenced within the field of the corneal limbal niche captured stem cell movement and their contributions to self-maintenance and post-injury repair. To accomplish this, an individual section will be dedicated to the limbal niche, encompassing its physical and chemical factors and common signaling pathways activated in the proliferation and movement of stem cells. Lastly, current therapeutic methods derived via discussed experiments will be showcased, along with possible future implications of such methods to combat conditions of the cornea and limbal niche more effectively.

2. The Limbal Niche

Although multiple interpretations exist regarding how the cornea maintains its structure and overall health, recent developments in live imaging and new studies have shed more light on the actual specifics of this question. Specifically, the debate has gone on regarding where stem cells required to maintain the cornea are housed and their regenerative capacity. The limbus is a cornea region recently found to house populations of slow-cycling stem cells capable of significant corneal maintenance [17] [18]. DiGirolamo *et al.* revealed that stem cell populations, known as limbal epithelial stem cells (LESCs), are largely absent in the central cornea and rather exist in the limbus, a vascularized region of the eye lying on the periphery of both the cornea and conjunctiva [19] [20] (**Figure 2**). The limbus can be characterized as a boundary line between the cornea and conjunctiva, and plays a role in corneal regeneration. Keratin 14 (K14) is a biochemical marker commonly expressed in epidermal tissues, and many experiments have been utilized to test for the presence of stem cells [15] [19] [21]. Taking samples of stem cells from tissue samples of both the limbus and central cornea and immunostaining allowed for the detection of K14. Immunostaining tissue sections derived from the corneal epithelium revealed K14 expression firmly centered near the limbal zone instead of the central cornea. The process of

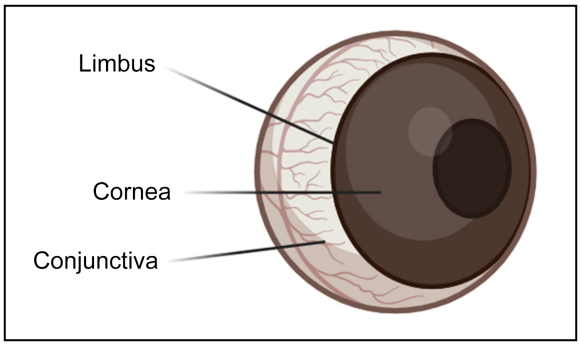

Figure 2. The limbus is located directly on the outer edge of the cornea. Specifically, it is a transition-region that is formed from the thinning of the conjunctival membrane whilst combining with the cornea (Created with BioRender.com).

corneal repair requires the migration of stem cells housed in the limbus across the surface of the corneal epithelium. This large distance contributes, in part, to the potential inability to harness stem cells as viable treatment options for corneal conditions. Further research might evaluate how different growth factors or molecules might affect these migration rates.

Initially, lineage tracing of stem cells and their progeny was a viable tool to track their direction and migration patterns. Amitai-Lange *et al.* demonstrated that stem cell populations collectively moved centripetally towards the center of the cornea, where self-maintenance was needed, using an inducible K14CreERT2; R26-Confetti mouse model. Through experimentation on mice cornea, stem cell populations collectively moved centripetally towards the center of the cornea, where self-maintenance was needed. Despite this, it is still unknown how migration rates might vary depending on the region of the corneal injury. For example, whether a central corneal injury necessitates more chemical self-repair from the limbal stem cell populations than a peripheral injury has yet to be answered, leaving significant room for further research.

In a separate experiment, Amitai-Lange *et al.* showed the presence of limbal streaks using a sample flattened cornea model [15]. Using a K14CreERT2 reporter, model corneas were observed following Tamoxifen induction after ten days and four months [15]. Results showed a cumulative movement of stem cell progeny towards the center of the cornea in the form of streaks. The ten-day observations reported small fragments of K14 expression compared to the four-month trial, which depicted clear banding patterns of stem cell movement. These findings ultimately reported that limbal stem cells move centripetally to contribute to the self-maintenance of the cornea. A recovery period of four months resulted in greater amounts of stem cell movement than the ten-day group. In the case of human applications to stem cell migratory rates, however, further research still needs to be done regarding the optimal amount of time given to stem cell therapeutics. Optimizing this time in ongoing clinical trials would help generate novel stem cell therapies for corneal injury.

Similarly, Di Girolamo *et al.* used lineage tracing to demonstrate the centripetal movement of stem cells from the limbus to the central cornea. Di Girolamo *et al.* demonstrated cells expressing K14 in the limbal epithelium as opposed to the corneal epithelium. Lineage tracing of the limbal stem cells also resulted in clear streaks leading towards the central cornea over time, along with their direct progeny. This revealed the centripetal motion of limbal stem cells, indicating their role in supplementing corneal repair procedures. In later experiments, the administration of Tamoxifen and monitoring of CFP, YFP, and RFP through K14 revealed a direct movement of limbal stem cells towards the cornea on a horizontal plane through observations made on fluorescence patterns. As time post-tamoxifen induction increased, streaks representing stem cell movement as displayed by fluorescence became increasingly prominent. Similar observations were made following the confocal imaging of a K14CreERT2-Confetti mouse

cornea, in which the same lineage tracing method tracked the progression of stem cells and their progeny. Lineage tracing, however, does not allow for the tracking of individual cells. Cell replication of traversing stem cells makes it impossible to differentiate between originally tagged cells and newly produced progeny, highlighting a key drawback of the lineage tracing method of stem cell observation. While lineage tracing provides important information about the general direction of stem cell movement, it may not provide a complete understanding of the dynamics of individual cell behavior within the corneal epithelium. Lastly, distinct confocal microscopy was performed using a K14CreERT2-Confetti model, revealing stem cells' presence in the limbus and their centripetal movement toward the central cornea [19].

Although these methods of observing the movement of stem cell populations were initially successful, they suffered from some drawbacks. Throughout lineage tracing, progenitor cells fluoresce to indicate how the entire ancestry of initially marked cells migrated. This drawback makes it impossible to tell which cells were originally marked and track those individual progressions. Likewise, identifying exactly when K14 expression occurred following Tamoxifen induction becomes difficult.

In recent years, experiments utilizing photoactivatable GFP reporters combined with live imaging have come to light, allowing the monitoring of individual cells through the same mouse cornea. For example, a photoactivatable H2B-GFP reporter can track the mitotic patterns of corneal stem cells. Briefly, this fluorescent histone protein entangles itself in the histones of chromosomes within the nuclei of cells, meaning that mitotic divisions resulting in the split of nuclei result in a division of H2B-GFP concentrations. These concentrations can be observed through photoactivation, with brighter cells indicating those cells maintained their nucleic concentrations by not dividing frequently. Farrelly and colleagues demonstrated the live imaging procedure by employing the R26 promoter to control the expression of photoactivatable H2B-GFP. In this study, Doxycycline inhibited the activation of H2B-GFP after the administration of Tamoxifen. This effectively reduced the continuous fluorescence of cells, enabling precise monitoring of individual stem cell movements. Since progenitor cells are located in the inner limbus, their movement was tracked based on fluorescence changes by repositioning the light source from the limbus to the central cornea. In their latter experiments, Farrelly *et al.* again showed the distinction between the inner limbal and outer limbal migratory patterns, specifically in that cells of the inner limbus selectively migrated towards the center of the cornea. In contrast, those of the outer limbus did not. The true reason for this strange occurrence is not understood, indicating a need to learn more about the biochemical mechanisms by which cells are signaled to enter migration. Selective Harmonic Generation (SHG) is a process in which collagen fibers reflect light without adding fluorophores. Farrelly *et al.* reveal the collagen formation and how a significant portion of collagen exists in the outer limbus, indicating it may have

some sort of role in affecting migratory signaling in stem cells.

The ability to trace the fate of individual stem cells has also come about through live imaging methods. Farrelly *et al.* demonstrated this concept by monitoring the directions of cell divisions an individual cell underwent to move towards the ocular surface. Since the cell moved towards the ocular surface, it underwent differentiation across all layers of the imaged epithelium.

Regarding the actual characteristics of LESCs, various experiments have been pursued to learn more about their proliferative capacities. Amitai Lange *et al.* displayed stem cell quiescence through lineage tracing. The stagnant population of labeled and fluorescing cells represents stem cells in a quiescent, dormant state. It is later displayed that stem cells within the limbus are additionally very long-lived and dormant in their replicative abilities. Farrelly *et al.* similarly showcased the slow-cycling capabilities of stem cells residing in the limbal niche through a more modern method of measuring photoactivatable GFP concentrations within the nuclei of cells. In essence, this experiment used the ability of the GFP protein to entangle itself within histone proteins of the nucleus, allowing for the monitoring of fluorescence levels following the administration of Doxycycline. It is expected that each time cells divide mitotically, their GFP concentrations lessen and lessen by intervals of 2, meaning they get dimmer. This phenomenon allows the general tracking of stem cell life spans in varying eye regions to determine if LESCs are more long-lived than their corneal and conjunctival counterparts. Observing fluorescence levels showed that LESCs divided significantly less than the cornea and limbus stem cells. Although cells of each region of the eye initially exhibited significant amounts of fluorescence, only those of the limbus had cells that did not divide often enough to lose the bulk of their GFP concentrations by day 50 following initial Doxycycline administration. Farrelly *et al.* later add to the previously mentioned concept of cellular quiescence within the limbal niche through photos taken via live imaging. Said images reveal stem cell populations that have not moved nor divided, meaning they have retained initial GFP concentrations, thus labeling them as quiescent or dormant. Additionally, dividing cells are highlighted to show that not all limbal stem cell niches are dormant. Even further images reiterate the general concept that limbal cells divide far less than those of the cornea and conjunctiva, resulting in increased fluorescence levels when imaged. The strategy of GFP monitoring proved helpful and revealed a long-lived and slow-cycling stem cell population within the limbus, but changes likely occur during migration into the cornea. These dormant populations have yet to be evaluated in studies regarding their differentiative potential. The application of growth hormones or unique factors might prove useful in triggering these cells to develop out of their dormant states. In addition, the best ways to trigger these stem cells to grow and differentiate in response to corneal injury are currently unknown, and their status following injury has not been effectively determined. Similarly, the studies do not extensively investigate the behavior of LESCs in response to injury or disease conditions, which are crucial for

understanding their regenerative potential and therapeutic applications. The potential changes in stem cell behavior and characteristics during migration from the limbus into the cornea could significantly impact their regenerative capacity and differentiation potential.

In addition to regenerating the cornea, the limbal niche is known to replenish its reserve of stem cells. Di Girolamo *et al.* demonstrated that the limbal stem cell population is positive for the proliferation marker Ki67 and actively cycling, producing progeny migrating towards the central cornea and indicating potential regarding maintenance and longevity.

To replenish corneal epithelial cells from their high turnover rate, the limbal niche consists of multiple biochemical factors aiding in LESC migration and growth. Some LESC-supportive cells include melanocytes, keratocytes, mesenchymal cells, and Langerhans cells, which aid cell growth, nutrition, and division [22]. Melanocytes are thought to play a role in the blockage of ultraviolet rays. Various stem cell markers have indicated that signaling predominantly occurs in the basal layer of the corneal-limbal junction. Stem basal layer cells within the limbus primarily express various proteins vital in maintaining structure, such as vimentin and cytokeratin 14, 15, and 19. Similarly, limbal stem cells express molecules used for cell adhesion, including integrin $\alpha 6$, $\beta 1$, $\beta 4$, P-cadherin, and N-cadherin. Certain enzymes such as α-enolase, aldehyde dehydrogenase, cytochrome oxidase, Na+/K+-ATPase, and carbonic anhydrase also function as essential signaling factors, along with growth factor receptors like KGF-R and NGF-R. Finally, cell fate regulators, including notch-1, Musashi-1, $\Delta Np63\alpha$, p75, Bmi-1, and C/EBPδ, all function in the basal limbal layer and an ATP binding transporter protein called ABCG2. It is believed that ABCG2 proteins relieve LSCs from oxidative stress by facilitating the transport of diffusion molecules associated with stem cell proliferation, differentiation, and controlled death. Further experiments regarding culturing these specific cells under oxidative conditions would corroborate these claims. Stem cells actively expressing ABCG2 proteins are labeled side population (SP) cells, which have been shown to up-regulate following wounding to the central cornea. The limbal epithelial basement membrane is compositionally distinct from the central cornea. The limbal membrane is known to test positive for type IV collagen $\alpha 1$ chain, laminin $\alpha 2$, $\beta 1$, $\beta 2$, $\gamma 1$, $\gamma 3$ chains, nidogen, agrin, BM40/SPARC, tenascin-C and thrombospondin-4. Separately, the corneal membrane demonstrated immunoreactivity to type IV collagen $\alpha 3$ chain, type V collagen, thrombospondin-1, and endostatin. It is believed that either cytokines or the difference in compositional values between the corneal and limbal epithelium initially signal centripetal migration [23]. Further research is required to specifically isolate the direct causes of centripetal stem cell migration. It might be pursued by adding a treatment of cytokines to an experimental group and observing differences in centripetal migration from control with no added cytokines.

Commonly expressed proteins within stem cell populations vary throughout

their migratory paths, ultimately exiting the limbus. One characteristic protein expressed within corneal epithelial cells is cytokeratin 3 and 12, connexin 43 and 50, involucrin, and CLED, an enzyme linked with early differentiation. Similarly, migrating LESCs lose their expression of α-enolase and melanin pigmentation as they enter the central cornea. The production of a high number of metabolic enzymes and proteins in the central corneal cells is suggested to contribute to the rise in cell size. Further research can be conducted on these enzymes concerning the differentiation potential of stem cells. Furthermore, increased cell size has been linked to reduced colony-forming efficiency in the limbus [23]. Various experiments have additionally shown high differentiation potential in a live setting once appropriate molecular signaling combinations were required following migration. Zhao *et al.* demonstrated stem cells' potential to transform into neuronal cells by signaling progenitors β-tubulin, nestin, and neurofilament [24]. Exploring how environmental cues and microenvironmental factors influence stem cell behavior and fate determination may uncover novel strategies for enhancing the therapeutic potential of stem cell-based therapies for corneal diseases. Moreover, integrating advanced imaging techniques and single-cell omics approaches could offer a comprehensive understanding of the heterogeneity within stem cell populations and their dynamic changes during migration and differentiation processes.

Anatomically, the limbus is structured based on a pattern known as the palisades of Vogt, making it exceptionally distinct from the rest of the corneal surface. The palisades are fluctuations in the stroma and Bowman's membrane, where the corneal epithelium extends to conjoin with the limbal niche. The palisades of Vogt are crucial to the limbus precisely because they form small crypts in which supportive cells and LESCs can be supported through close contact. The basement and neurovasculature also contribute growth factors, nutrients, and structural support to promote stem cell division and proliferation. Two especially significant components of the limbus are the stroma and mesenchymal stromal cells, or MSCs. MSCs have previously been located underlying the basal epithelium and regulate signaling pathways, intracellular contact, and cytokine expression, all of which are imperative in the functioning of LESCs [22]. Research on the role of MSCs is lacking, particularly in how they relate to uptakes in regeneration following corneal injury. In addition, one more prominent anatomical structure exists within the limbus: focal stromal projections. Focal stromal projections provide a large surface to which supportive cells may attach themselves and mediate central blood vessels reaching up to the epithelium needed to transport nutrients [23]. More research still needs to be performed regarding other potential effects of focal stromal projections on the regenerative capacity of the cornea. Understanding the interactions between focal stromal projections, supportive cells, and central blood vessels could unveil novel strategies for promoting corneal tissue repair and regeneration. Integrating advanced imaging techniques, such as high-resolution microscopy and three-dimensional

reconstruction, with molecular profiling approaches could offer a comprehensive understanding of the complex microenvironment within the limbus and its role in corneal homeostasis and regeneration.

3. Corneal Repair

Limbal response to corneal injury has recently been of keen interest in the context of the corneal niche. It is currently known that during the event of corneal damage, levels of cytokines and other inflammatory mediators, such as interferon-γ, IL-1α, IL-1β, IL-6, and vascular endothelial growth factor (VEGF), are known to increase, resulting in a disruption of the conditions required for LESC proliferation. Inflammation in the scarred region then attracts T-lymphocytes, neutrophils, and macrophages and increases vasculature formation, which may disrupt the limbal niche. Ultimately, inflammatory injury results in a significantly altered limbal environment, resulting in an inability to support a limbal stem cell population and consequently severely hampering corneal regenerative ability. A process known as conjunctivalization is known to follow, in which an abnormal deposition of goblet cells, paired with vascularization and pathological fibrosis, covers the cornea in a vascularized epithelium, leading to eventual vision loss [22]. The connection between a weakened limbal stem cell population and conjunctivalization is currently unknown, leaving a large portion of research to address all functions of the limbal stem cell population aside from simple regeneration following wounding.

Similar to how general limbal stem cell patterns were tracked, their movement following different degrees of corneal injury was observed. Amitai-Lange *et al.* observed this concept by inflicting a series of injuries ranging in severity onto model corneas of the R26R-Confetti/K14CreERT mice relative to a control cornea. Afterward, lineage tracing was performed to reveal that the rate of stem cell turnover was proportional to the severity of injury in the wounded region, resulting in increased migration. Lineage tracing was performed following the administration of Tamoxifen onto the model corneas, and the presence of streaks was isolated from the experimental model labeled "Severe," indicating that stem cell populations and progeny heavily migrated centripetally. The process of harnessing limbal stem cell capabilities is currently a field of ongoing research. Combining LESCs with known growth factors might open avenues to create tissue-engineered substances similar to their original donor samples, thus providing potential alternatives for the traditional corneal transplant.

Farrelly *et al.* displayed experimental techniques that similarly evaluated limbal stem cells' movement during corneal injury through a different method. Following laser ablation of stem cells of the limbus, inner corneal stem cells slowly shifted towards the central cornea without proper growth. This indicates that these corneal stem cells were migrating to replace the function of ablated progenitor cells, which would have, under normal circumstances, produced progeny to travel centripetally. There is potential for this characteristic to be utilized in

developing corneal therapies for stem cell deficiencies or response to injury. Determining what factors might elucidate this replacement function would be important in advancing the current understanding of the limbal niche. Additionally, such a response translates to the fact that LESC populations directly contribute to the functionality of migrating cells, even though corneal stem cells typically do not express migratory patterns further inward. In further experiments, Farrelly *et al.* observed the movement of outer limbal stem cells towards the cornea following severe injury to contribute to the lost inner limbal stem cell population. Corneal cells move mainly because a mild to moderate injury of the LESC population is likely recoverable on its own. They act as light substitutes, fulfilling the same purpose. On the other hand, severe injury to the LESC population requires migration of outer limbal stem cells to rapidly replace cells that would typically produce progenitors and corneal cells to migrate towards the central cornea. While the study provides valuable insights into the potential regenerative capacity of limbal stem cells in response to injury, it does not extensively investigate the specific factors that regulate their migratory behavior or the functional consequences of their migration on corneal tissue repair and regeneration. Further research is still needed to comprehensively understand the mechanisms underlying limbal stem cell migration and their role in corneal tissue repair and explore potential therapeutic interventions that can enhance their regenerative potential in clinical settings.

Inflammation has been known to contribute to LESC dysfunction in a variety of ways previously. The inflammatory environment produces positive feedback secretion of inflammatory cytokines, decreased or damaged macrophage function, and angiogenesis. Similarly, LESCs show a decreased ability to assemble and work cohesively, resulting in a decreased ability to form colonies [25].

4. Therapeutics & Future Directions

One of the central disorders affecting the limbal niche is limbal stem cell deficiency, or LCSD, a condition with many treatment options. LCSD occurs following traumatic, immunologic, or chemical sources of injury and, in some cases, may simply recover by itself by treating said causes [22]. Some common symptoms of LCSD include the following: conjunctivalization, corneal vascularisation, pain, tear, redness, edema, poor vision, and blindness [23]. During inflammation in areas within or surrounding the limbus leading to limbal damage, pursuing methods of anti-inflammatory therapies is imperative. In cases of severe LCSD or other corneal diseases, transplantation of new stem cells is required and is typically most effective when administered in the early stages of progression [22].

Limbal transplantation is arguably the most common method of treating LSCD and involves surgically adding limbus tissue onto the ocular surface [22]. A new technique called simple limbal epithelial transplantation has recently gained popularity in the scientific community. It involves a section of limbal tissue from

a donor's eye. It is ultimately grafted on the corneal surface, thus reducing the risk of injury to the donor eye, as may occur in typical transplantation procedures. Unilateral LSCD is typically treated through a conjunctival limbal autograft (CLAU), where the unaffected eye of the host is used to provide limbal stem cells to replenish the missing population of the other eye [22] [26]. In more severe, bilateral cases of LSCD, treatments mainly encompass allogeneic limbal grafts donated from living humans or cadavers and the administration of long-term immunosuppression [22]. This transplant technique is estimated to have a 76% success rate, ultimately ranging from 50% to 100% across varying studies [23]. In addition, advances in understanding stem cell regulation within the limbus have allowed progress in developing primary organoid models. Organoid models have become increasingly important in modeling cornea diseases [27] [28]. Experiments have also revealed organoid transplants contributing to the successful combat against LSCD in host corneas [28]. Since organoid models have been shown capable of interacting with host corneas and successfully replacing lost limbal stem cells, employing their use as disease and stem cell models in the future would be beneficial to further the current understanding of limbal stem cell contributions to corneal repair.

Specific methods to target LCSD from a medical perspective might be considering the application of drugs capable of promoting stem cell differentiation within the limbus and observing their regenerative effects. Various studies have demonstrated the ability of the drug metformin to promote stem cell proliferation and differentiation into neural cells. Examining the effects of the drug metformin in the context of the limbal niche and corneal repair might help formulate different alternatives to current, existing therapies [29].

One treatment for LSCD is *ex-vivo* epithelial cell cultivation, or CLET, in which a small sample of donated limbal tissue is expanded within a culture and ultimately administered to the patient's eye. This method promotes the regeneration of the limbal niche while minimizing the region's exposure to potential harm. To fully maintain a microenvironment similar to that within the limbal niche, various growth factor components compose the Petri culture. Following complete development, cells are transplanted through a scaffold, typically made from an amniotic membrane and fibrin gel. When the patient suffers from bilateral LCSD, meaning the traditional method of employing CLET is not feasible, other autologous cells have been presented as viable alternatives. While studies have shown conjunctival epithelial cells, along with types of oral mucosal epithelial transplantation, to provide temporary stability to the ocular surface, neither have been genuinely effective in replenishing the limbal niche [22]. Recently, various strategies have presented themselves as means to repopulate the cornea's limbal epithelial stem cell population. Using ex-vivo cultured cells is a growing practice, developed to maintain limbal stem cell populations by cultivating a microenvironment. Following growth within a culture, cells are typically used for transplantation via cell carriers. In current clinical studies, human am-

niotic membranes have been used, but various other synthetic materials, like collagens, can also be used.

A slightly more promising type of cell observed in the context of the regeneration of the limbal niche is the mesenchymal stromal stem cell or MSC. MSCs are multipotent variations of stem cells that reside in the bone marrow, fat, and, in this case, the limbus. These cells are known to aid in replenishing damaged stem cell populations locally, supporting immunosuppression via the secretion of cytokines with anti-inflammatory properties. MSCs administered into the limbal niche would provide beneficial anti-inflammatory effects to damaged LESC populations and produce soluble factors to contribute against angiogenic activity. Another benefit bolstering the use of these cells is that MSCs derived from bone marrow can differentiate into MSCs of the limbus, which would thus provide an additional source to draw donor cells. It is plausible that combinations of MSC and LESC populations interacting within the limbal niche would form interdependent interactions, increasing the growth and development of the limbus and, thus, corneal regenerative capacity [22].

The lack of corneal stem cell tissue has resulted in attempts to develop alternate sources of epithelial transplant tissue. Oral mucosal and conjunctival cells have previously been introduced, along with amniotic epithelial cells, embryonic stem cells, induced pluripotent stem cells, and immature human dental pulp stem cells. Oral mucosal epithelial cells have expressed fairly high success rates in developing ocular surface stability in clinical studies. Still, the phenotype is more opaque and thick, refusing to convert to a corneal character from its oral one. Further research should be conducted regarding converting oral epithelium into corneal, possibly by introducing molecules present in traditional corneal cells absent in oral epithelial tissue. Similarly, patient-derived conjunctival epithelial cells have been successful in clinical studies, exhibiting decreased corneal opacification and conjunctivalization. Human-induced pluripotent stem cells have been tested due to the unavailability of conjunctival and oral tissue. These pluripotent stem cells regenerate within a stem cell deficiency model of the cornea. However, pluripotent stem cells have not been extensively researched, and further investigations are continuing regarding their potential therapeutic applications [25].

Congenital corneal dystrophies are another condition that could benefit significantly from a further understanding stem cell interactions. Essentially, corneal dystrophies occur following the abnormal buildup of material in the cornea, leading to ultimate vision loss or blurriness. Although little laboratory research has been employed regarding the limbal niche's role in replacing opaque tissue, the implications for future treatments are still fairly significant. The primary treatment for severe congenital corneal dystrophy is currently Descemet stripping automated endothelial keratoplasty (DSAEK), in which the stroma, Descemet's membrane, and endothelium from a donor are implanted into the eye. Recently, a new therapy method, Descemet membrane endothelial keratoplasty

(DMEK), has been shown to act even faster than DSAEK since it does not involve the transplantation of the stromal layer [30]. Due to the lack of constant accessible corneas, using the limbal niche to regenerate as many layers as possible might lessen the immediate demand for corneal transplant donors. Rat corneal transplantation has previously revealed that the peripheral corneal endothelium can regenerate itself, as displayed by its migration to grafts [31]. There is potential to remove the need to transplant the corneal endothelium into therapies for congenital corneal dystrophies should drugs arise capable of increasing stem cell turnover within the limbus.

5. Summary and Conclusion

The world is in dire need of corneal donor tissue. Conditions such as limbal stem cell deficiency that result in diminishing levels of corneal stem cells are growing in numbers. The cornea exhibits significant regeneration due to the limbal stem cell niche. Not only does the presence of stem cells help to replenish expiring epithelial populations, but it also responds actively to both disease and physical trauma. Various studies have previously monitored the movement of cells from the limbus. Lineage tracing is a common model used to track stem cell movement but suffers from the drawback that it cannot identify individual cells but only their progeny. Live imaging is a technique to solve this problem, as it can identify dormant populations of stem cells within the limbus and monitor individual cells' movements. Therapeutic measures involving the limbal stem cell niche might prove helpful in resolving the lack of accessible donor tissue available for transplant. The administration of certain drugs, such as metformin, might promote stem cell proliferation following trauma to the corneal epithelium to result in regeneration ultimately. Similarly, the growth and application of distinct lines of stem cells taken from distinct body regions, such as the oral epithelium or dental pulp, are currently being tested in clinical trials for traditional corneal stem cells. Despite drawbacks, cornea's advances in stem cell monitoring tech are crucial in developing more commercially available and seamless corneal therapies.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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