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Current development of alternative treatments for endothelial decompensation: Cell-based therapy

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ABSTRACT

Current treatment for corneal endothelial dysfunction consists in the replacement of corneal endothelium by keratoplasty. Owing to the scarcity of donor corneas and the increasing number of transplants, alternative treatments such as cell-based therapies are necessary. In this article, we highlight the biological aspects of the cornea and the corneal endothelium, as well as the context that surrounds the need for new alternatives to conventional keratoplasty. We then review some of those experimental treatments in more detail, focusing on the development of the *in vitro* and preclinical phases of two cell-based therapies: tissue-engineered endothelial keratoplasty (TE-EK) and cell injection. In the case of TE-EK graft construction, we analyse the current progress, considering all the requirements it must meet in order to be functional. Moreover, we discuss the inherent drawbacks of endothelial keratoplasties, which TE-EK grafts should overcome in order to make surgical intervention easier and to improve the outcomes of current endothelial keratoplasties. Finally, we analyse the development of preclinical trials and their limitations in terms of performing an optimal functional evaluation of cell-based therapy, and we conclude by discussing early clinical trials in humans.

1. Introduction

The cornea is the first eye lens that participates in light refraction onto the retina, allowing for the collection of visual information from the external environment. The internal organization of its components, together with its avascularity, provides the physical basis for that function. Among its main corneal layers (epithelium, stroma and endothelium), the stroma is the most important one from an optical point of view. This layer accounts for two thirds of the total refractive power of the eye and is mainly composed of overlapping lamellae constituted of collagen I and proteoglycans, which attract water from the aqueous humor. In normal stromal deturgescence conditions, i.e. in a relative state of stromal dehydration, the homogeneous collagen fiber arrangement confers the required transparency and curvature for vision. However, the stroma cannot properly fulfill that role without the endothelium, which controls stromal dehydration and, therefore, ensures the correct distance among stromal collagen fibers to maintain

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Abbreviations

BK	bullous keratopathy
CI	cell injection
DM	Descemet membrane
DMEK	Descemet membrane endothelial keratoplasty
DSAEK	Descemet stripping automated endothelial keratoplasty
DWEK	descemetorhexis without endothelial keratoplasty
EC	endothelial cell
ECD	endothelial cell density
ECM	extracellular matrix
EK	endothelial keratoplasty
EPC	endothelial progenitor cell
FED	Fuchs' endothelial dystrophy
GMP	good manufacturing practice
hEC	human endothelial cell
IOP	intraocular pressure
iPSc	induced pluripotent stem cell
PK	penetrating keratoplasty
ROCK	rho-associated protein kinase
ROCKi	ROCK inhibitor
TE	tissue engineering
TE-EK	tissue-engineered endothelial keratoplasty
ZO-1	zonula occludens-1

corneal features (Meek and Knupp, 2015).

If endothelium fails, vision can be impaired and, in severe cases, surgical intervention may be required to replace the damaged tissue with a new one by keratoplasty. Here, we review some alternative treatments for endothelial dysfunction, which otherwise often ends in corneal or endothelial transplantation. We particularly focus on the development of two cell-based therapies: cell injection (CI) and tissue-engineered endothelial keratoplasty (TE-EK), considering the biological and functional aspects of the endothelium and the cornea. Regarding TE-EK, we analyse the engineered graft keeping in mind the drawbacks of conventional endothelial keratoplasty, which this future treatment should match or exceed, and we conclude by providing an overview of clinical trials, which come into play to assess the actual value of these new treatments.

1.1. Corneal endothelium: function, aging and disease

Corneal endothelium is constituted by hexagonal-like endothelial cells (ECs) that form a packed cell monolayer on a specialized basal membrane called Descemet membrane (DM). A strong cohesion among ECs is generated via adhesion complexes mainly formed by adherens junctions, localized to the lateral-basal membrane, and tight junctions, localized to the lateral-apical membrane (He et al., 2016b). Tight junctions make it possible for the endothelium to play the role of a leaky barrier, as they control transmembrane circulation of molecules such as bicarbonate ions, sodium, chloride and glucose between the aqueous humor and the stroma (Bonanno, 2012; Srinivas, 2012), together with different transporters, such as the Na^+/K^+ ATPase pump and the sodium bicarbonate cotransporter, which counteract the flux of water into the stroma by pumping ions and other solutes into the aqueous humor. Thus, the endothelium, as a leaky-pump barrier, keeps the water content of the stroma at adequate levels so that it remains transparent (Bonanno, 2012; Edelhauser, 2006; Srinivas, 2012).

Human ECs (hECs) show a limited capacity for division *in vivo*. They are mostly arrested in phase G1 of the cell cycle, and a decrease in proliferation potential from the periphery to the central part of the cornea can be observed (Joyce, 2012; Joyce et al., 1996; Mimura and Joyce, 2006; Senoo and Joyce, 2000). Without the possibility to replace dead ECs, endothelial cell density (ECD) decreases at a rate of 0.3% per year, with an average ECD around 3000 cells/mm^2 in adult individuals (Hollingsworth et al., 2001). Thus, the strategies used by the endothelium to maintain a functional cell monolayer are cell migration and contiguous cell spread (Matsuda et al., 1985). Due to this, the adult endothelium appears as a sheet of cells distributed following a cobblestone pattern generated by variations in shape (pleomorphism) and size (polymegathism) (Hollingsworth et al., 2001).

If the rate of EC loss is unusually high and ECD decreases down to values below a threshold of approximately 500 cells/mm^2, the endothelium cannot execute its function properly, subsequently leading to corneal edema, visual impairment and even blindness (Mishima, 1982). This extreme situation can be observed, for example, in endothelia affected by Fuchs' endothelial dystrophy (FED). FED has an estimated prevalence of 4–9% in some American and European regions and 3–7% in Asian regions (Soh et al., 2019). This dystrophy normally begins at middle age and progressively develops for years. Signals of FED consist of fast ECD decrease, changes in ECs morphology and formation of guttae in DM (Feizi, 2018; Soh et al., 2019). In its later stages, endothelial decompensation can result in epithelial bullae as a consequence of stromal edema (Eghrari et al., 2015). The presence of epithelial bullae can also be due to endothelial trauma caused, for example, during intraocular surgeries such as cataract extraction and can lead to bullous keratopathy (BK). Elderly individuals are the ones who most frequently develop BK as a consequence of trauma (Feizi, 2018).

1.2. Corneal transplant for endothelial decompensation

Corneal transplant is the conventional treatment for irreversible endothelial decompensation (Feizi, 2018). In the 20th century, transplantation of the full-thickness cornea, or penetrating keratoplasty (PK), was the gold standard procedure; however, nowadays, endothelial keratoplasty (EKs) disputes their place as the surgery of choice for providing a healthy endothelium for visual recovery. EK consists in the selective replacement of injured endothelium without substituting the remaining, non-damaged corneal layers. Among the different types of EK, Descemet stripping automated endothelial keratoplasty (DSAEK) (Gorovoy, 2006; Price and Price, 2006) and Descemet membrane endothelial keratoplasty (DMEK) (Melles et al., 2002) are the most successful and widely performed. In DSAEK, the graft consists of a curved cut from the donated posterior cornea with a thin piece of stroma. In DMEK, the replacement is structurally more exact, as the graft is only composed of endothelium on DM. In both cases, the graft is inserted after descemetorhexis (DM-endothelium removal) (Maharana et al., 2017).

The obvious transition from PK to EK in developed countries (Flockerzi et al., 2018; Nishino et al., 2019; Park et al., 2015) is widely justified by the benefits of EK over PK. EK is less invasive and allows maintaining the structural integrity of the eyeball while avoiding the complications associated with open-sky PK, including sutures and infections (Boynton and Woodward, 2015). Moreover, the reduction or absence of stroma minimizes the risk of a graft rejection episode, as the graft contains fewer antigen-presenting cells (Hos et al., 2019). Furthermore, patient visual acuity recovery is better and more quickly achieved after EK than after PK (Boynton and Woodward, 2015). All these advantages greatly compensate for the main drawbacks of EK. These are related to the highly demanding DSAEK and DMEK techniques, whose correct performance entails a long and difficult learning curve due to the issues associated with graft handling. Severe graft EC loss, detected during the early postsurgical period within one, six, or twelve months after surgery and also described for PK (Ku et al., 2017; Price et al., 2016; Woo et al., 2019), has also been reported as a

disadvantage of EK, along with graft detachment. Moreover, EK is not free of refractive aberrations due to irregularities in the graft or the host-graft interface, although they are less severe and more predictable than in the case of PK (Boynton and Woodward, 2015; Price et al., 2017). However, all these disadvantages have been partially solved by using grafts with high ECD, improving surgical techniques and developing new instruments for specific keratoplasty steps. Among these are the improvement of the graft collection process (Parekh et al., 2017), the use of graft-insertion devices minimize graft handling (Khor et al., 2011; Soma et al., 2019), and easy re-attachment approaches in case of graft detachment (Parekh et al., 2018a).

The safety and excellent results of EK, together with the aging of the population and the subsequent increase in late-onset eye diseases such as cataracts or glaucoma, whose treatments can lead to endothelial damage (Feizi, 2018), encourage surgeons and patients to perform this surgery at earlier stages of endothelial dysfunction (Flockerzi et al., 2018). This situation leads to an imbalance between the increasing number of corneal transplants and the current stock of donated cadaveric corneas, the raw material for all types of keratoplasty (Gain et al., 2016). For EK, the graft is excised from a cornea with high-quality endothelium. Considering donor selection criteria (European Directorate for the Quality of Medicines and Healthcare, 2017) and the decrease in ECD with aging, compliance with all the requirements for EK grafts is difficult to achieve.

2. Experimental treatments for endothelial decompensation

Several alternatives to conventional PK or EK have been proposed for the treatment of endothelial dysfunction. These experimental therapies aim to regenerate the corneal endothelium using different methods and are currently in different states of development and understanding. Here, we classify them according to the origin of the ECs used to regenerate the endothelium.

2.1. Treatments with autologous ECs

Descemetorhexis without endothelial keratoplasty (DWEK) (Garcerant et al., 2019) and acellular DM transplantation (Soh and Mehta, 2018) are experimental treatments based on the migration and enlargement capacity of hECs *in vivo*. Hence, after these surgeries, host ECs located in the endothelial periphery should migrate centripetally and either cover the bare stroma generated by DWEK or colonize the transplanted denuded DM. Sometimes, DWEK is combined with the administration of a rho-associated protein kinase (ROCK) inhibitor (ROCKi) formulated as eye drops to promote endothelial regeneration (Garcerant et al., 2019). The ROCKi protein family has shown positive modulation of EC migration and cell cycle and has been demonstrated to promote cell adhesion (Okumura et al, 2009, 2017) and to contribute to the conservation of a normal EC morphology and phenotype (Okumura et al, 2012, 2017). The huge potential of ROCKi has not only been used in this type of alternative treatments, but also in cell-based therapies (Okumura et al., 2017), which are later reviewed in section 2.2.2. as well as in the form of experimental eye drops to treat certain endothelial dysfunctions (Okumura et al., 2013).

Considering the low number of studies on DWEK and acellular DM transplantation, as well as other clinical cases related to *in vivo* endothelium regeneration after a failed EK, some authors have suggested the possibility of a certain degree of hEC division, which could explain the improvements in endothelial cell density and in visual acuity of patients (Van den Bogerd et al., 2018a). Nonetheless, the relative success of these treatments varies among patients and among clinical case reports, and it is higher in the case of FED due to the presence of a large cell reservoir at the periphery, which is sometimes lacking in BK (Garcerant et al., 2019; Soh and Mehta, 2018). Further research is needed to understand this variability in the results and to turn DWEK and DM transplantation into reliable treatments for endothelial decompensation.

2.2. Treatments with allogenic sources of ECs

Cell-based therapies that use allogenic ECs are the most developed and, consequently, the most promising experimental treatments for endothelial decompensation compared with the above-mentioned treatments. These include cell injection (CI) for *in vivo* formation of endothelium and tissue engineering (TE) for *in vitro* construction of EK grafts.

2.2.1. Cell sources for cell-based therapies

The collection of a sufficient number of hECs to be used in cell-based therapies must avoid any ethical issues and guarantee the safety of the engrafted patient. At present, these premises reduce the possibility of employing three unlimited hEC sources: embryonic stem cells, induced pluripotent stem cells (iPSc) and immortalized hECs. While the first one involves the destruction of an embryo, which is ethically questionable, both iPS and immortalized cells are linked to tumorigenesis (Maqsood et al., 2013; Yoshihara et al., 2017) in relation to the genetic reprogramming used to generate them.

Endothelial progenitor cells (EPCs) represent a cell source with a certain capacity for division. EPCs have been suggested to be present in Schwalbe's line at the endothelial periphery and/or in the trabecular meshwork. These hypotheses are supported by the expression of stem, neural crest and proliferation markers in cells from those areas; by the capacity of these cells to form spheres under culture conditions, which is characteristic of neural crest-derived cells with proliferative abilities; and by successful endothelial differentiation assays (Hara et al., 2014; Katikireddy et al., 2016; McGowan et al., 2007; Mimura et al., 2010; Yam et al., 2019; Yokoo et al., 2005; Zhang et al., 2018). With respect to the use of these cell sources for cell-based therapies, Mimura et al. employed sphere-forming progenitor cells for CI therapy, while Hara et al. used progenitor cell-derived ECs to build an endothelial graft using atelocollagen as a carrier; both were used in an animal model with considerable success (Hara et al., 2014; Mimura et al., 2005b, 2005c). However, as far as we know, this source of ECs has not been further used in any other cell-based therapy studies, probably because of their heterogeneous proliferative potential, which in turn is due to the regulation of pathways related to their cell differentiation process (Hirata-Tominaga et al., 2013), to donor age (Hara et al., 2014) or to other donor-related variables. Further efforts are clearly warranted to understand the mechanisms of migration and differentiation of EPCs and to characterize them, as well as to develop an effective protocol for their isolation and expansion that can provide ECs for cell-based therapies (Sie et al., 2020).

Primary hECS, obtained directly from corneal endothelium, are the most commonly used type of cells in TE and CI therapy studies. For this purpose, basic research has provided the knowledge required for hEC isolation and culture, as well as for the progressive understanding of their biology both *in vivo* and *in vitro*. Thus, by using culture media supplemented with serum and mitogenic factors that force hEC division (Fig. 1), a sufficient number of cells with the desirable properties of hECs can be obtained (Soh et al., 2016). These properties are, on one hand, an adequate hexagonal morphology and, on the other, endothelial biomarkers, such as the typically used ZO-1 of tight junctions and Na^+/K^+ ATPase, among others, whose expression and protein cytolocalization resemble that of the *in vivo* phenotype of hECs (Frausto et al., 2016; He et al., 2016b).

Nevertheless, primary hECs are not free of limitations. They can dedifferentiate in culture, lose their recognizable biomarkers and acquire a mesenchymal-like morphology and phenotype, frequently after the third passage (Peh et al., 2011). This change occurs within a process known as endothelial-mesenchymal transition (EMT) (Roy et al., 2015), which renders these hECs invalid for cell-based therapies and thus reduces the possibility of obtaining a large amount of cells from a single donor. Moreover, variability among donors can lead to a reduction in the number of acceptable hECs because even if cells have been cultured

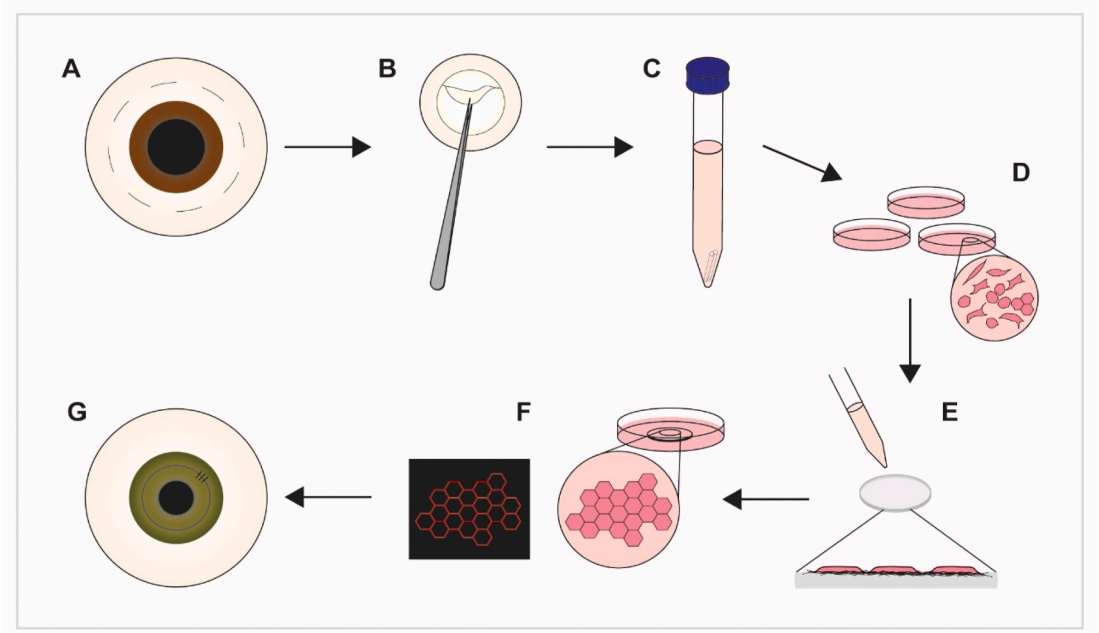

Fig. 1. Example of the workflow of an *in vitro* phase of tissue engineered-endothelial keratoplasty (TE-EK) therapy. Donor cornea is excised from an eyeball (A). The Descemet membrane-endothelium complex is manually peeled off (B) and incubated with collagenase or other enzymes for endothelial cell (EC) isolation (C). ECs are expanded (D), and an adequate number of cells is seeded on a flat thin transparent carrier, previously functionalized with a coating to enhance cell adhesion and proliferation (E). ECs on the carrier are cultured to allow for the formation of a tightly packed endothelium. Quality control of the process is performed to ensure the adequate hexagonal morphology of cells at the end of culture (F, right) and expression of EC biomarkers, such as those that participate in the cell-cell adhesion complex, which would be cytolocalizable to their membranes (F, left). The engineered graft is transplanted using the current EK techniques to restore the visual acuity of a patient (G).

under similar conditions, they can behave differently due to, for example, the donor's age or health condition (Soh et al., 2016).

At present, the best available source of primary hECs is found in eye banks. On one hand, healthy endothelium from donated corneas discarded either due to pathologies in other corneal layers or to low ECD can be used for hEC isolation and culture. On the other hand, the endothelial periphery that remains in a donated cornea after trephination for PK, as proposed by Vázquez et al. (2016), or after stripping the central area of the DM-endothelium complex for DMEK could be recycled for the same purpose.

2.2.2. Cell injection for in vivo endothelium regeneration

Comparing CI and TE, CI is the less invasive and less technically demanding treatment. In addition, the *in vitro* phase of CI therapy is short, as it only requires the expansion of hECs. A suspension of these cells in a vehicle solution is then injected into the anterior chamber. This constitutes another advantage of CI over TE, as CI does not require the presence of a carrier. Conversely, TE graft transplantation requires a carrier, which could affect light refraction and visual acuity, as explained below. In CI, hECs should adhere to the inner surface of the cornea, which can be either empty stroma or acellular DM. The presence of DM seems to lead to better results (Xia et al., 2019), which suggests that this therapy could be limited to those endothelial dysfunctions with a healthy DM, such as some BK cases, while it could not be used, for example, in late-stage FED.

One of the crucial points of CI is the control of the amount and fate of injected ECs, because excess or non-adhered cells could obstruct trabecular meshwork and, in a worst-case scenario, could block the outflow of aqueous humor, consequently elevating intraocular pressure (IOP). Moreover, those cells could deposit ectopically in other tissues of the eye or body (Mimura et al., 2003). To ensure correct cell disposition, the simplest technique for leading ECs to the inner surface is by gravity, with the engrafted host adopting a prone position after cell injection, thus allowing for cell adhesion (Mimura et al., 2007). Several researchers have demonstrated that endocytosis of non-cytotoxic concentrations of iron particles (Mimura et al, 2003, 2005a) or superparamagnetic microspheres (Patel et al., 2009; Xia et al., 2019) during the *in vitro* phase of CI therapy helped lead ECs to the recolonization area, either using a magnet to attract them in combination with gravity (Xia et al., 2019) or not (Mimura et al, 2003, 2005a).

Additionally, to enhance cell adhesion, ROCKi Y-27632 is usually added to the EC suspension for cell injection (Table 1). With this supplement, the correct expression of EC functional biomarkers and the normal hexagonal-like morphology has also been observed to improve and yielded substantially better outcomes in preclinical studies (Okumura et al., 2016b).

2.2.3. Tissue engineering of an EK graft

TE offers the possibility of creating EK grafts with customized characteristics, which could be used for all types of endothelial decompensations. Tissue-engineered EK (TE-EK), with ECD values above the eye banks' thresholds for EK surgeries, could replace conventional EK by maintaining and simplifying the well-known EK surgical technique while matching or exceeding its patient outcomes. Considering the drawbacks of EK (as discussed in section 1.3.), which can summarize as difficult graft handling, graft detachment, initially detected severe EC loss and refractive aberrations, tissue engineering should solve or reduce these complications.

Building a TE-EK graft requires a biocompatible carrier for the regenerated endothelium. Endothelium can be developed directly on said carrier or be transferred to it after its formation on a different surface, such as a thermo-responsive plastic (Lai et al., 2015). Carriers whose composition is essentially collagen, such as corneal stromal lamellae (Arnalich-Montiel et al., 2019; He et al., 2016a; Peh et al, 2017, 2019), basal cell membranes (Liu et al., 2019; Van den Bogerd et al., 2018b), fish scales (Parekh et al., 2018b) and collagen or collagen-derived sheets (Li et al., 2017; Vázquez et al., 2016), have been the most extensively tested *in vitro* and/or in animal models, together with carriers of other

Table 1
Attempts for cell injection therapy in animal models. Thickness should be assessed relative to the normal (untreated) corneal thickness in the corresponding animal model, which is approximately 400 μm for rabbits, over 470 μm for monkeys and around 578 μm for cats. ECD: endothelial cell density; fEC: feline endothelial cells; hEC: human endothelial cells; mEC: monkey endothelial cells; N/A: no data available; rEC: rabbit endothelial cells; ROCKi: ROCK inhibitor; w/: with; w/o: without.

Reference	Preclinical phase					
	Injected cells	Animal	Follow-up	*Final follow-up*		
				ECD (cells/mm^2)	Thickness (μm)	Transparency
Xia et al. (2019)	Magnetic endocytosing rECs	Rabbit	3 months	N/A	N/A	Yes
Peh et al. (2019)	hEC w/ROCKi	Rabbit	3 weeks	1409 ± 128	582.5 ± 171.5	Yes
Okumura et al. (2018)	rEC w/ROCKi	Rabbit	2 weeks	1602 ± 241	<600	Yes
Okumura et al. (2016b)	mEC w/and w/o ROCKi	Monkey	1 year	>2000 (w/ROCKi)	~600	Yes
	hEC w/and w/o ROCKi	Monkey	3 months	2890 (w/ROCKi)	~850	Yes
Okumura et al. (2016a)	rEC w/ROCKi	Rabbit	2 weeks	>2000	~400	Yes
Bostan et al. (2016)	fEC w/ROCKi	Cat	1 month	1015	781	Yes
Okumura et al. (2012)	mEC w/ROCKi	Monkey	3 months	2208	<600	Yes
	rEC w/ROCKi	Rabbit	2 weeks	N/A	409	Yes
Mimura et al. (2005c)	Spheres w/progenitor cells of hEC	Rabbit	3 weeks	2781 ± 92	394 ± 26	Yes
Mimura et al. (2005b)	Spheres w/progenitor cells of rEC	Rabbit	3 weeks	2963 ± 302	~450 - 350	Yes
Mimura et al. (2005a)	Iron-endocytosing rEC	Rabbit	12 months	2581 ± 230	407 ± 11	Yes
Mimura et al. (2003)	Iron-endocytosing rEC	Rabbit	5 weeks	N/A	~500 - 400	Yes

materials such as silk fibroin (Vázquez et al., 2017) or synthetic polymers (Kim et al., 2017; Kruse et al., 2018; Salehi et al., 2017).

Nonetheless, not all these carriers are valid to engineer an EK graft, as several physical properties are required to ensure functional endothelium regeneration and correct vision. The characteristics of these carriers can sometimes be complemented by culture techniques that promote the formation of the endothelial layer.

2.2.3.1. Carrier transparency, thickness and shape. Transparency is the first optical parameter intuitively sought in a carrier. However, it is not sufficient to guarantee correct visual acuity. Irregularities in carrier structure or in the junction between the host and the TE-EK graft can lead to refractive aberrations. To minimize them, thickness and shape should be controlled.

According to clinical studies, thin grafts such as those used for DMEK decrease the probabilities of refractive aberrations (Dickman et al., 2016). This supports the use of very thin carriers with thickness values close to those of DMEK grafts, as some research groups are currently attempting by using silk fibroin and collagen-derived sheets (Vázquez et al, 2016, 2017; Yoshida et al., 2017).

As for shape, flat carriers could cause wrinkles when attached to the inner face of the cornea. However, carriers with a curvature similar to that of the host stroma could fit and adhere properly across its whole surface, thus decreasing the chance of graft detachment (Kimoto et al., 2014) and fibrosis in wrinkled spaces, which would otherwise lead to refractive aberrations (Müller et al., 2016). Taking this issue into account, researchers gave a curved shape to their porcine atelocollagen (Yoshida et al, 2014, 2017), collagen I (Li et al., 2017) and gelatin carriers (Kimoto et al., 2014) or directly used decellularized corneal lamellae cut by femtosecond laser, the same method used to generate ultra-thin DSAEK grafts (Arnalich-Montiel et al., 2019; He et al., 2016a; Peh et al, 2017, 2019).

2.2.3.2. Carrier strength, elasticity and permeability. Carriers should tolerate IOP and act as a structural barrier that allows molecules to cross between the stroma and the endothelium or the aqueous humor. For these purposes, they should have sufficient strength and elasticity, as well as a certain degree of permeability, without disrupting their structural internal organization.

The two aforementioned mechanical properties are also involved in TE-EK graft handling. As the unfolding step in DSAEK is easier and faster than in DMEK, thanks to the slight rigidity that the stromal layer confers to grafts, the ideal TE-EK graft should have a strength and elasticity similar to those of DSAEK grafts, thus involving a shorter learning curve for surgeons (He et al., 2016a). These characteristics occur naturally in corneal stromal lamellae, as noted by several research groups (Arnalich-Montiel et al., 2019; He et al., 2016a; Honda et al., 2009; Peh et al, 2017, 2019). Interestingly, some authors demonstrated the handleability of their manufactured TE-EK grafts without carrying out a pre-clinical trial. They performed a simple but effective assay consisting in loading the constructed graft with the current EK graft insertion instruments, introducing it in an excised eyeball, and unfolding it (He et al., 2016a; Vázquez et al., 2015).

2.2.3.3. Topography and hydrophilicity of carrier. Moving on to the *in vitro* step of endothelial regeneration, carriers should show high hydrophilicity to allow for initial cell adhesion, usually confirmed by a low contact angle of a water drop on its surface (Kim et al, 2015, 2017, 2018). In addition, a slightly homogeneous rough topography promotes the development of a confluent and packed endothelium without gaps among cells (Muhammad et al., 2015; Rizwan et al., 2017).

2.2.3.4. Functionalization of carriers. Functionalization of carriers with coatings is a culture technique used to improve EC adhesion and endothelial regeneration (Fig. 1) (Arnalich-Montiel et al., 2019; Parekh et al., 2018b; Salehi et al., 2017; Van den Bogerd et al., 2018b; Vázquez et al., 2017). Coatings composed of proteins or glycoproteins, such as laminin, collagen or fibronectin, which normally constitute basal membranes such as DM, provide an increasing number of binding points for EC integrins. They are widely used *in vitro* for EC expansion (Okumura et al., 2015; Peh et al., 2015). When using them, EC usually showed better expression of functional endothelial biomarkers and better EC morphology (Arnalich-Montiel et al., 2019; Okumura et al., 2015; Vázquez et al., 2017). Additionally, functionalization can be performed by incorporating other types of substances that promote cell proliferation and differentiation, such as β-carotene, to carrier surfaces (Kim et al., 2018).

2.2.3.5. Bioreactors for endothelial regeneration. Endothelial regeneration and TE-EK graft maturation are usually performed under static conditions and without pressure (Fig. 1). Several studies have developed perfusion devices that offer the possibility of recreating the physiological corneal environment, adding pressure and an exchange rate of a medium simulating aqueous humor outflow in the cornea. Natural corneal conditions were demonstrated to improve expression of functional biomarkers of ECs and their recognizable morphology (Li et al., 2017; Thériault et al., 2019), thus providing excellent results for endothelial regeneration on a collagen carrier for TE-EK (Li et al., 2017).

2.2.4. Preclinical trials on cell-based therapies

In recent years, numerous research groups have tested their designed TE-EK grafts in animal models, while additional studies on CI therapy have been carried out (briefly summarized in Tables 1 and 2). These studies share some particularities in terms of their performance and evaluation.

2.2.4.1. Animal models. The selection of an animal model for preclinical trials is significant, as those animals should be as close to humans as possible to allow extrapolating results. Thus, some researchers have carried out their preclinical trials on cats (Bostan et al., 2016) and monkeys (Koizumi et al., 2008; Okumura et al, 2012, 2016b), whose corneal and/or corneal endothelial characteristics are the most comparable to those of humans (Proulx and Brunette, 2012). However, these have been a minority, while the use of rabbits has been notable. The tendency to use this rodent model is explained by the fact that rabbits are small, manageable and affordable (Proulx and Brunette, 2012). Nevertheless, they show several drawbacks, such as their narrow anterior chamber, which complicates TE-EK graft testing (Yoshida et al., 2017) and requires training to perform the surgery, and the fast *in vivo* division of rabbit ECs (rECs) (Van Horn et al., 1977). One strategy that has been thoroughly employed to reduce the likelihood of host rEC division and migration to the engrafted area of the cornea consists in limiting the duration of the assessment to 2–6 weeks (Peh et al., 2019) (Tables 1 and 2). However, this strategy prevents the generation of long-term results. Hsiue et al. and Lai et al. supplied a mitosis inhibitor (mitomycin-C) to the transplanted cornea (Hsiue et al., 2006; Lai et al, 2007, 2015) to prevent any EC growth and, therefore, to allow prolonging follow-up for several months.

Regardless of the selected animal model, EC labeling with cell trackers such as quantum dots (Toda et al., 2019) or membrane fluorescent dies (Bostan et al., 2016; Okumura et al., 2016b; Yoshida et al., 2017) before injection or keratoplasty is a widely used technique to verify the origin of ECs. Thus, it is possible to confirm that ECs in the engrafted area are those incorporated by the selected cell-based therapy rather than host ECs, as well as to ensure that injected ECs are not deposited on any other structure within the eyeball. With the same aim, Xia et al. used transduced ECs, which express green fluorescent protein, for CI therapy; similarly, Peh et al. carried out an immunofluorescence assay with a human-specific nuclei antibody on the endothelium of the excised TE-transplanted cornea (Peh et al., 2017; Xia et al., 2019).

2.2.4.2. Functional evaluation of therapy and endothelium. During and after the follow-up period of cell-based therapy, several clinical parameters are usually recorded to provide information about the overall functionality of the treatment. Thickness and transparency, both related to each other, are the most important ones, although measuring IOP in CI therapy is required to ensure the lack of presence of cells in the trabecular meshwork (Bostan et al., 2016; Okumura et al., 2018; Xia et al., 2019). Immediately after the intervention, an increase in corneal thickness due to stromal edema and a loss of transparency are observed. If the treatment succeeds and the regenerated endothelium accomplishes its role, the cornea should become thinner and more transparent after a few days, and its final thickness should be as close as possible to that of a healthy non-transplanted cornea of the animal model. For example, the ideal engrafted corneal thickness should be around 400 μm in rabbits (Chan et al., 1983) and around 470 μm for primates (Zurawski et al., 1989), while for cats, thickness should be approximately 578 μm (Gilger et al., 1993). Despite the short follow-up periods, many researchers have succeeded in achieving the normal corneal thickness of

Table 2
Attempts for tissue-engineered endothelial keratoplasty in animal models. Research was limited to those carriers that have been tested in pre-clinical trials using EK techniques or, in the case of those marked by an asterisk (*), using the predecessor of EK: deep lamellar endothelial keratoplasty. Thickness should be assessed relative to the normal (untreated) corneal thickness of the corresponding animal model, which is approximately 400 μm for rabbits and approximately 470 μm for monkeys. DM: Descemet membrane; ECD: endothelial cell density; hEC: human endothelial cells; mEC: monkey endothelial cells; N/A: no data available; PCL: polycaprolactone; PEG: poly(ethylene glycol); rEC: rabbit endothelial cells; sEC: sheep endothelial cell; w/: with.

	In vitro phase			Preclinical phase				
Reference	Carrier	Cells	ECD (cells/mm^2)	Animal	Follow-up	*Final follow-up*		
						ECD (cells/mm^2)	Thickness (μm)	Transparency
Peh et al. (2019)	Human decellularized stromal lamella	hEC	N/A	Rabbit	3 weeks	1248 ± 64	484.3 ± 73.7	Yes
Arnalich-Montiel et al. (2019)	Human decellularized stromal lamella w/FNC coating®	hEC	2300	Rabbit	4 weeks	N/A	747	Relative
Kim et al. (2018)	Bovine decellularized amniotic membrane	RNase 5 vector-transfected hEC	1244	Rabbit	4 weeks	N/A	426.3 ± 153.9	Yes
Yoshida et al. (2017)	Porcine atelocollagen	hEC	N/A	Rabbit	2 weeks	N/A	997.3 ± 134.9	Relative
Vázquez et al. (2017)	Fibroin silk w/FNC coating®	rEC	N/A	Rabbit	6 weeks	N/A	366	Yes
Peh et al. (2017)	Decellularized stromal lamella and DM	hEC	2380 ± 192; 2680 ± 438	Rabbit	3 weeks	1321 ± 23	517.3 ± 151.3	Yes
Vázquez et al. (2016)	Human collagen I	rEC	N/A	Rabbit	6 weeks	N/A	Close to native	Yes
Lai et al. (2015)	Hyaluronic acid	rEC	3289 ± 70	Rabbit	4 weeks	3216 ± 95	412.7 ± 25.0	Yes
Hara et al. (2014)	Atelocollagen	Progenitor-derived hEC	N/A	Rabbit	4 weeks	2263 ± 215	369	Yes
Ozcelik et al. (2014)	PEG + hydrogel + PCL	sEC	3150 ± 459	Sheep	4 weeks	N/A	N/A	Yes
Kimoto et al. (2014)	Gelatin (collagen-derived) w/ atellocolagen coating	mEC	2944 ± 350	Monkey	4 weeks	2300 ± 100	>700	Yes
Honda et al. (2009)	Stromal lamella	hEC	1656	Rabbit	4 weeks	N/A	776	Yes
(Koizumi et al, 2007, 2008)	Collagen I (Vitrigel)	mEC	2240 ± 30.9	Monkey	6 months; 2 years	1992-2475; 1642	Close to native	Yes
(Lai et al., 2007)*	Degradable gelatin (collagen-derived)	hEC	2587 ± 272	Rabbit	6 months	N/A	504.4 ± 24.7	Yes
Hsiue et al. (2006)	Gelatin (collagen-derived)	hEC	~2500	Rabbit	3 months	N/A	N/A	Yes
(Mimura et al., 2004)*	Collagen I sheet	hEC	N/A	Rabbit	4 weeks	2531 ± 290	~400	Mild opacity

their animal model (Kim et al., 2018; Lai et al., 2015; Okumura et al., 2016a; Vázquez et al., 2017).

The quality and functionality of the regenerated endothelium at the end of the follow-up period must also be assessed. Some researchers have used the ECD parameter for this purpose. An ECD over the endothelium function impairment threshold indicates a correct endothelial function, which is related to the recovery of transparency and relatively normal thickness of the treated cornea. In the case of TE-EK, some authors have recorded graft ECD before and after transplantation, which provides information about initially detected severe EC loss caused by the intrinsic characteristics of the surgical technique, which is one of the main problems of EKs, as explained in section 1.3 above (Kimoto et al., 2014; Koizumi et al, 2007, 2008; Lai et al., 2015; Peh et al., 2017). Likewise, corneal and endothelial evaluation is performed on excised engrafted corneas using histological techniques, e.g. basic staining techniques such as alizarin red, Masson's trichrome staining, electron microscopy or immunofluorescence. These are usually employed to observe the morphology and/or expression of functional biomarkers of ECs such as ZO-1, N-cadherin or Na^+/K^+ ATPase and, in the case of TE-EK, to provide information about graft adhesion to the recipient cornea (Arnalich-Montiel et al., 2019; Kim et al., 2018; Okumura et al., 2016b; Peh et al, 2017, 2019; Vázquez et al, 2016, 2017; Xia et al., 2019; Yoshida et al., 2017).

With respect to visual acuity experiments, while they could provide information about possible refractive aberrations, they are generally overlooked in preclinical trials. This is understandable because the most commonly used animal model is rabbit, on which the test required to assess visual acuity cannot be performed, as they are not usually trained to focus on a target to measure aberrations. Furthermore, their optical aberrations are different from those found in the human cornea and would therefore be difficult to extrapolate (Chen et al., 2014).

2.2.5. *Clinical trials of cell-based therapies*

Although preclinical trials can be successful, the possibility of offering a real alternative treatment for endothelial decompensation can only be supported by optimal results from clinical trials. This entails guaranteeing quality and patient safety across the whole TE-EK and CI procedure. All the steps of TE-EK graft construction and CI injection preparation, from the *in vitro* phase to the intervention itself, must comply with good manufacturing practices (GMP) and with regional quality and safety regulations and guidelines for the clinical use of cell-based therapies. The current common protocols for EC culture do not fulfill these requirements, since they use animal-derived materials, which could cause xenorejection or infection with animal pathogens. Therefore, Peh et al. reformulated their culture protocol for TE-EK, testing different substitutes to replace animal-derived serum and coatings (Peh et al., 2019). Okumura et al. created a new vehicle solution for CI to avoid injecting culture media into the patient's eyeball (Okumura et al., 2016a).

Once safety and quality can be ensured, it is necessary to verify the efficacy of the new treatment, which should be equal to or better than that of the standard treatment. So far, only one clinical trial on CI therapy has been initiated by Kinoshita et al. The initial results after a two-year period showed a recovery of transparency and normal thickness, as well as an improvement in visual acuity in some patients (Kinoshita et al., 2018). Regarding tissue engineering, the preclinical trials by Peh et al. (Peh et al, 2017, 2019) will be followed by the first approved clinical trial for TE-EK (Peh et al., 2019), where their TE-EK graft with decellularized stromal lamellae as carriers will be assessed and applied on humans.

Other requirements in order to implement a cell-based therapy in centers are reproducibility and cost-effectiveness. The new treatment should be affordable for health care systems or patients. In addition, the new cell-based therapy should have an impact on health equal to or better than that of the standard treatment for endothelial dysfunctions. CI is clearly less expensive than TE, as neither a carrier nor a long *in vitro* phase are necessary. As far as we know, only one economic analysis of TE-EK has been performed. In said study, Tan et al. calculated the cost of TE-EK therapy using a collagen sheet and concluded that it would be four times cheaper than conventional EK (Tan et al., 2014).

The selection of an affordable and easily reproducible carrier, such as collagen sheets or stromal lamellae from discarded corneas, could contribute to decreasing the cost. Moreover, the use of existing equipment routinely used in eye banks, especially in those where EK grafts are prepared and delivered and corneal organ cultures are stored, could reduce the investment costs. Nevertheless, long-term clinical outcomes must be obtained for both cell-based therapies in order to accurately assess their overall advantages and disadvantages.

3. Conclusions and future perspectives

We are currently closer than ever to offering a real alternative treatment for endothelial decompensation. Many successful advances have been made in the development of TE-EK grafts and in the performance of preclinical TE-EK and CI therapy trials, which have already allowed moving on to clinical research. However, further information is needed about some aspects of the adaptation of culture protocols to GMP, as well as about the economic cost of these cell-based therapies, which should be included as additional objective in future studies on cell-based therapies.

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References

Arnalich-Montiel, F., Moratilla, A., Fuentes-Julián, S., Aparicio, V., Martin, M.C., Peh, G., Mehta, J.S., Adnan, K., Porrua, L., Pérez-Sarriegui, A., de Miguel, M.P., 2019. Treatment of corneal endothelial damage in a rabbit model with a bioengineered graft using human decellularized corneal lamina and cultured human corneal endothelium. PloS One 14, 1–16. https://doi.org/10.1371/journal.pone.0225480.

Bonanno, J.A., 2012. Molecular mechanisms underlying the corneal endothelial pump. Exp. Eye Res. 95, 2–7. https://doi.org/10.1016/j.exer.2011.06.004.

Bostan, C., Thériault, M., Forget, K.J., Doyon, C., Cameron, J.D., Proulx, S., Brunette, I., 2016. In vivo functionality of a corneal endothelium transplanted by cell-injection therapy in a feline model. Investig. Ophthalmol. Vis. Sci. 57, 1620–1634. https://doi.org/10.1167/iovs.15-17625.

Boynton, G.E., Woodward, M.A., 2015. Evolving techniques in corneal transplantation. Curr. Surg. Reports 3, 1–13. https://doi.org/10.1007/s40137-014-0079-5.

Chan, T., Payor, S., Holden, B., 1983. Corneal thickness profiles in rabbits using an ultrasonic pachometer. | IOVS | ARVO Journals. Invest. Ophthalmol. Vis. Sci. 24, 1408–1410.

Chen, L., Huang, L.C., Gray, B., Chernyak, D.A., 2014. Comparison of wavefront aberrations in rabbit and human eyes. Clin. Exp. Optom. 97, 534–539. https://doi.org/10.1111/cxo.12184.

Dickman, M.M., Kruit, P.J., Remeijer, L., van Rooij, J., Van der Lelij, A., Wijdh, R.H.J., van den Biggelaar, F.J.H.M., Berendschot, T.T.J.M., Nuijts, R.M.M.A., 2016. A randomized multicenter clinical trial of ultrathin descemet stripping automated endothelial keratoplasty (DSAEK) versus DSAEK. Ophthalmology 123, 2276–2284. https://doi.org/10.1016/j.ophtha.2016.07.036.

Edelhauser, H.F., 2006. The balance between corneal transparency and edema: the proctor lecture. Investigative Ophthalmology and Visual Science. The Association for Research in Vision and Ophthalmology, pp. 1755–1767. https://doi.org/10.1167/iovs.05-1139.

Eghrari, A.O., Riazuddin, S.A., Gottsch, J.D., 2015. Fuchs Corneal Dystrophy. In: Progress in Molecular Biology and Translational Science, first ed. Elsevier Inc. https://doi.org/10.1016/bs.pmbts.2015.04.005.

European Directorate for the Quality of Medicines and Healthcare, 2017. In: Guide to the Quality and Safety of Tissues and Cells for Human Application, third ed. Council of Europe, Strasbourg.

Feizi, S., 2018. Corneal endothelial cell dysfunction: etiologies and management. Ther. Adv. Ophthalmol. 10 https://doi.org/10.1177/2515841418815802, 251584141881580.

Flockerzi, E., Maier, P., Böhringer, D., Reinshagen, H., Kruse, F., Cursiefen, C., Reinhard, T., Geerling, G., Torun, N., Seitz, B., 2018. Trends in corneal transplantation from 2001 to 2016 in Germany: a report of the DOG–section cornea and its keratoplasty registry. Am. J. Ophthalmol. 188, 91–98. https://doi.org/10.1016/j.ajo.2018.01.018.

Frausto, R.F., Le, D.J., Aldave, A.J., 2016. Transcriptomic analysis of cultured corneal endothelial cells as a validation for their use in cell replacement therapy. Cell Transplant. 25, 1159–1176. https://doi.org/10.3727/096368915X688948.

Gain, P., Jullienne, R., He, Z., Aldossary, M., Acquart, S., Cognasse, F., Thuret, G., 2016. Global survey of corneal transplantation and eye banking. JAMA Ophthalmol 134, 167–173. https://doi.org/10.1001/jamaophthalmol.2015.4776.

Garcerant, D., Hirnschall, N., Toalster, N., Zhu, M., Wen, L., Moloney, G., 2019. Descemet's stripping without endothelial keratoplasty. Curr. Opin. Ophthalmol. 30, 275–285. https://doi.org/10.1097/ICU.0000000000000579.

Gilger, B., Wright, J., Whitley, R.D., McLaughlin, S.A., 1993. Corneal thickness measured by ultrasonic pachymetry in cats - PubMed. Am. J. Vet. Res. 54, 228–230.

Gorovoy, M.S., 2006. Descemet-stripping automated endothelial keratoplasty. Cornea 25, 886–889. https://doi.org/10.1097/01.ico.0000214224.90743.01.

Hara, S., Hayashi, R., Soma, T., Kageyama, T., Duncan, T., Tsujikawa, M., Nishida, K., 2014. Identification and potential application of human corneal endothelial progenitor cells. Stem Cell. Dev. 23, 2190–2201. https://doi.org/10.1089/scd.2013.0387.

He, Z., Forest, F., Bernard, A., Gauthier, A.S., Montard, R., Peoc'h, M., Jumelle, C., Courrier, E., Perrache, C., Gain, P., Thuret, G., 2016a. Cutting and decellularization of multiple corneal stromal lamellae for the bioengineering of endothelial grafts. Investig. Ophthalmol. Vis. Sci. 57, 6639–6651. https://doi.org/10.1167/iovs.16-20256.

He, Z., Forest, F., Gain, P., Rageade, D., Bernard, A., Acquart, S., Peoc'h, M., Defoe, D.M., Thuret, G., 2016b. 3D map of the human corneal endothelial cell. Sci. Rep. 6, 29047. https://doi.org/10.1038/srep29047.

Hirata-Tominaga, K., Nakamura, T., Okumura, N., Kawasaki, S., Kay, E.P., Barrandon, Y., Koizumi, N., Kinoshita, S., 2013. Corneal endothelial cell fate is maintained by LGR5 through the regulation of hedgehog and Wnt pathway. Stem Cell. 31, 1396–1407. https://doi.org/10.1002/stem.1390.

Hollingsworth, J., Perez-Gomez, I., Mutalib, H.A., Efron, N., 2001. A population study of the normal cornea using an in Vivo, slit-scanning confocal microscope. Optom. Vis. Sci. 78, 706–711. https://doi.org/10.1097/00006324-200110000-00010.

Honda, N., Mimura, T., Usui, T., Amano, S., 2009. Descemet stripping automated endothelial keratoplasty using cultured corneal endothelial cells in a rabbit model. Arch. Ophthalmol. 127, 1321–1326. https://doi.org/10.1001/archophthalmol.2009.253.

Hos, D., Matthaei, M., Bock, F., Maruyama, K., Notara, M., Clahsen, T., Hou, Y., Le, V.N. H., Salabarria, A.C., Horstmann, J., Bachmann, B.O., Cursiefen, C., 2019. Immune reactions after modern lamellar (DALK, DSAEK, DMEK) versus conventional penetrating corneal transplantation. Prog. Retin. Eye Res. 73 https://doi.org/10.1016/j.preteyeres.2019.07.001, 0–1.

Hsiue, G.H., Lai, J.Y., Chen, K.H., Hsu, W.M., 2006. A novel strategy for corneal endothelial reconstruction with a bioengineered cell sheet. Transplantation 81, 473–476. https://doi.org/10.1097/01.tp.0000194864.13539.2c.

Joyce, N.C., 2012. Proliferative capacity of corneal endothelial cells. Exp. Eye Res. 95, 16–23. https://doi.org/10.1016/j.exer.2011.08.014.

Joyce, N.C., Meklir, B., Joyce, S.J., Zieske, J.D., 1996. Cell cycle protein expression and proliferative status in human corneal cells. Investig. Ophthalmol. Vis. Sci. 37, 645–655.

Katikireddy, K.R., Schmedt, T., Price, M.O., Price, F.W., Jurkunas, U.V., 2016. Existence of neural crest–derived progenitor cells in normal and Fuchs endothelial dystrophy corneal endothelium. Am. J. Pathol. 186, 2736–2750. https://doi.org/10.1016/j.ajpath.2016.06.011.

Khor, W.B., Mehta, J.S., Tan, D.T.H., 2011. Descemet stripping automated endothelial keratoplasty with a graft insertion device: surgical technique and early clinical results. Am. J. Ophthalmol. 151 https://doi.org/10.1016/j.ajo.2010.08.027.

Kim, D.K., Sim, B.R., Kim, J.I., Khang, G., 2018. Functionalized silk fibroin film scaffold using β-Carotene for cornea endothelial cell regeneration. Colloids Surf. B Biointerfaces 164, 340–346. https://doi.org/10.1016/j.colsurfb.2017.11.052.

Kim, E.Y., Tripathy, N., Cho, S.A., Joo, C.K., Lee, D., Khang, G., 2015. Bioengineered neo-corneal endothelium using collagen type-I coated silk fibroin film. Colloids Surf. B Biointerfaces 136, 394–401. https://doi.org/10.1016/j.colsurfb.2015.09.041.

Kim, E.Y., Tripathy, N., Cho, S.A., Lee, D., Khang, G., 2017. Collagen type I-PLGA film as an efficient substratum for corneal endothelial cells regeneration. J. Tissue Eng. Regen. Med. 11, 2471–2478. https://doi.org/10.1002/term.2145.

Kimoto, M., Shima, N., Yamaguchi, M., Hiraoka, Y., Amano, S., Yamagami, S., 2014. Development of a bioengineered corneal endothelial cell sheet to fit the corneal curvature. Investig. Ophthalmol. Vis. Sci. 55, 2337–2343. https://doi.org/10.1167/iovs.13-13167.

Kinoshita, S., Koizumi, N., Ueno, M., Okumura, N., Imai, K., Tanaka, H., Yamamoto, Y., Nakamura, T., Inatomi, T., Bush, J., Toda, M., Hagiya, M., Yokota, I., Teramukai, S., Sotozono, C., Hamuro, J., 2018. Injection of cultured cells with a ROCK inhibitor for bullous keratopathy. N. Engl. J. Med. 378, 995–1003. https://doi.org/10.1056/NEJMoa1712770.

Koizumi, N., Sakamoto, Y., Okumura, N., Okahara, N., Tsuchiya, H., Torii, R., Cooper, L. J., Ban, Y., Tanioka, H., Kinoshita, S., 2007. Cultivated corneal endothelial cell sheet transplantation in a primate model. Investig. Ophthalmol. Vis. Sci. 48, 4519–4526. https://doi.org/10.1167/iovs.07-0567.

Koizumi, N., Sakamoto, Y., Okumura, N., Tsuchiya, H., Torii, R., Cooper, L.J., Ban, Y., Tanioka, H., Kinoshita, S., 2008. Cultivated corneal endothelial transplantation in a primate: possible future clinical application in corneal endothelial regenerative medicine. Cornea 27, 48–55. https://doi.org/10.1097/ICO.0b013e31817f2298.

Kruse, M., Walter, P., Bauer, B., Rütten, S., Schaefer, K., Plange, N., Gries, T., Jockenhoevel, S., Fuest, M., 2018. Electro-spun membranes as scaffolds for human corneal endothelial cells. Curr. Eye Res. 43, 1–11. https://doi.org/10.1080/02713683.2017.1377258.

Ku, B.I., Hsieh, Y.T., Hu, F.R., Wan, I.J., Chen, W.L., Hou, Y.C., 2017. Endothelial cell loss in penetrating keratoplasty, endothelial keratoplasty, and deep anterior lamellar keratoplasty. Taiwan J. Ophthalmol 7, 199–204. https://doi.org/10.4103/tjo.tjo_55_17.

Lai, J., Cheng, H., Ma, D.H., 2015. Investigation of Overrun-Processed Porous Hyaluronic Acid Carriers in Corneal Endothelial Tissue Engineering 1–20. https://doi.org/10.1371/journal.pone.0136067.

Lai, J.Y., Chen, K.H., Hsiue, G.H., 2007. Tissue-engineered human corneal endothelial cell sheet transplantation in a rabbit model using functional biomaterials. Transplantation 84, 1222–1232. https://doi.org/10.1097/01.tp.0000287336.09848.39.

Li, S., Han, Y., Lei, H., Zeng, Y., Cui, Z., Zeng, Q., Zhu, D., Lian, R., Zhang, J., Chen, Z., Chen, J., 2017. In vitro biomimetic platforms featuring a perfusion system and 3D spheroid culture promote the construction of tissue-engineered corneal endothelial layers. Sci. Rep. 7 https://doi.org/10.1038/s41598-017-00914-1.

Liu, C., Tang, S., Niu, G., Zhang, J., Huang, X., Zhang, Y., Bi, Y., 2019. Ex vivo construction of rabbit corneal endothelial cell sheets on a porcine descemet membrane graft. Exp. Ther. Med. 18, 242–252. https://doi.org/10.3892/etm.2019.7573.

Maharana, P., Sahay, P., Singhal, D., Garg, I., Titiyal, J., Sharma, N., 2017. Component corneal surgery: an update. Indian J. Ophthalmol. 65, 658. https://doi.org/10.4103/ijo.IJO_582_17.

Maqsood, M.I., Matin, M.M., Bahrami, A.R., Ghasroldasht, M.M., 2013. Immortality of cell lines: challenges and advantages of establishment. Cell Biol. Int. https://doi.org/10.1002/cbin.10137.

Matsuda, M., Sawa, M., Edelhauser, H.F., Bartels, S.P., Neufeld, A.H., Kenyon, K.R., 1985. Cellular migration and morphology in corneal endothelial wound repair. Investig. Ophthalmol. Vis. Sci. 26, 443–449.

McGowan, S.L., Edelhauser, H.F., Pfister, R.R., Whikehart, D.R., 2007. Stem cell markers in the human posterior limbus and corneal endothelium of unwounded and wounded corneas. Mol. Vis. 13, 1984–2000.

Meek, K.M., Knupp, C., 2015. Corneal structure and transparency. Prog. Retin. Eye Res. 49, 1–16. https://doi.org/10.1016/j.preteyeres.2015.07.001.

Melles, G.R.J., Lander, F., Rietveld, F.J.R., 2002. Transplantation of Descemet's membrane carrying viable endothelium through a small scleral incision. Cornea 21, 415–418. https://doi.org/10.1097/00003226-200211000-00024.

Mimura, T., Joyce, N.C., 2006. Replication competence and senescence in central and peripheral human corneal endothelium. Investig. Ophthalmol. Vis. Sci. 47, 1387–1396. https://doi.org/10.1167/iovs.05-1199.

Mimura, T., Shimomura, N., Usui, T., Noda, Y., Kaji, Y., Yamgami, S., Amano, S., Miyata, K., Araie, M., 2003. Magnetic attraction of iron-endocytosed corneal endothelial cells to Descemet's membrane. Exp. Eye Res. 76, 745–751. https://doi.org/10.1016/S0014-4835(03)00057-5.

Mimura, T., Yamagami, S., Usui, T., Ishii, Y., Ono, K., Yokoo, S., Funatsu, H., Araie, M., Amano, S., 2005a. Long-term outcome of iron-endocytosing cultured corneal endothelial cell transplantation with magnetic attraction. Exp. Eye Res. 80, 149–157. https://doi.org/10.1016/j.exer.2004.08.021.

Mimura, T., Yamagami, S., Usui, T., Seiichi, Honda, N., Amano, S., 2007. Necessary prone position time for human corneal endothelial precursor transplantation in a rabbit endothelial deficiency model. Curr. Eye Res. 32, 617–623. https://doi.org/10.1080/02713680701530589.

Mimura, T., Yamagami, S., Yokoo, S., Usui, T., Amano, S., 2010. Selective isolation of young cells from human corneal endothelium by the sphere-forming assay. Tissue Eng. C Methods 16, 803–812. https://doi.org/10.1089/ten.tec.2009.0608.

Mimura, T., Yamagami, S., Yokoo, S., Usui, T., Tanaka, K., Hattori, S., Irie, S., Miyata, K., Araie, M., Amano, S., 2004. Cultured human corneal endothelial cell transplantation with a collagen sheet in a rabbit model. Investig. Ophthalmol. Vis. Sci. 45, 2992–2997. https://doi.org/10.1167/iovs.03-1174.

Mimura, T., Yamagami, S., Yokoo, S., Yanagi, Y., Usui, T., Ono, K., Araie, M., Amano, S., 2005b. Sphere therapy for corneal endothelium deficiency in a rabbit model. Investig. Ophthalmol. Vis. Sci. 46, 3128–3135. https://doi.org/10.1167/iovs.05-0251.

Mimura, T., Yokoo, S., Araie, M., Amano, S., Yamagami, S., 2005c. Treatment of rabbit bullous keratopathy with precursors derived from cultured human corneal endothelium. Investig. Ophthalmol. Vis. Sci. 46, 3637–3644. https://doi.org/10.1167/iovs.05-0462.

Mishima, S., 1982. Clinical investigations on the corneal endothelium. Ophthalmology 89, 525–530. https://doi.org/10.1016/S0161-6420(82)34755-7.

Muhammad, R., Peh, G.S.L., Adnan, K., Law, J.B.K., Mehta, J.S., Yim, E.K.F., 2015. Micro- and nano-topography to enhance proliferation and sustain functional markers of donor-derived primary human corneal endothelial cells. Acta Biomater. 19, 138–148. https://doi.org/10.1016/j.actbio.2015.03.016.

Müller, T.M., Verdijk, R.M., Lavy, I., Bruinsma, M., Parker, J., Binder, P.S., Melles, G.R.J., 2016. Histopathologic features of descemet membrane endothelial keratoplasty graft remnants, folds, and detachments. Ophthalmology 123, 2489–2497. https://doi.org/10.1016/j.ophtha.2016.08.014.

Nishino, T., Kobayashi, A., Yokogawa, H., Mori, N., Sugiyama, K., 2019. Changing indications and surgical techniques for keratoplasty during a 16-year period

(2003–2018) at a tertiary referral hospital in Japan. Clin. Ophthalmol. 13, 1499–1509. https://doi.org/10.2147/OPTH.S214515.
Okumura, N., Kakutani, K., Inoue, R., Matsumoto, D., Shimada, T., Nakahara, M., Kiyanagi, Y., Itoh, T., Koizumi, N., 2016a. Generation and feasibility assessment of a new vehicle for cell-based therapy for treating corneal endothelial dysfunction. PloS One 11, 1–14. https://doi.org/10.1371/journal.pone.0158427.
Okumura, N., Kakutani, K., Numata, R., Nakahara, M., Schlötzer-Schrehardt, U., Kruse, F., Kinoshita, S., Koizumi, N., 2015. Laminin-511 and -521 enable efficient in vitro expansion of human corneal endothelial cells. Investig. Ophthalmol. Vis. Sci. 56, 2933–2942. https://doi.org/10.1167/iovs.14-15163.
Okumura, N., Kinoshita, S., Koizumi, N., 2017. Application of rho kinase inhibitors for the treatment of corneal endothelial diseases. J. Ophthalmol. https://doi.org/10.1155/2017/2646904.
Okumura, N., Koizumi, N., Kay, E.D.P., Ueno, M., Sakamoto, Y., Nakamura, S., Hamuro, J., Kinoshita, S., 2013. The ROCK inhibitor eye drop accelerates corneal endothelium wound healing. Investig. Ophthalmol. Vis. Sci. 54, 2439–2502. https://doi.org/10.1167/iovs.12-11320.
Okumura, N., Koizumi, N., Ueno, M., Sakamoto, Y., Takahashi, H., Tsuchiya, H., Hamuro, J., Kinoshita, S., 2012. ROCK inhibitor converts corneal endothelial cells into a phenotype capable of regenerating in vivo endothelial tissue. Am. J. Pathol. 181, 268–277. https://doi.org/10.1016/j.ajpath.2012.03.033.
Okumura, N., Matsumoto, D., Fukui, Y., Teramoto, M., Imai, H., Kurosawa, T., Shimada, T., Kruse, F., Schlötzer-Schrehardt, U., Kinoshita, S., Koizumi, N., 2018. Feasibility of cell-based therapy combined with descemetorhexis for treating Fuchs endothelial corneal dystrophy in rabbit model. PloS One 13, 1–14. https://doi.org/10.1371/journal.pone.0191306.
Okumura, N., Sakamoto, Y., Fujii, K., Kitano, J., Nakano, S., Tsujimoto, Y., Nakamura, S. I., Ueno, M., Hagiya, M., Hamuro, J., Matsuyama, A., Suzuki, S., Shiina, T., Kinoshita, S., Koizumi, N., 2016b. Rho kinase inhibitor enables cell-based therapy for corneal endothelial dysfunction. Sci. Rep. 6, 1–11. https://doi.org/10.1038/srep26113.
Okumura, N., Ueno, M., Koizumi, N., Sakamoto, Y., Hirata, K., Hamuro, J., Kinoshita, S., 2009. Enhancement on primate corneal endothelial cell survival in vitro by a rock inhibitor. Investig. Ophthalmol. Vis. Sci. 50, 3680–3687. https://doi.org/10.1167/iovs.08-2634.
Ozcelik, B., Brown, K.D., Blencowe, A., Ladewig, K., Stevens, G.W., Scheerlinck, J.P.Y., Abberton, K., Daniell, M., Qiao, G.G., 2014. Biodegradable and biocompatible poly (ethylene glycol)-based hydrogel films for the regeneration of corneal endothelium. Adv. Healthc. Mater. 3, 1496–1507. https://doi.org/10.1002/adhm.201400045.
Parekh, M., Baruzzo, M., Favaro, E., Borroni, D., Ferrari, S., Ponzin, D., Ruzza, A., 2017. Standardizing descemet membrane endothelial keratoplasty graft preparation method in the eye bank-experience of 527 descemet membrane endothelial keratoplasty tissues. Cornea 36, 1458–1466.
Parekh, M., Leon, P., Ruzza, A., Borroni, D., Ferrari, S., Ponzin, D., Romano, V., 2018a. Graft detachment and rebubbling rate in Descemet membrane endothelial keratoplasty. Surv. Ophthalmol. 63, 245–250. https://doi.org/10.1016/j.survophthal.2017.07.003.
Parekh, M., Van Den Bogerd, B., Zakaria, N., Ponzin, D., Ferrari, S., 2018b. Fish scale-derived scaffolds for culturing human corneal endothelial cells. Stem Cell. Int. https://doi.org/10.1155/2018/8146834.
Park, C.Y., Lee, J.K., Gore, P.K., Lim, C.Y., Chuck, R.S., 2015. Keratoplasty in the United States: a 10-year review from 2005 through 2014. Ophthalmology 122, 2432–2442. https://doi.org/10.1016/j.ophtha.2015.08.017.
Patel, S.V., Bachman, L.A., Hann, C.R., Bahler, C.K., Fautsch, M.P., 2009. Human corneal endothelial cell transplantation in a human ex vivo model. Investig. Ophthalmol. Vis. Sci. 50, 2123–2131. https://doi.org/10.1167/iovs.08-2653.
Peh, G.S.L., Ang, H.P., Lwin, C.N., Adnan, K., George, B.L., Seah, X.Y., Lin, S.J., Bhogal, M., Liu, Y.C., Tan, D.T., Mehta, J.S., 2017. Regulatory compliant tissue-engineered human corneal endothelial grafts restore corneal function of rabbits with bullous keratopathy. Sci. Rep. 7, 1–17. https://doi.org/10.1038/s41598-017-14723-z.
Peh, G.S.L., Chng, Z., Ang, H.-P., Cheng, T.Y.D., Adnan, K., Seah, X.-Y., George, B.L., Toh, K.-P., Tan, D.T., Yam, G.H.F., Colman, A., Mehta, J.S., 2015. Propagation of human corneal endothelial cells: a novel dual media approach. Cell Transplant. 24, 287–304. https://doi.org/10.3727/096368913X675719.
Peh, G.S.L., Ong, H.S., Adnan, K., Ang, H.P., Lwin, C.N., Seah, X.Y., Lin, S.J., Mehta, J.S., 2019. Functional evaluation of two corneal endothelial cell-based therapies: tissue-engineered construct and cell injection. Sci. Rep. 9, 1–13. https://doi.org/10.1038/s41598-019-42493-3.
Peh, G.S.L., Toh, K.-P., Wu, F.-Y., Tan, D.T., Mehta, J.S., 2011. Cultivation of human corneal endothelial cells isolated from paired donor corneas. PloS One 6, e28310. https://doi.org/10.1371/journal.pone.0028310.
Price, M.O., Calhoun, P., Kollman, C., Price, F.W., Lass, J.H., 2016. Descemet stripping endothelial keratoplasty ten-year endothelial cell loss compared with penetrating keratoplasty. Ophthalmology 123, 1421–1427. https://doi.org/10.1016/j.ophtha.2016.03.011.
Price, M.O., Gupta, P., Lass, J., Price, F.W., 2017. EK (DLEK, DSEK, DMEK): new frontier in cornea surgery. Annu. Rev. Vis. Sci. 3, 69–90. https://doi.org/10.1146/annurev-vision-102016-061400.
Price, M.O., Price, F.W., 2006. Descemet's stripping with endothelial keratoplasty. Comparative outcomes with microkeratome-dissected and manually dissected donor tissue. Ophthalmology 113, 1936–1942. https://doi.org/10.1016/j.ophtha.2006.05.034.
Proulx, S., Brunette, I., 2012. Methods being developed for preparation, delivery and transplantation of a tissue-engineered corneal endothelium. Exp. Eye Res. 95, 68–75. https://doi.org/10.1016/J.EXER.2011.06.013.
Rizwan, M., Peh, G.S.L., Ang, H.P., Lwin, N.C., Adnan, K., Mehta, J.S., Tan, W.S., Yim, E. K.F., 2017. Sequentially-crosslinked bioactive hydrogels as nano-patterned substrates with customizable stiffness and degradation for corneal tissue engineering applications. Biomaterials 120, 139–154. https://doi.org/10.1016/j.biomaterials.2016.12.026.
Roy, O., Beaulieu Leclerc, V., Bourget, J.-M., Theriault, M., Proulx, S., 2015. Understanding the process of corneal endothelial morphological change in vitro. Invest. Ophthalmol. Vis. Sci. 56, 1228–1237. https://doi.org/10.1167/iovs.14-16166.
Salehi, S., Czugala, M., Stafiej, P., Fathi, M., Bahners, T., Gutmann, J.S., Singer, B.B., Fuchsluger, T.A., 2017. Poly (glycerol sebacate)-poly (ε-caprolactone) blend nanofibrous scaffold as intrinsic bio- and immunocompatible system for corneal repair. Acta Biomater. https://doi.org/10.1016/j.actbio.2017.01.013.
Senoo, T., Joyce, N.C., 2000. Cell cycle kinetics in corneal endothelium from old and young donors. Investig. Ophthalmol. Vis. Sci. 41, 660–667.
Sie, N.M., Yam, G.H.F., Soh, Y.Q., Lovatt, M., Dhaliwal, D., Kocaba, V., Mehta, J.S., 2020. Regenerative capacity of the corneal transition zone for endothelial cell therapy. Stem Cell Res. Ther. https://doi.org/10.1186/s13287-020-02046-2.
Soh, Y., Peh, G.S., Mehta, J., 2016. Translational issues for human corneal endothelial tissue engineering. J Tissue Eng Regen Med. https://doi.org/10.1002/term.2131.
Soh, Y.Q., Kocaba, V., Pinto, M., Mehta, J.S., 2019. Fuchs endothelial corneal dystrophy and corneal endothelial diseases : east meets West. Eye. https://doi.org/10.1038/s41433-019-0497-9.
Soh, Y.Q., Mehta, J.S., 2018. Regenerative therapy for Fuchs endothelial corneal dystrophy. Cornea 37, 523–527. https://doi.org/10.1097/ICO.0000000000001518.
Soma, T., Koh, S., Oie, Y., Maruyama, K., Tsujikawa, M., Kawasaki, S., Maeda, N., Nishida, K., 2019. Clinical evaluation of a newly developed graft inserter (NS endo-inserter) for descemet stripping automated endothelial keratoplasty. Clin. Ophthalmol. 13, 43–48. https://doi.org/10.2147/OPTH.S182628.
Srinivas, S.P., 2012. Cell signaling in regulation of the barrier integrity of the corneal endothelium. Exp. Eye Res. 95, 8–15. https://doi.org/10.1016/j.exer.2011.09.009.
Tan, T.E., Peh, G.S.L., George, B.L., Cajucom-Uy, H.Y., Dong, D., Finkelstein, E.A., Mehta, J.S., 2014. A cost-minimization analysis of tissue-engineered constructs for corneal endothelial transplantation. PloS One 9. https://doi.org/10.1371/journal.pone.0100563.
Thériault, M., Roy, O., Brunette, I., Proulx, S., 2019. Physiological pressure enhances the formation of tight junctions in engineered and native corneal endothelium. Exp. Eye Res. 179, 102–105. https://doi.org/10.1016/j.exer.2018.11.004.
Toda, M., Yukawa, H., Yamada, J., Ueno, M., Kinoshita, S., Baba, Y., Hamuro, J., 2019. In vivo fluorescence visualization of anterior chamber injected human corneal endothelial cells labeled with quantum dots. Investig. Ophthalmol. Vis. Sci. 60, 4008–4020. https://doi.org/10.1167/iovs.19-27788.
Van den Bogerd, B., Dhubhghaill, S.N., Koppen, C., Tassignon, M.J., Zakaria, N., 2018a. A review of the evidence for in vivo corneal endothelial regeneration. Surv. Ophthalmol. 63, 149–165. https://doi.org/10.1016/j.survophthal.2017.07.004.
Van den Bogerd, B., Ní Dhubhghaill, S., Zakaria, N., 2018b. Characterizing human decellularized crystalline lens capsules as a scaffold for corneal endothelial tissue engineering. J. Tissue Eng. Regen. Med. 12, e2020–e2028. https://doi.org/10.1002/term.2633.
Van Horn, D.L., Sendele, D.D., Seideman, S., Buco, P.J., 1977. Regenerative capacity of the corneal endothelium in rabbit and cat. Investig. Ophthalmol. Vis. Sci. 16, 597–613.
Vázquez, N., Chacón, M., Meana, Á., Menéndez-Menéndez, Y., Ferrero-Gutierrez, A., Cereijo-Martín, D., Naveiras, M., Merayo-Lloves, J., 2015. Keratin-chitosan membranes as scaffold for tissue engineering of human cornea. Histol. Histopathol. 30, 813–821. https://doi.org/10.14670/HH-11-585.
Vázquez, N., Chacón, M., Rodríguez-Barrientos, C.A., Merayo-Lloves, J., Naveiras, M., Baamonde, B., Alfonso, J.F., Zambrano-Andazol, I., Riestra, A.C., Meana, Á., 2016. Human bone derived collagen for the development of an artificial corneal endothelial graft. in Vivo results in a rabbit model. PloS One 11, 1–18. https://doi.org/10.1371/journal.pone.0167578.
Vázquez, N., Rodríguez-Barrientos, C.A., Aznar-Cervantes, S.D., Chacón, M., Cenis, J.L., Riestra, A.C., Sánchez-Avila, R.M., Persinal, M., Brea-Pastor, A., Fernández-Vega Cueto, L., Meana, Á., Merayo-Lloves, J., 2017. Silk fibroin films for corneal endothelial regeneration: transplant in a rabbit descemet membrane endothelial keratoplasty. Investig. Ophthalmol. Vis. Sci. 58, 3357–3365. https://doi.org/10.1167/iovs.17-21797.
Woo, J.H., Ang, M., Htoon, H.M., Tan, D., 2019. Descemet membrane endothelial keratoplasty versus descemet stripping automated endothelial keratoplasty and penetrating keratoplasty. Am. J. Ophthalmol. 207, 288–303. https://doi.org/10.1016/j.ajo.2019.06.012.
Xia, X., Atkins, M., Dalal, R., Kuzmenko, O., Chang, K.C., Sun, C.B., Benatti, C.A., Rak, D. J., Nahmou, M., Kunzevitzky, N.J., Goldberg, J.L., 2019. Magnetic human corneal endothelial cell transplant: delivery, retention, and short-term efficacy. Investig. Ophthalmol. Vis. Sci. 60, 2438–2448. https://doi.org/10.1167/iovs.18-26001.
Yam, G.H.F., Seah, X., Yusoff, N.Z.B.M., Setiawan, M., Wahlig, S., Htoon, H.M., Peh, G.S. L., Kocaba, V., Mehta, J.S., 2019. Characterization of human transition zone reveals a putative progenitor-enriched niche of corneal endothelium. Cells 8. https://doi.org/10.3390/cells8101244.
Yokoo, S., Yamagami, S., Yanagi, Y., Uchida, S., Mimura, T., Usui, T., Amano, S., 2005. Human corneal endothelial cell precursors isolated by sphere-forming assay. Investig. Ophthalmol. Vis. Sci. 46, 1626–1631. https://doi.org/10.1167/iovs.04-1263.
Yoshida, J., Oshikata-Miyazaki, A., Yokoo, S., Yamagami, S., Takezawa, T., Amano, S., 2014. Development and evaluation of porcine atelocollagen vitrigel membrane with

a spherical curve and transplantable artificial corneal endothelial grafts. Investig. Ophthalmol. Vis. Sci. 55, 4975–4981. https://doi.org/10.1167/iovs.14-14211.

Yoshida, J., Yokoo, S., Oshikata-Miyazaki, A., Amano, S., Takezawa, T., Yamagami, S., 2017. Transplantation of human corneal endothelial cells cultured on bio-engineered collagen vitrigel in a rabbit model of corneal endothelial dysfunction. Curr. Eye Res. 42, 1420–1425. https://doi.org/10.1080/02713683.2017.1351568.

Yoshihara, M., Hayashizaki, Y., Murakawa, Y., 2017. Genomic instability of iPSCs: challenges towards their clinical applications. Stem Cell Rev. Reports. https://doi.org/10.1007/s12015-016-9680-6.

Zhang, Y., Cai, S., Tseng, S.C.G., Zhu, Y.T., 2018. Isolation and expansion of multipotent progenitors from human trabecular meshwork. Sci. Rep. 8 https://doi.org/10.1038/s41598-018-21098-2.

Zurawski, C.A., McCarey, B.E., Rij, G., Fernandes, A., 1989. Corneal biometrics of the rhesus monkey (Macaca mulatta). J. Med. Primatol. 18, 461–466. https://doi.org/10.1111/j.1600-0684.1989.tb00413.x.