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Review Article

Progenitors for the Corneal Endothelium and Trabecular Meshwork: A Potential Source for Personalized Stem Cell Therapy in Corneal Endothelial Diseases and Glaucoma

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Several adult stem cell types have been found in different parts of the eye, including the corneal epithelium, conjunctiva, and retina. In addition to these, there have been accumulating evidence that some stem-like cells reside in the transition area between the peripheral corneal endothelium (CE) and the anterior nonfiltering portion of the trabecular meshwork (TM), which is known as the Schwalbe's Ring region. These stem/progenitor cells may supply new cells for the CE and TM. In fact, the CE and TM share certain similarities in terms of their embryonic origin and proliferative capacity *in vivo*. In this paper, we discuss the putative stem cell source which has the potential for replacement of lost and nonfunctional cells in CE diseases and glaucoma. The future development of personalized stem cell therapies for the CE and TM may reduce the requirement of corneal grafts and surgical treatments in glaucoma.

1. Introduction

Rapid progress in stem cell research in recent years provides new hope for the treatment of various previously incurable diseases. The basic treatment principle is to replace lost or damaged cells with healthy ones derived from stem cells and/or stimulate endogenous regeneration via paracrine effects mediated by the transplanted stem cells [1, 2]. Stem cells can reasonably be categorized into three main types according to their origins: embryonic stem (ES) cells, induced pluripotent stem cells (iPSCs), and adult stem cells. The focus of this paper will concern a specific region of the eye, namely, the adult stem cells of the human corneal endothelium (CE) and neighbouring trabecular meshwork (TM), there will be considerable overlap in the techniques applied to the differentiation of cells and obstacles to be addressed before regeneration therapies are available.

2. Stem Cell Overview

Whilst ES cells have numerous advantages in research such as their unlimited capacity to self-renew and pluripotency allowing them to differentiate into any cell type in the body, the fear of teratoma formation, immune-rejection issues, and ethical concerns regarding the destruction of embryos have slowed their progress towards clinical trials.

The research field of iPSCs has rapidly gained momentum since the discovery by Takahashi and Yamanaka [3]. iPSCs afford an advantage in that an autologous approach may be possible and as such circumventing the ethical and immunological disadvantages of ES cells. However, there are major safety concerns that involvement of retroviral or lentiviral vector integration in iPSCs engineering may cause genomic disruption and oncogenesis [4–6]. Besides, after the reports proving iPSCs retained epigenetic memory from the somatic cell of origin [7, 8], questions have been raised whether iPSCs are completely pluripotent like ES cells, as how far back they are reprogrammed will influence their directed differentiation potential. Despite the bright future iPSCs may have, extensive efforts and a measured scientific approach are required to guarantee safety and production quality, to understand more about the molecular signaling and pathways, and to find out reliable differentiation protocols of iPSCs before transplantation can be done in patients.

Adult stem or progenitor cells are also referred to as somatic stem cells. They reside in many adult tissues such as the bone marrow, skeletal muscle, heart, brain, skin, and limbus (summarized in [9]). Although they are not pluripotent, they still retain high plasticity. Their ethical superiority over embryonic stem cells and autologous origin avoiding immunological suppression after surgery makes them a popular stem cell source for small-scale clinical application [10, 11]. Adult stem cells are enriched in locations that are very close to the target tissue, as such, they may have more direct and faster access to the site of injury when compared with other stem cell types [9]. In addition, they have already undergone critical developmental stages, which render them comparatively easier to commit to the cell types desired [12, 13]. Understandably, difficulties such as their isolation, expansion efficiency due to limited cell numbers and integration and survival in the host tissue still remain to be unravelled. Adult stem cells, albeit with their own limitations, may be a relatively safer and more ethical alternative cell source for therapeutic use at present.

In the eye, the most successful stem-cell-based therapy to date has been the use of limbal epithelial stem cells to regenerate the corneal epithelium [14]. Apart from limbal epithelial stem cells, intensive research has been done on different niches of adult ocular stem cells, such as conjunctival epithelial and retinal stem cells, aiming for ocular repair and regeneration [13, 15, 16]. One niche of cells that has had relatively limited attention and may be of considerable clinical value (which is the focus of this paper) are the progenitor cells located in the transition zone between the periphery of the CE and the anterior extension of the TM, which is known as the Schwalbe's Ring region. In the recent decade, more and more evidence emerged to support the idea that these progenitors may be able to provide new cells for the CE, TM, or possibly both. This opens up a new prospect on research using these intriguing progenitors to treat CE diseases and glaucoma. In this paper, we will review the biological properties of CE and TM cells, summarize and discuss the evidence suggesting the presence of stem-like cells in the transition area, and, in addition, outline how these cells can be used for regeneration.

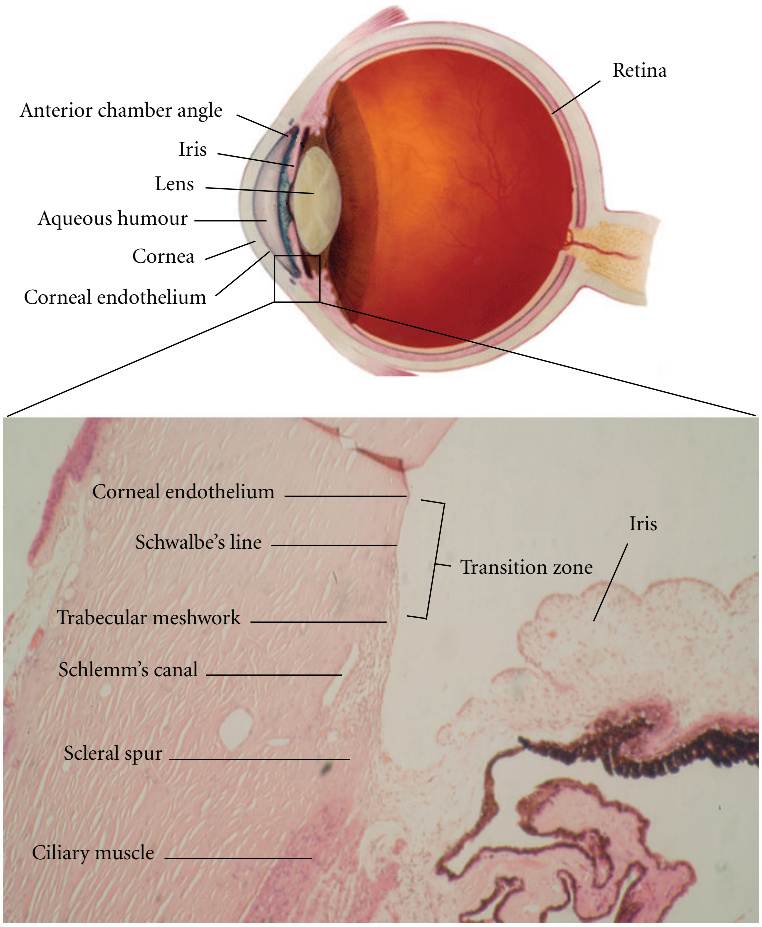
3. CE and TM: Structure, Function, and Embryology

3.1. Corneal Endothelium. Cornea is the transparent tissue located at the front of the eye which provides approximately two-thirds of the total ocular refractive power (Figure 1(a)). It consists of five layers: the multilayered epithelium, Bowman's membrane, the stroma, Descemet's membrane, and the endothelium. CE is on the posterior surface of the cornea facing the anterior chamber. It is composed of a single layer of regularly arranged hexagonal and pentagonal cells which are around 5 μm thick and 20 μm in diameter [17]. The crucial function of the CE is to maintain corneal transparency by regulating corneal hydration while allowing nutrients from the aqueous to diffuse back to the avascular cornea. The endothelium accomplishes this by a pump-leak model. It serves as a "leaky" barrier to permit selective permeability of the nutrients but prevents bulk fluid flow into the stroma. At the same time, it actively removes excess fluid from the stroma into the anterior chamber through ionic fluid pumps to prevent corneal swelling. In addition to the barrier and pump functions, the endothelial cells are responsible for the synthesis of Descemet's membrane, which is the basement membrane where the endothelium resides [18, 19].

3.2. Trabecular Meshwork. The anterior chamber of the eye is bordered anteriorly by the CE and posteriorly by the iris. At the periphery of the chamber, there lie the TM, scleral spur, ciliary body, and iris root, which form the anterior chamber angle (Figure 1(b)). The TM extends from an anatomical position called Schwalbe's line, which marks the end of Descemet's membrane, to the ciliary body and iris root at their junction. There is a specific cell population near the transition area (Schwalbe's line) between cornea and meshwork, and this will be discussed in detail in Section 5. Together with Schlemm's canal, the collector channels, and aqueous veins, the TM forms the major structure for aqueous humour outflow [17]. Aqueous humour is produced by the ciliary body and passes through the pupil into the anterior chamber. It subsequently leaves the eye through the TM into Schlemm's canal, then from there to the intrascleral plexus, and finally to the episcleral venous system [20].

The TM is a porous tissue comprised of three regions: the innermost uveal meshwork which is chord-like in structure, the deeper corneoscleral meshwork with flattened sheet-like trabeculae, and the juxtacanalicular connective tissue (also called cribriform layer or endothelial meshwork) which links the corneoscleral trabeculae with the inner wall endothelium of Schlemm's canal [17, 21]. The trabecular lamellae or beams contain collagenous cores surrounded by endothelial cells, and the lamellae are bridged by the TM cells [22]. The corneoscleral and uveal meshwork do not provide much resistance to aqueous outflow and Grant showed that aqueous outflow facility was not affected even if the inner parts of the TM were excised [23]. The outflow resistance resides primarily at the region near the juxtacanalicular connective tissue and the endothelial lining of Schlemm's canal [22]. Since the Schlemm's canal is shorter than the TM in the anteroposterior direction, the TM can also be divided into the anterior nonfiltering and posterior filtering portions.

3.3. Embryology. During embryogenesis, the neural ectoderm, the surface ectoderm, the neural crest, and, to a lesser extent, the mesoderm are involved in the development of



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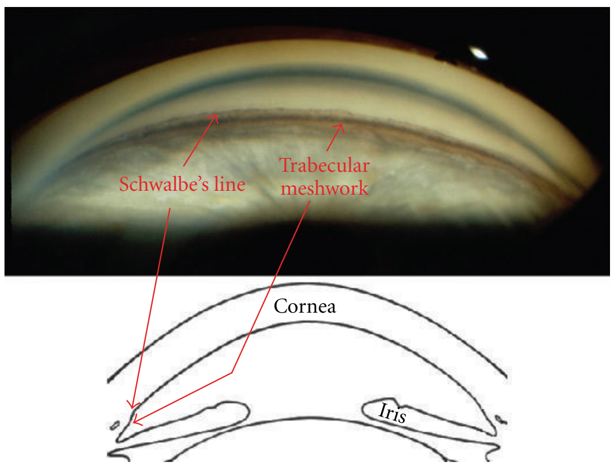


(b)

Figure 1: (a) Schematic diagram of the human eye and transverse section of haematoxylin and eosin stained chamber angle tissues (×100). (b) Anterior chamber angle as viewed in gonioscopy.

the eye. The CE and TM are both derived from the neural crest. They are formed from the first wave of neural crest-derived mesenchymal cells migrating between the surface ectoderm and the lens. The development of the cornea begins at approximately 33 days of postfertilization [24]. At around the 40th day, a double row of flattened cells posterior to the basal lamina of the corneal epithelium is produced by the mesenchyme and it develops into the monolayer of CE by the 18th week [18, 24, 25]. At this time, the CE extends nearly to the angle recess. This endothelial membrane covering the angle recess starts to regress at around 15 weeks of gestation [25].

The primitive TM is formed at around the fourth month. It consists of a triangular mass of undifferentiated mesenchymal cells. During the seventh month, these cells flatten and become slightly separated from each other, and the cavities are filled with extracellular fibers. The fibers are then organized to form the trabecular lamellae or beams. Some cells with a stellate phenotype form the juxtacanalicular layer of the TM. The complete morphogenesis of the TM is finished around birth [24–27].

These tissue developments require specific gene regulatory networks in which many transcription factors and molecular signals are involved. Although the detailed developmental networks are still not well defined, some contributing factors are known. Cvekl and Tamm performed a comprehensive review of the transcription factors that are associated with the anterior segment morphogenesis [26]. They include PAX6, PITX2, PITX3, FOXC1, FOXE3, LMX1B, and MAF, where PAX6 is the essential regulator for eye development in different organisms [26, 28, 29]. It is involved in controlling neural crest migration and thus has a critical role in early formation of the CE and TM [30, 31]. The CE did not develop in *Pitx2*$^{-/-}$ and *Foxc1*$^{-/-}$ mice and the TM was abnormally formed [32–35]. LMX1B was shown to have a direct link to the dysgenesis of the TM [36]. Whilst these transcription factors clearly have an important role, some other transcription factors also influence CE and TM development [37–41]. In addition, specific signaling molecules also play a key role in coordinating the anterior segment growth. This is borne out with transforming growth factor (TGF)-beta 2 knockout mice which developed a much thinner cornea with CE failing to develop [42, 43]. Moreover, heterozygous deficiency of BMP4 resulted in absent or hypoplastic TM and Schlemm's canal, and profound extracellular matrix deficiencies in the TM [44]. For the role of different growth factors during embryogenesis and differentiation of the eye, readers are referred to the review by Tripathi et al. [45].

4. Biological Properties of CE and TM Cells

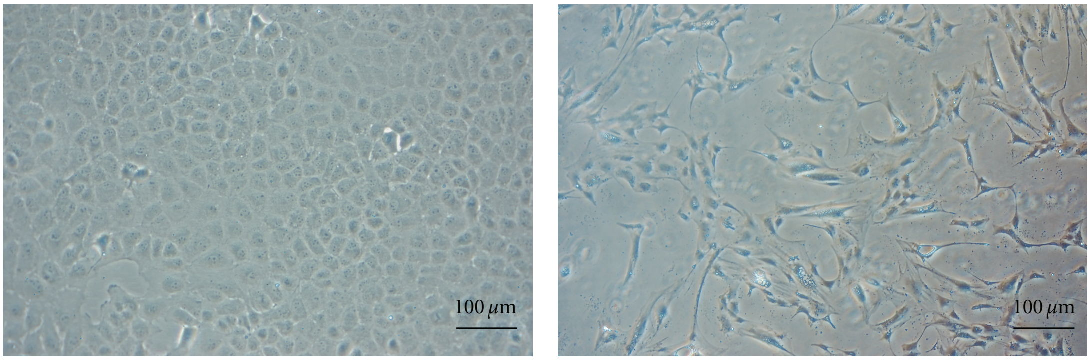
4.1. Cellular Characteristics and Markers Identification. CE cells adjoin one another with extensive interdigitations and are interconnected by tight and gap junctions. The tight junctions do not completely encircle the cells so that the endothelium can allow selective permeability for nutrients. The apical sides of the cells contain a band of actin filaments which helps maintain cell shape and barrier function and facilitate cell migration in wound healing [46]. Ultrastructurally, the endothelial cells have a large nucleus and contain numerous mitochondria, a prominent endoplasmic reticulum and Golgi apparatus, which are the characteristic features for cells metabolically active in transport, synthesis, and secretory function [18].

Although the CE is named "endothelium," it differs from the vascular endothelium. The CE is derived from the neural crest, whereas the vascular endothelium is from the mesoderm [25]. Additionally, the CE does not contain Weibel-Palade bodies nor express factor VIII, which are the typical vascular endothelium markers [47]. The CE displays an unusual combination of immunoreactions to antibodies against intermediate filaments vimentin and neurofilaments, as well as neural markers including neural cell adhesion molecule, neuron specific enolase, and S-100 protein [47–50]. These observations are not surprising when we trace back the origin of the CE. However, there have been controversies about the cytokeratin (CK) profile in the CE. CK8 and CK18 expressions in human CE cells were described in several studies [51–53] but not in others [54, 55]. Merjava et al. proposed that the variable results were due to different processing approach and antibody sensitivities [53]. The labeling of CK7 and CK19 in the CE is even more controversial [51, 55, 56]. To date, there has not been a specific marker for the CE. One can mainly distinguish them by their hexagonal morphology and evaluate their function using an Ussing chamber to measure the transport activity [57–59].

The TM cells bridge the intertrabecular spaces through cytoplasmic extensions, and adjacent cells are firmly connected to each other by desmosomes [22]. Electron microscopic studies revealed that gap junctions form the main intercellular connection between the TM cells [60]. The major actin distributions in the TM cytoplasm are straight stress fibers [61]. However, cross-linked actin networks (CLANs) have also been detected in human and bovine TM tissues [61, 62]. Similar to CE cells, there are no specific biomarkers to identify TM cells. It has been shown that the TM cells express vimentin, non-muscle actin, aquaporin-1, acetylated and acetoacetylated low-density lipoproteins, and the alpha-2 adrenergic receptor [49, 63–66]. The expression of myocilin in TM cells was increased after dexamethasone treatment [67]. Nevertheless, these proteins are also present in other cell types, making it difficult to use a single marker to identify TM cells. Some other potential TM markers including the matrix GLA protein and chitinase-3-like-1 were reported by other groups [68, 69].

Despite the lack of specific marker proteins, the TM cells possess some typical physiological characteristics. Rohen and Van der Zypen was the first to show that TM cells have phagocytic capacity [70]. It is believed that the phagocytosis helps remove debris in the circulating aqueous humour [71]. Besides, meshwork cells can synthesize a variety of extracellular materials including collagens, glycoproteins, and glycosaminoglycans (see [72] for review). The replacement and modification of the extracellular matrix compensates the gradual washout of materials during aqueous perfusion, so that the necessary outflow resistance is maintained. Moreover, the presence of contractile filaments in the TM cell cytoplasm indicates their contractility [73, 74]. It was found that substances that contract meshwork cells decrease the aqueous outflow facility and vice versa [75].

Both the CE and TM cells are exposed to continuous workload throughout lifetime, yet, they have limited proliferative capacity in situ to replace lost cells under normal circumstances [76, 77]. In the CE, the surrounding cells spread and slide to fill the gaps caused by cell loss. The endothelial cells are arrested in G1-phase of the cell cycle [76]. Bovine TM cells were also shown to be locked in



(a) (b)

Figure 2: (a) Corneal endothelial and (b) trabecular meshwork cells cultured *in vitro*.

G0/G1-phase [77]. Although both cell types can be grown in culture, they are contact inhibited [76]. The division rate of bovine TM cells decreased to negligible amounts when they were in contact and formed gap junctions [78].

4.2. Consequence of Cell Loss or Malfunction. There are approximately 4,000 CE cells per mm^2 at birth, but the cell density decreases with age at a rate of 0.6% per year throughout life [79]. The cell number is usually adequate to maintain normal corneal function for a lifetime. However, besides the factor of ageing, endothelial cell loss can also occur due to disease, trauma, and surgery. These may result in a higher cell depletion rate than normal, leading to endothelial failure and hence, loss of visual acuity. In order to maintain adequate corneal function, a minimum level of 400 to 700 endothelial cells per mm^2 is required and the cells need to be of uniform size and shape [18]. Hence, corneas for grafting need to be screened for endothelial health and cell numbers before they are accepted. Some ocular diseases are manifested by abnormal endothelial cells. Fuch's endothelial dystrophy is a corneal disease involving malfunction of the endothelial cells, in which irregular warts or excrescences of Descemet's membrane are secreted. The excrescences are collagenous secretions (known as guttata) deposited at the posterior surface of the membrane, causing disruption of the overlying endothelial cells and thus compromise endothelial function [18, 80]. Another disease attributable to aberrant CE is the iridocorneal endothelial (ICE) syndrome. The endothelium proliferates and migrates outward to the TM and across the angle onto the surface of the iris, which may progress to glaucoma, corneal decompensation, or both [81, 82].

The TM cellularity decreases with age as well. Alvarado et al. reported a cell loss rate of 0.58% per year [83]. This is comparable to that seen in the CE. It was estimated that there were 750,000 cells in the meshwork at 20 years of age but the number decreased down to around 400,000 by 80 years [84]. Other age-related changes in the TM include trabecular thickening, trabecular fusions, and alterations to the extracellular material in the juxtacanalicular meshwork; all of which would increase the aqueous outflow resistance and subsequently the intraocular pressure (IOP) [71]. Pathologically elevated intraocular pressure is the major risk factor in primary open angle glaucoma (POAG). Indeed, these age-related changes are intimately linked to the glaucomatous alterations found in POAG patients. The glaucomatous eyes were found to have significantly more cellular losses compared with age-matched normal eyes [85]. This is believed to precipitate the decrease in drainage facility. When cell loss is progressive, trabecular thickening and fusion may develop due to adhesions of the denuded portions of the trabeculae. Furthermore, accumulation of extracellular materials and meshwork cell hyperplasia in glaucomatous TM that are believed to obstruct the outflow pathway was also documented [71]. Hence, TM cells are essential to maintain a healthy meshwork for aqueous drainage.

4.3. Culture In Vitro. In spite of the restricted replication capacity *in vivo*, the CE and TM cells can be grown in culture under appropriate conditions. Figure 2 shows the *in vitro* culture of bovine CE and TM cells. It has been demonstrated that fibroblast growth factor (FGF) stimulates the proliferation of CE and TM cells [86–88]. Hepatocyte growth factor (HGF) is also a competent mitogen for both CE and TM cells in a dose-dependent manner [89, 90]. Culturing of CE cells on dishes coated with collagen type IV, laminin, or fibronectin favoured the formation of a typical hexagonal monolayer [86]. Hyldahl reported that the addition of insulin-like growth factor-1 (IGF-1) and epidermal growth factor (EGF) stimulates CE cells to initiate DNA synthesis [91]. Treatment of TM cells with platelet-derived growth factor (PDGF) can also increase their cell division. Besides, it enhances the phagocytic activity and promotes extracellular matrix secretion [92]. IGF-1 was shown to promote the incorporation of [3H] thymidine in TM cells, whereas vascular endothelial cell growth factor (VEGF) restrained cell growth [93]. Studies have revealed that TGF-beta, which is present in the aqueous humour, inhibits TM cell proliferation and suppresses S-phase entry of CE cells [94, 95]. Table 1 shows a summary of the mentioned growth factors effects on CE and TM cell proliferation *in vitro*.

Table 1: The influence of growth factors on CE and TM cell proliferation.

Growth factor	Corneal endothelium	Trabecular meshwork
EGF	++	++
bFGF	+	++
HGF	+	++
PDGF	++	+
IGF-1	+	+
TGF	−	− −
VEGF		−

Both the CE and TM cells are unique cell types in the eye. They do not normally replace themselves once they are lost in aging or diseases. To date, there has been no cell therapy for the treatment of CE and TM related diseases. A lot of unknown facts about the regenerative capacity of TM or CE still exist. Thus, the potential to repair or replace the CE and TM is an important area that needs to be explored.

5. Evidence of Stem-Like Cells in the Schwalbe's Ring and Their Therapeutic Implication

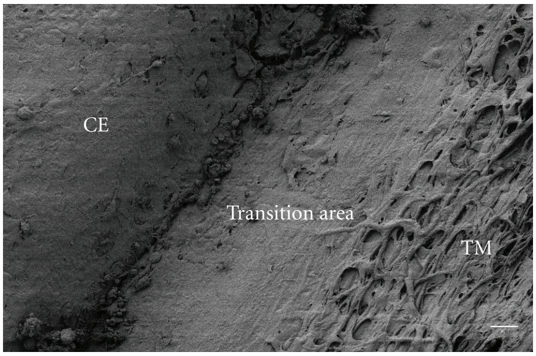
Stem cells are undifferentiated cells which can renew themselves indefinitely and produce one or more progenies through symmetric and asymmetric division, whereas progenitor cells have relatively limited self-renewal capacity and more restricted differentiation abilities. As mentioned earlier, stem/progenitor cells have been identified in various adult mammalian tissues. They are crucial for tissue renewal and regeneration. These adult stem cells have some key characteristics, including small cell size and high nucleus to cytoplasm ratio, high proliferative potential, slow cell cycle, and poor differentiation capacity with primitive cytoplasm [96]. They reside within a specialized microenvironment called niche, which offers protection and nourishment to the cells [97]. It is believed that adult stem cells have huge ethical and immunological advantages over embryonic stem cells as a future therapeutic option.

In the eye, accumulating evidence reveals that there is a population of stem-like cell located in the transition area between the periphery of the CE and the anterior nonfiltering portion of the TM (Figure 3). This transition region is referred to as Schwalbe's Ring. Schwalbe's line marks the peripheral termination of the Descemet's membrane and can be viewed clinically in gonioscopy (Figure 1(b)). In 1982, Raviola identified a population of unusual cells located just beneath the Schwalbe's line in rhesus monkey, which she called Schwalbe's line cells [98]. These cells are different from the typical CE and TM cells and have distinct ultrastructural features. As described, these cells "form a discontinuous cord, oriented circumferentially at the corneal periphery and deep to the CE lining of the anterior chamber." They morphologically resembled whorled multilamellar bodies of type II alveolar epithelial cells of the lung and were proposed to be secretory. Stone et al. found that these cells were immunoreactive to neuron-specific enolase, suggesting that they may have neuroregulatory function in the anterior segment [99]. Rittig and colleagues later reported intense staining of the enzyme hyaluronan synthase in Schwalbe's line cells, indicating their hyaluronan production ability [100]. Samuelson et al. documented Schwalbe's line cells in canine eyes as well [101]. In general, there seems to be a distinct cell population in the transition area, while their function is still unclear.

Not much attention was paid to Schwalbe's line cells until there was more evidence supporting the presence of stem/progenitor cells in this transition zone. The idea came primarily from the observation of an increase in TM cell division localized to the anterior nonfiltering portion of the TM after argon laser trabeculoplasty (ALT) [102]. ALT is a glaucoma therapy which aims at lowering the IOP. The principle of this laser procedure is not to make drainage holes through the TM, but to "blanch" the tissue which creates superficial burn restricted to the uveal meshwork [103]. The exact mechanism by which this treatment lowers IOP is not known, however, one of the possible mechanisms of action is the repopulation of the TM by stimulating cell division [104]. Indeed, several studies have shown marked tritiated thymidine incorporation into the TM cells following ALT in different species [102, 105, 106]. Acott and colleagues demonstrated a four-fold increase in TM cell division in human laser-treated explants compared with untreated controls [102]. They found that more than 60% of the cell division was initially localized to the anterior nonfiltering region of the TM and these proliferative cells migrated to repopulate the burn lesions afterwards. It appears that these cells are putative stem cells that are invigorated after ALT to repopulate the TM, possibly through the release of growth factors and cytokines. Due to their location at the insertion region into the cornea just beneath Schwalbe's line, Kelley have named them the "TM insert cells" [107].

Although ALT can lower the IOP successfully and, to some extent, repopulate the cell-deficient TM in glaucoma, uncontrolled repair process that occurred in the tissue may become a detrimental consequence. An abnormal corneal and/or trabecular endothelial cell sheet covering the anterior uveal meshwork was observed in some glaucoma patients after ALT [108, 109]. In some cases, they can grow extensively and block the aqueous outflow subsequently, leading to the failure of the surgery. Alexander et al. observed this aberrant endothelial membrane as well in normal human TM which was subjected to ALT [110]. They found that laser placed close to Schwalbe's line advanced the endothelial extension. It was believed that these repopulating processes after ALT involve migration of a specialized population of cells extending from the Schwalbe's line region [109, 110].

In addition to the observations in the TM, a significantly higher cell density at the peripheral CE also suggests that stem-like cells may be present in the peripheral transition region to provide differentiated CE cells [111, 112]. Otherwise, the cell density should be uniform all over the CE. It has been documented in the literature that at least some CE cells have the ability to divide under specific circumstances [113–116]. It was found that the peripheral CE cells retained higher replication competence than those in the central and this



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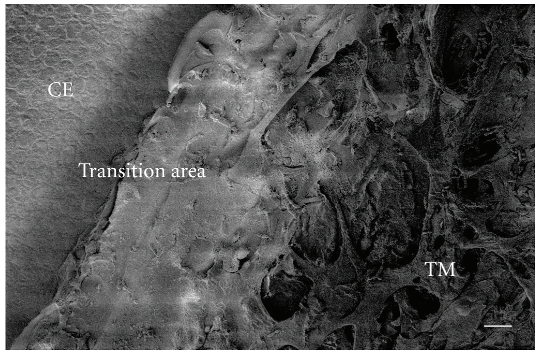

(b)

FIGURE 3: Scanning electron micrographs of (a) human and (b) bovine eyes, showing the transition between the peripheral cornea and anterior portion of the trabecular meshwork. There is a distinct transition area in the human tissue but the transition is more abrupt in the bovine one. The samples were coated with gold and imaged at an accelerating voltage of 4 kV and a working distance of 8 mm using a SE2 detector (Gemini LEO 1550 SEM). Scale bar = 20 μm.

was independent on the donor age [117]. Moreover, corneal grafts in hosts who retain the peripheral endothelium survive much longer than grafts in hosts with CE cell loss [118]. Persistent precursors from the host cornea may explain the enhanced long-term survival of grafts. Interestingly, Balachandran et al. reported in a case series that in spite of complete graft detachment after Descement membrane endothelial keratoplasty, spontaneous recovery of corneal transparency was observed in two patients [119]. They suggested that "endothelial transfer, migration, regeneration, or a combination thereof from either the donor or the recipient may explain the visual recovery." Schwartzkopff et al. later reported *in vivo* re-endothelialization following complete endothelial cell loss of the grafted donor cornea in rats and suggested that peripheral CE cells in recipients can support the regeneration [120]. These findings indicate that CE may have some sort of regenerative capacity under specific conditions, which is not consistent with the long-term belief that they do not divide *in vivo* [76]. In particular, the peripheral CE seems to be the regenerative zone in these conditions. As such, research of the Schwalbe's Ring region has become even more interesting, as the precursor cells in this transition area may be able to supply new cells for both the TM and CE.

In recent years, molecular marker studies supply more supportive data for the stem cell niche at the transition zone. Whikehart et al. detected telomerase activity, which is a stem cell maker, in the peripheral cornea [121]. They also observed bromodeoxyuridine (BrdU) labeling, which is a marker for cell division, in the TM and posterior limbus. The BrdU incorporation increased and extended into the CE in response to mechanical wounding. McGowan et al. showed that cells at the transition region express stem cell makers nestin, alkaline phosphatase, and telomerase [122]. Following corneal wounding, additional putative stem cell markers (Oct3/4, Wnt1) and differentiation markers (Pax6, Sox2) were observed. It was suggested that the putative stem cells in the transition area migrated to renew the wounded CE. To date, there has been no specific marker for this population of putative stem cells despite the observation of a different immunohistochemical profile in the CE, TM insert cells, and TM cells. Neuron-specific enolase was found to locate at the anterior but not posterior portion of the human TM [49]. Ankyrin G and human milk fat globule protein (HMFG, also known as breast antigen 46) were highly expressed in the insert cells. On the contrary, YKL-40 (also known as chitinase-3-like-1 or cartilage glycoprotein-39) had lower expression levels when compared to the CE and TM [107, 123].

Ideally, if the molecular signature of the stem-like cells is known, one can isolate and enrich the stem cell pool relatively easily using fluorescence-activated cell sorting (FACS) or magnetic immunosorting. However, the search of the specific stem cell signature will involve a laborious process and screening of a huge amount of putative markers. Hence, attempts have been made to isolate and propagate undifferentiated progenitor cells using a sphere culture protocol [124–126]. Precursors from human and rabbit CE have been successfully isolated using the sphere-forming assay and it was found that the peripheral CE contained significantly more precursors than the central region [127–130]. Mimura et al. proved that this culture assay selectively isolated younger progenies [131]. Huang and colleagues showed that bovine CE cells resembled bovine aorta in its content of endothelial colony forming cells [132]. Our sphere culture of primary peripheral bovine CE cells revealed the presence of undifferentiated precursor cells with self-renewal capacity and their potential to differentiate into neuronal lineages (Figures 4 and 5). Besides the CE, progenitor spheres were also isolated from human TM primary cultures [133]. It is likely that these isolated precursors from the CE and TM are from the transition zone in between them. It remains to be determined whether "Schwalbe's Line cells", "TM insert cells," and precursors having been isolated are the same cell type. For our convenience we have called the progenitor cells "PET cells" (Progenitors for Endothelium and Trabeculum) so not to presume until proven that we have the exact same cell population previously described.

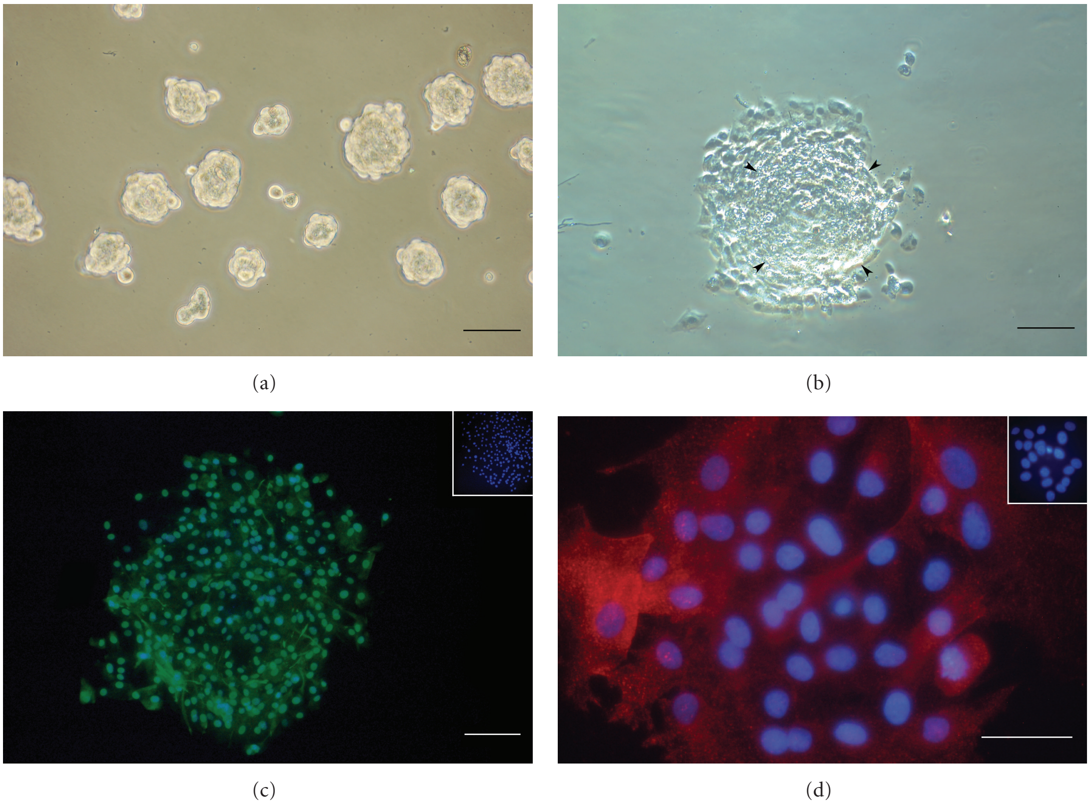

FIGURE 4: Sphere culture of bovine peripheral corneal endothelial cells. (a) Floating spheres on day 7 in defined serum-free media. Aggregation and development of dark cores can occur when spheres are left over the optimal culturing period of 5–7 days. (b) Cells migrating from an attached sphere on adherent substrate. The arrowheads show the contour of the sphere. (c) Nestin (green: undifferentiated cell marker) and (d) β-III tubulin (red: neuronal marker) staining were detected in the cells that migrated from the primary spheres. Nuclei were counterstained with DAPI (blue). Insets are negative controls with nonimmunized IgG. (a–c) Scale bar = 100 μm; (d) Scale bar = 50 μm.

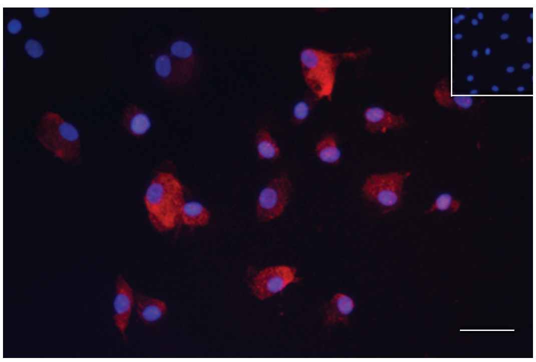

FIGURE 5: After 7 days of differentiation, cells derived from the spheres also expressed β-III tubulin. Nuclei were counterstained with DAPI (blue). The inset is a negative control with nonimmunized IgG. Scale bar = 50 μm.

The studies of stem cells in the eye have important implications in ocular health and disease treatment. Animal models using the isolated CE precursors for regeneration have been documented in several studies [59, 134, 135]. The PET cells within the transition area may be a promising cell source for replacing worn-out endothelium *in vivo* or boosting the number of endothelial cells *in vitro* on potential corneal graft materials. As mentioned in the previous section, ICE syndrome is manifested by abnormal proliferative CE cells that grow and cover the angle. It is tempting to speculate that the aberrant cells are metaplastic progenitors residing in the endothelium. Treatments can possibly be developed by targeting these cells. Besides, we know that the number of TM cells drops significantly in glaucoma patients, which precipitates the blockage of the aqueous outflow pathway. Thus, repopulating the cell-deficient TM using the PET cells may be a useful treatment to enhance drainage in glaucoma.

6. Summary and Future Directions

Recent progress in stem cell research provides an optimistic prospect on their use in regenerative medicine and tissue engineering. Specifically, advances in iPSCs and adult stem cells research raise hope for personalized cell replacement therapies. However, before iPSCs can be clinically applied, extensive efforts are needed to devise reliable production methods to address the safety concerns. In this paper, we summarized the accumulating evidence of the presence of putative stem cells in the transition zone between the peripheral CE and the anterior extension of the TM. We also discussed the origin and biological properties of both CE and TM cells. Up to now, there has been no clear definition of the progenitor cells located in the transition area. We have called the putative stem cells "PET cells" as they have the potential to replenish both the CE and TM. It remains to be determined whether the previously described "Schwalbe's Line cells" and "TM insert cells", as well as the precursors having been isolated, are of the same cell type; if they are, exactly what proliferation and differentiation potential do they retain, why do they not seem to repopulate the TM in glaucoma or the CE in age and disease when these populations are sorely depleted and finally how can they be used therapeutically?

Further research is required to establish the protocol to regulate cell division and differentiation of the PET cells towards appropriate lineages for repopulation of the diseased CE and TM. We need to identify which factors and signals govern their division and differentiation. Another challenge is the specific biomarker identification of the PET cells, which would facilitate stem cell isolation and enrichment. Furthermore, a better understanding of the migration and settlement properties of the PET cells is also important for the use of possible bioengineered materials. CE and TM cell loss is central to a number of ocular conditions including corneal diseases and glaucoma. In spite of the challenges, PET cells represent an attractive therapeutic stem cell source for the regeneration of the CE and TM. It is hoped that future research will ultimately lead to the development of stem-cell-based therapies for CE diseases and glaucoma, which can reduce the requirement for corneal grafts and laser or surgical treatments in glaucomatous patients.

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