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Review

Status and prospects for the development of regenerative therapies for corneal and ocular diseases

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ABSTRACT

Among the regenerative therapies being put into clinical use, the field of corneal regenerative therapy is one of the most advanced, with several regulatory approved products. This article describes the progress from initial development through to clinical application in the eye field, with a particular focus on therapies for corneal epithelial and endothelial diseases that have already been regulatory approved as regenerative therapy products. The applications of regenerative therapy to the corneal epithelium were attempted and confirmed earlier than other parts of the cornea, following advancements in basic research on corneal epithelial stem cells. Based on these advances, four regenerative therapy products for corneal epithelial disease, each employing distinct cell sources and culture techniques, have been commercialized since the regulatory approval of Holoclar® in Italy as a regenerative therapy product for corneal epithelial disease in 2015. Corneal endothelial regenerative therapy was started by the development of an *in vitro* method to expand corneal endothelial cells which do not proliferate in adults. The product was approved in Japan as Vyznova® in 2023. The development of regenerative therapies for retinal and ocular surface diseases is actively being pursued, and these therapies use somatic stem cells and pluripotent stem cells (PSCs), especially induced pluripotent stem cells (iPSCs). Accordingly, the eye field is anticipated to play a pioneering role in regenerative therapy development going forward.

Contents

Abbreviations: PSCs, pluripotent stem cells; iPSCs, induced pluripotent stem cells; SJS, Stevens–Johnson syndrome; LSCD, Limbal stem cell deficiency; AM, Amniotic membrane; SEAM, Self-formed ectoderm autonomous multizone; HLA, Human leukocyte antigen; ROCK, Rho-associated protein kinase; ESCs, embryonic stem cells; RPE, retinal pigment epithelium.

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1. Introduction

In the field of regenerative therapy for ocular diseases, several products have been developed in recent years, with five (Holoclar®, Nepic®, Ocural®, Sakracy®, Vyznova®) receiving regulatory approval (Table 1). The reasons for the progress of ocular regenerative therapy are thought to be that the therapeutic effectiveness is clear to assess and the long-established practice of transplant medicine, including corneal transplantation. Additionally, that epithelial stem cell research has long been active, particularly with regards to the corneal epithelium, is also considered to be a major reason. This review summarizes the historical background and prospects for corneal regenerative therapy, including basic research, product developments and clinical applications.

2. Regenerative therapy for the corneal epithelium

Currently, cell-based regenerative therapy products for corneal disorders include those for corneal epithelial and endothelial diseases. In this section, regenerative therapy for the corneal epithelium developed earlier is described.

2.1. Biology and pathobiology of the corneal epithelium

The cornea is a transparent, avascular tissue located in the front of the eye. It consists of three major layers: the epithelium, stroma and endothelium (Fig. 1). The corneal epithelium envelopes the superficial surface of the cornea and is composed of multiple layers of epithelial cells. It protects the eye by preventing the entry of foreign substances and preserves the transparency of the cornea. Corneal epithelial stem cells are located at the limbus, which is the border region between the cornea and conjunctiva, and they maintain corneal epithelial homeostasis by providing a continuous supply of daughter cells. When corneal epithelial stem cells are lost due, for example to Stevens-Johnson syndrome (SJS) or a chemical or thermal burn, the conjunctival tissue invades the central cornea, together with blood vessels, resulting in severe vision loss. This is called a limbal stem cell deficiency (LSCD). To treat such a severe condition, corneal transplantation has long been performed. However, there are challenges surrounding this approach, including a shortage of donors and poor outcomes due to immune rejection. Therefore, new therapies have been designed to overcome these problems.

2.2. Development of regenerative therapy for corneal epithelial disease

Transplantation of autologous limbal tissue was initially proposed as an alternative LSCD treatment to conventional corneal transplantation using allogenic donor corneas [1]. This treatment can replenish corneal epithelial stem cells but is not applicable for bilateral disease due to the absence of remaining undamaged corneal limbal tissue. Additionally, it requires an invasive procedure, the removal of limbal tissue from the healthy eye of patients with unilateral disease. In 1997, a novel approach to treating an LSCD was proposed in which small sections of a patient's corneal limbal tissues were harvested and cultured in a dish to expand the corneal epithelial stem/progenitor cells. They were then transplanted onto the cornea using a contact lens or petrolatum gauze as a transplant carrier [2]. Subsequent methods were then devised in which epithelial cells were cultured on a transplant carrier and transplanted along with it. A fibrin matrix had been used as a carrier for the transplantation of cultured epidermal keratinocytes to treat severe skin burns [3], and this procedure was adopted to transplant cultured corneal limbal epithelial cells. Similarly, amniotic membrane (AM) has been used as a carrier for the cultivation and transplantation of corneal limbal epithelial cells [4]. With these advances in cell culture technology, the clinical application of corneal epithelial regenerative therapy for LSCD has progressed rapidly toward the increasing number of cases [5]. These early developments served as a foundation for the increase in regenerative therapy products utilizing cell culture technologies.

In 2015, cultured corneal epithelial cells, using fibrin matrix as a carrier, received regulatory approval in Italy as a regenerative therapy product (Holoclar®) for LSCD treatment and have now been approved in the EU (Table 1). Meanwhile, autologous cultured corneal epithelial cell sheet transplantation, using temperature-responsive culture dishes without a carrier, was developed [6] and received regulatory approval in Japan (Nepic®) in 2020 for the

Table 1
Regulatory approved regenerative therapies for corneal treatment.

Product	Composition (Auto/Allo)	Indication	Manufacturing and sales companies	Regulatory Approval date	Area and Countries
Holoclar®	Human corneal limbal epithelium with FM (Auto)	LSCD due to burns	Holostem and Chiesi Farmaceuti	17 February 2015	EU
Nepic®	Human corneal limbal epithelium (Auto)	LSCD −unilateral	J-TEC and Nidek	19 March 2020	Japan
Ocural®	Oral mucosal epithelium (Auto)	LSCD −bilateral	J-TEC and Nidek	11 June 2021	Japan
Sakracy®	Oral mucosal epithelium with AM (Auto)	LSCD with adhesions	Hirosaki Lifescience Innovation	20 January 2022	Japan
Vyznova®	Human corneal endothelium (Allo)	Bullous keratopathy	Aurion Biotech Japan and S-RACMO	17 March 2023	Japan

Auto: autologous transplant.
Allo: allogeneic transplant.
FM: fibrin matrix.
AM: amniotic membrane.
LSCD: limbal stem cell deficiency.

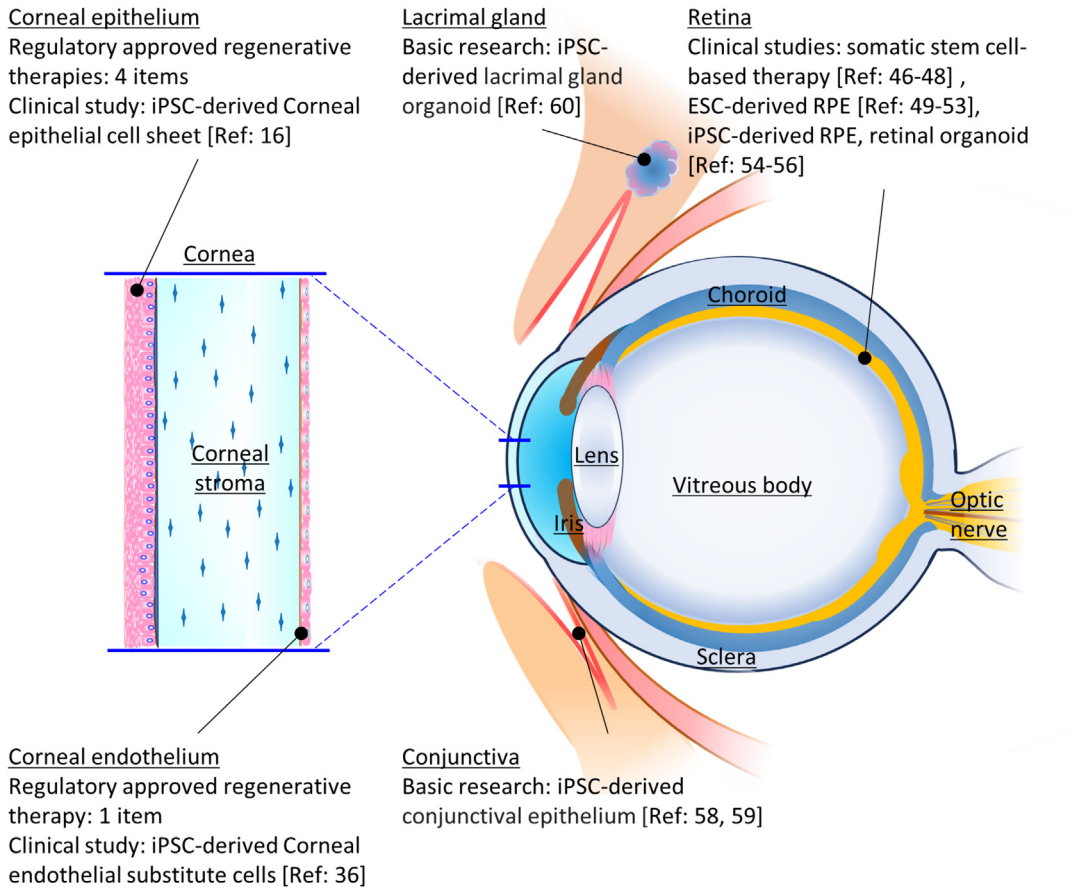

Fig. 1. Schematic diagram of the eye, including the cornea, and the regenerative therapies being developed in each part. The right panel represents a full image of the eye, and the left panel represents a magnified view of the cornea. Several regenerative therapy products were approved for corneal epithelial and corneal endothelial diseases, while clinical studies for them using iPSCs are also in progress. Several clinical studies of regenerative therapies targeting the retina using somatic stem cells and PSCs are underway. iPSC-based reconstitution techniques have also been reported for the conjunctiva and lacrimal gland.

treatment of LSCD (Table 1). New approaches using alternative cell sources have also been developed to treat bilateral corneal epithelial disease. In 2003, preclinical studies indicated the possibility of treating LSCD by culturing oral mucosal epithelial cells on AM as an alternative for corneal epithelial cells and transplanting them onto the ocular surface [7,8]. Then, in 2004, the results of clinical studies of autologous oral mucosal epithelial cell transplantation were reported [9,10]. These regenerative therapy products subsequently received regulatory approval in Japan as Ocular® and Sakracy® following clinical trials, in 2021 and 2022 (Table 1). Although transplantation of oral mucosal epithelial cells to treat an LSCD tends to have better results than conventional corneal transplantation [11], severe inflammatory ocular surface diseases, such as SJS, remain difficult to treat because of corneal epithelial defects, recurrent infections and neovascular invasion from the corneal periphery.

It has been suggested that intrinsic differences between corneal and oral mucosal epithelium may underlie the neovascular invasion after oral mucosal epithelial transplantation for an LSCD [12,13]. Therefore, the development of a novel corneal epithelial regenerative therapy using iPSCs as a cell source to reconstruct corneal epithelium has started. In 2016, the generation of two-dimensional eye-like organoids, named SEAMs (Self-formed Ectoderm Autonomous Multizone), derived from human iPSCs was first reported [14,15]. These multi-zonal organoids consist of several types of ocular primordium, including corneal epithelial stem cells, from which functional iPSC-derived corneal epithelial cell sheets could be fabricated following the isolation of corneal epithelial cells by cell-sorting (Fig. 2 a). In 2019, the transplantation of iPSC-derived corneal epithelial cell sheets (Fig. 2 b) onto the corneas of patients with an LSCD was conducted (Provision Plan: jRCTa050190084 [16]), with the cell sheets manufactured from iPSCs derived from homozygous human leukocyte antigen (HLA) donors using the SEAM technology. This approach enables the treatment of patients with bilateral LSCD, however, because of using allogeneic iPSCs as a cell source, it might be necessary to deal with immunological reactions to retain a long-term stable outcome when HLA haplotypes are not matched. To overcome this, iPSCs in which the HLA genes predominantly involved in immune rejection are knocked out by genome-editing technology, are being developed [17–22] and are expected to be a universal cell source for regenerative therapy in the coming years.

3. Regenerative therapy for the corneal endothelium

Regenerative therapies for corneal endothelial disease are still in the early stages of development compared to those for corneal epithelial diseases and there are limited products in development. Nevertheless, considerable progress has been made in recent years, which we review in the following section.

3.1. Biology and pathobiology of the corneal endothelium

The corneal endothelium is a cellular monolayer that lines the inner surface of the cornea, which regulates the hydration of the

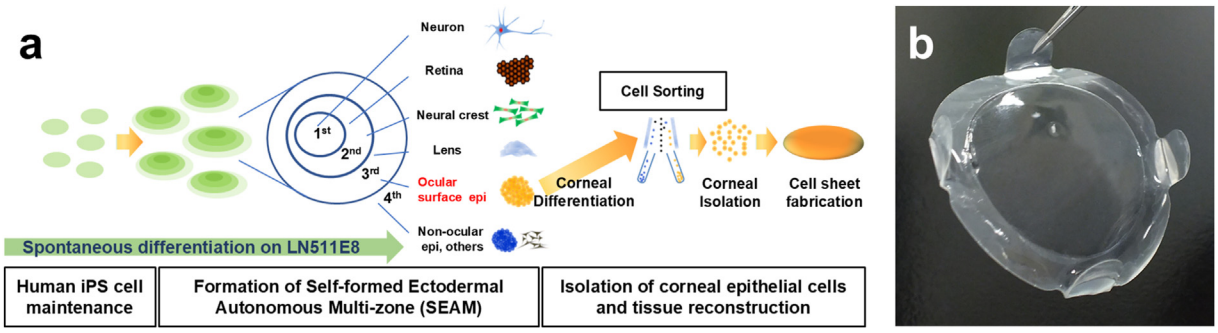

Fig. 2. a) Flow diagram illustrating the production of corneal epithelial cell sheets from iPSCs through the self-formed ectodermal autonomous multizone (SEAM) formation process. Cells seeded in the "Human iPS cell maintenance" are processed into the "Formation of Self-formed Ectodermal Autonomous Multi-zone" to form SEAMs containing cells of various ocular lineages. The next step, "Isolation of corneal epithelial cells and tissue reconstruction," involves purification of corneal epithelial stem/progenitor cells and preparation of corneal epithelial cell sheets. This process produces corneal epithelial cell sheets composed of corneal epithelial stem/progenitor cells. epi, epithelial cells [15]. Copyright 2017, The Authors. b) An iPSC-derived corneal epithelial cell sheet, cultured and fabricated on support rings, is shown. The cell sheet is composed of homogeneous cells and is highly transparent.

cornea through its pump and barrier functions, thereby maintaining corneal transparency (Fig. 1). Corneal endothelial function is majorly affected by the density of corneal endothelial cells, and when this drops to less than 500 cells/mm^2, corneal edema and opacity occur. Adult corneal endothelial cells do not proliferate *in vivo*, and once reduced, corneal endothelial cell density cannot recover. The only existing treatment for damaged corneal endothelium is corneal transplantation using donor corneas. However, corneal transplantation has challenges because of donor tissue shortages, rejection following the transplantation and side effects associated with steroid administration that include glaucoma, infections, and irregular astigmatism. The corneal endothelium, however, is considered well-suited for transplantation because it faces the immunologically privileged anterior chamber inside the eye [23], thus the development of regenerative therapies with cell transplantation for corneal endothelial dysfunction is being intensively pursued.

3.2. Development of regenerative therapies for corneal endothelial disease

As mentioned, replenishing corneal endothelial cells through regenerative therapy holds promise as a novel approach to treating corneal endothelial diseases. A significant issue, however, surrounds the need to amplify corneal endothelial cells, which do not proliferate *in vivo* except during ocular development, and to do so in sufficient quantity and with stable quality for use in regenerative therapy. Various culture conditions have been investigated to address this challenge, and it has been discovered that the use of Rho-associated protein kinase (ROCK) inhibitors has made a significant contribution to the amplification of corneal endothelial cells [24–26]. This approach cleared the way for the development of regenerative therapies using cultured corneal endothelial cells. Indeed, a clinical trial of a treatment in which endothelial cells from donor corneas were cultured, amplified and injected as a single cell suspension into the patient's anterior chamber was started in 2013 [27]. The treatment received regulatory approval in Japan in 2023 as the first regenerative therapy product (Vyznova®) for corneal endothelial disease (Table 1). As is the case for the corneal epithelium, PSCs are now being investigated as a source of corneal endothelial cells without reliance on donor corneas. Developmentally, the corneal endothelium is derived from neural crest cells, which generate the periocular mesenchyme. Thereby, a series of multi-step differentiation methods, which recaptures corneal endothelial development, have been adopted to obtain corneal endothelial cells [28–32], and regenerative therapies to treat corneal endothelial dysfunction have been devised. These induce differentiation of corneal endothelial-like cells – or functionally equivalent corneal endothelial substitutional cells – from human iPSCs or embryonic stem cells (ESCs), which are then transplanted into the anterior chamber of non-human primates with similar characteristics to human corneal endothelium [33,34]. Recently, a clinical study of the transplantation of iPSC-derived corneal endothelial substitutional cells into the eyes of patients with bullous keratopathy, a corneal endothelial disease, was conducted in Japan [35] (Provision plan: jRCTa031210199 [36]).

4. Perspectives and conclusions

As discussed above, regenerative therapy has made a great impact in the field of corneal disease. However, issues remain to be addressed and hurdles to be overcome.

Primary diseases causing LSCD are often accompanied by severe inflammation, the control of which is key to its long-term therapeutic efficacy and vision restoration. Therefore, as well as developing new regenerative therapies, it is also important to establish effective post-transplant management procedures. For example, immunological reactions need to be further controlled in the case of allogeneic regenerative therapy. In fact, long-term efficacy has been demonstrated in the case of allogeneic limbal transplantation for severe LSCD with combinations of appropriate immunosuppressive agents [37]. As a new attempt at regenerative therapy for severe LSCD, it was reported that the development and use of limbal-supported rigid-type contact lens, which can reduce corneal surface irregularities and symptoms of dry eye, for maintenance after oral mucosal epithelial transplantation, improved the outcomes [38].

Regenerative therapy with autologous corneal epithelium has been reportedly effective in the long term post-transplantation [5,39,40]. This suggests that the replenishment of corneal epithelial stem cells themselves serves for the long-term reconstruction of corneal epithelial tissue. On the other hand, some studies of allogeneic limbal transplantation also reported that even when transplanted donor cells only partially remain, the corneal epithelium is successfully reconstructed and maintained for an extended period [41,42]. Indeed, regarding regenerative therapies using autologous corneal epithelial cells, few studies have directly examined the remaining transplanted corneal epithelial cells after transplantation. Thus, it has been suggested that the therapeutic efficacy of regenerative therapy for corneal epithelial disease might

be attributable, not only to direct tissue recovery by the transplanted stem cells, but also to indirect effects such as the restoration of the microenvironment around stem cells by therapeutic interventions, including surgery [2,43,44]. It is currently unclear whether the direct or indirect therapeutic effects predominate and what impact a particular primary disease has on the healing process. Establishing scientific evidence on the mode of action in regenerative therapies is a common challenge in all fields. As the number of cases of regenerative therapy for corneal diseases is increasing with the commercialization of regenerative therapy products, it is expected that the accumulation of evidence on the mode of action will accelerate even more in the coming years.

Generally, the costs of the four regulatory approved regenerative therapy products for corneal epithelial disease tend to be higher comparing regular treatments such as corneal transplantation because autologous regenerative therapy requires individual manufacturing and testing in addition to two surgeries for cell source isolation and transplantation. High cost, in terms of social insurance burden, healthcare equality and cost-effectiveness, is a common issue for regenerative therapy products using autologous cells. Meanwhile, if products using allogeneic cell sources, such as those using iPSCs or ESCs, were put to practical use a significant cost reduction would be possible. Added to this, there is potential to increase production volume per batch and improve productivity through combining automation and efficient inventory management and transportation [45]. Thus, optimizing the manufacturing environment is expected to be essential for the future popularization of regenerative therapies.

In addition to the cornea, novel regenerative therapies using PSCs have been developed in the eye field with progress in the regenerative therapy for retinal diseases (Fig. 1). Regenerative therapies using somatic cells were first developed and clinically applied for treating retinal diseases, mainly for their paracrine effects [46–48]. Regarding regenerative therapies using PSCs, clinical trials of ESC-derived retinal pigment epithelial (RPE) transplantation have already been started, including the world's first clinical application of ESCs [49–53]. Moreover, the transplantation of an autologous iPSC-derived RPE cell sheet was conducted as the world's first clinical application of iPSCs [54]. Since then, the transplantation of allogeneic iPSC-derived RPE cell suspensions and retinal organoids containing photoreceptor cells have progressed to clinical application [55,56]. Regenerative therapies using PSCs have the flexibility to generate intended cell types and forms such as cell suspensions, sheets and organoids, and these can be generated by optimizing culture conditions. Actually, in the above retinal regeneration therapies, various graft forms are used depending on the disease and transplantation method, taking these advantages of PSCs. Importantly, PSCs, as starting materials, can be amplified almost infinitely, unlike somatic cells, resulting in significant scalability. This advantage allows us to obtain genome-edited PSCs, leading to the development of PSC-derived cells with low-immunogenicity by HLA knockout as alluded to above [20–22] as well as functionally enhanced PSC-derived cells [57]. Other recent innovations in ocular regenerative medicine include the development of conjunctival epithelial tissues and three-dimensional lacrimal gland-like organoids using SEAMs generated from human iPSCs [58–60] (Fig. 1). The development of new technologies related to regenerative medicine is active and ongoing, therefore, integrating these technologies such as PSC manipulation, genome editing and bioinformatics will allow us to treat previously incurable diseases by developing improved therapy methodologies for ocular tissue reconstruction.

The eye is highly suitable for the application of innovative regenerative therapies because of its small size and the small number of cells required for transplantation. In the case of cornea in particular, these advantages are enhanced by minimally invasive observation of the treatment process, and its low immunogenicity due to avascularity. Regenerative therapies for eye diseases have consistently been at the forefront of regenerative therapy product development, and the accumulated knowledge in this field will contribute to the advancement of new regenerative therapies for other tissues and organs.

Author contributions

Both authors contributed to the analysis of information and writing of this manuscript.

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Data availability statement

Not applicable.

Declaration of competing interest

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