# The impact of biomechanics on corneal endothelium tissue engineering

*Meng-Chen Tsai*[*]*, Julie T Daniels*

*UCL Institute of Ophthalmology, University College London, London, UK*

## Abstract

The integrity of innermost layer of the cornea, the corneal endothelium, is key to sustaining corneal transparency. Therefore, disease or injury causing loss or damage to the corneal endothelial cell population may threaten vision. Transplantation of corneal tissue is the standard treatment used to replace malfunctioning corneal endothelial cells. However, this surgery is dependent upon donor tissue, which is limited in supply. Hence, tissue engineers have attempted to construct alternative transplantable tissues or cell therapies to alleviate this problem. Nevertheless, the intrinsic non-dividing nature of corneal endothelial cells continues to foil scientists in their attempts to yield large numbers of cells in the laboratory for use in such novel therapies. Interestingly, the contribution of the biomechanical properties of the underlying extracellular matrix (ECM) on cell division, tissue development and maintenance has been extensively investigated in other many cell types. However, the impact of biomechanics on corneal endothelial cell behaviour is relatively unexplored.

Here, we describe contemporary tissue engineering solutions aimed at circumventing donor tissue scarcity. We review the ECM structure and biomechanical features of corneal endothelial cells. We discuss the alterations of ECM in endothelial disease development and progression and point out the role of ECM in developing a tissue-engineered corneal endothelium. We highlight the main biomechanical cues, including topographical and mechanical features, that impact cellular behaviors. Finally, we discuss the influence of biomechanical cues on cell and tissue development, and how corneal endothelial cells response to individual biomechanical stimuli in tissue engineering, which have implications for designing an engineered endothelium and maintaining cell function.

*Keywords: Corneal endothelial cell, Corneal endothelium, Extracellular matrix, Biomechanics, Descemet's membrane, Tissue engineering*

[*] This is to indicate the corresponding author.

Email address: meng-chen.tsai.19@ucl.ac.uk

Permanent address: 3/33, UCL Institute of Ophthalmology, 11-43 Bath Street, London, EC1V 9EL, UK

## 1. Structure and function of the cornea

The cornea is a clear and avascular tissue covering the anterior one sixth of the total surface of the eye. Adjoining with the sclera, the cornea constructs an architectural shell protecting and sustaining the tectonic integrity of the whole eyeball. Also, the cornea is the essential refractive medium of the optical system allowing light to transmit and focus onto the retina to assure visual clarity. Indeed, the cornea accounts for more than two-thirds of the eye's refractive power. The majority of the cornea is composed of cornea stroma, representing around 90% of the thickness. Thus, the biomechanical properties and the transparency of the cornea is largely governed by the extracellular matrix (ECM) of the stroma. Specifically, the highly organized arrangement of collagen fibers with uniform inter-fibrillar spacing, and the well-interwoven layers of collagen lamellae. To protect stroma from the external environment, corneal epithelial cells cover the anterior surface of the stroma as a cellular barrier which integrates with the tear film and conjunctiva to complete the ocular surface. On the posterior surface, corneal endothelial cells anchor to the Descemet's membrane, providing a barrier to separate the stroma and the aqueous humour, and to control fluid imbibition into the stroma.

## 2. Corneal endothelial cell loss

Corneal endothelium is a single layer of cells covering the posterior surface of the cornea in a honeycomb pattern. Its major function is to maintain the optimal hydration of the cornea by actively maintaining an osmotic gradient via the activity of Na+/K+ ATPase pumps (Klyce, 2020). Thus, sufficient cell density has crucial importance for sustaining transparent cornea. However, cell density diminishes with ageing at an annual reduction rate of 0.6% (Armitage et al., 2003). This is because corneal endothelial cells have limited proliferative capacity so cannot regenerate cells to replace dead or injured cells (Joyce, 2003). To compensate for this gradual cell loss, migration and expansion of neighboring cells occurs in order to maintain the functional integrity. As a result, an increase in overall cell size and an alteration from a hexagonal to a pleomorphic shape can be seen for natural or acquired (e.g. injury) cell density loss. Once the cell number can no longer support the cornea (cell density below 500 cells/mm$^2$), decompensation of the endothelium with permanent and sight threatening corneal edema results (Armitage et al., 2003).

## 3. Biomechanics changes of the ECM in diseased and ageing corneal endothelial cells

One of the intriguing issues is the influence of corneal biomechanics and its connection to diseased and ageing corneal endothelial cells. As the cornea is the matrix for the optical media and the outer tunic of the eye, the biomechanical properties of cornea, including cornea curvature, stiffness, clarity and regularity, not only shape the integrity structure of the eye, but also have impact on the eye's refractive performance. Previous results have shown that corneal stiffness increases with ageing as cross-linking between stroma collagen fibres increases (Elsheikh et al., 2007; Sharifipour et al., 2016). This results in dynamic changes to the biomechanics of the cornea, which are also associated with corneal disease development and progression in some individuals

(Chansangpetch et al., 2017; Kotecha, 2007; Zhao et al., 2019). Therefore, it is necessary to investigate the relationship between cornea biomechanical properties and disease. In the following paragraphs, focus is placed on the mechanical changes of the ECM related to corneal endothelial cells, including cell ageing, Fuchs' endothelial dystrophy and high intraocular pressure.

Cell ageing contributes to the changes in biomechanical properties of ECM. For example, the major components of Descemet's membrane are changed due to aged cell protein synthesis (Kabosova et al., 2007). Cell ageing also has an impact on the thickness of Descemet's membrane. After birth, corneal endothelial cells keep synthesizing a nonstriated and nonlamellar material on their basal side to form a new layer of ECM, resulting in a continuous growth of Descemet's membrane throughout the lifetime (Murphy et al., 1984a; Murphy et al., 1984b). In addition, previous research has showed that the mechanical property of the cornea changed with age. The cornea become stiffer as age increased (Elsheikh et al., 2010) and has a positive correlation coefficient between the ocular rigidity and age (Pallikaris et al., 2005). Similar stiffness change with age was also observed in Descemet's membrane where the elastic modulus of Descemet's membrane increased as age increased (Last et al., 2009; Last et al., 2012). In view of this, it is reasonable to assume that there is a connection between the mechanical properties of ECM and ageing.

Many studies have indicated the pathology changes of Descemet's membrane in Fuchs' endothelial dystrophy. Drop-like bumps on the posterior surface of the cornea, known as guttae, are the major feature in clinical diagnosis of Fuchs' endothelial dystrophy (Adamis et al., 1993). Other manifestations also include thicker Descemet's membrane (Levy et al., 1996), abnormal depositions of collagen IV, laminin, and fibronectin (Weller et al., 2014). In a rare form of early onset of Fuchs' endothelial dystrophy, such abnormal depositions on Descemet's membrane have been considered to be associated with the mutation of collagen VIII gene, including two autosomal dominant mutations, L450W (Gottsch et al., 2005) and Q455K in COL8α2 (Biswas et al., 2001). This reflects similar results seen in animal research in that COL8α2 knock-in mouse manifested ECM depositions and cell morphology changes on the corneal endothelium (Meng et al., 2013).

To understand the biomechanical impact of altered ECM on corneal endothelial cells, transgenic animal models have been used to investigate in Fuchs' endothelial dystrophy. For example, a COL8α2 gene mutation mice model has been used to recapitulate a rare form of early onset of Fuchs' endothelial dystrophy in previous research. The results showed that compared to wildtype control, there is a significant decrease of the stiffness of Descemet's membrane and a reduction of endothelial density in COL8α2 knock-in mice. More importantly, it was found that the stiffness alteration on Descemet's membrane precedes endothelial cell density loss and morphology changes (Leonard et al., 2019). This provided evidence to support the pathogenesis of Fuchs' endothelial dystrophy in that accumulated depositions on Descemet's membrane build up abnormal ECM structures causing biomechanical changes. Meanwhile, it also affects cell synthesis and ECM remodeling (Davis and Senger, 2005; Gasiorowski et al., 2013; Schwarz and Gardel, 2012).

Intraocular pressure is another factor contributing to corneal endothelial cell damage and biomechanical changes of the cornea. Under normal circumstances, in order to assure the transparency and the normal physiologic water content of the cornea, corneal endothelial cells provide a barrier to divide the corneal stroma from aqueous humor, and to prevent the excessive water imbibition of stroma. Abnormal resistance to the outflow of aqueous humour can continuously build up the intraocular pressure. In acute primary angle-closure glaucoma, a sudden closure of anterior angle can rapidly increase the intraocular pressure and break the endothelium barrier eventually, leading to corneal edema and endothelial cell density loss (Bigar and Witmer, 1982; Chen et al., 2012; Sihota et al., 2003). Likewise, corneal endothelial cell damage can also be witnessed in primary open-angle glaucoma with long-term mild intraocular pressure increase (Cho et al., 2009; Gagnon et al., 1997; Korey et al., 1982; Yu et al., 2019).

Recently, Li et al. found that a sudden increase in intraocular pressure can directly disrupt the integrity of endothelial tight junction barrier and the Na/K ATPase pump function, increasing endothelial permeability and causing cornea edema (Li et al., 2017). Also, previous studies have shown that there is a connection between intraocular pressure and the cornea biomechanical property changes (Jung et al., 2020; Miki et al., 2019; Salvetat et al., 2015; Tian et al., 2016). Wang et al. indicated that the resistance of the cornea to deformation is stronger in glaucoma patients than in healthy controls, and the cornea mechanical changes are correlated to intraocular pressure (Wang et al., 2015).

## 4. Clinical treatment of endothelium decompensation

Penetrating keratoplasty (PKP), where a full thickness donor cornea is transplanted to replace that of the recipient's own diseased cornea, used to be the primary surgical procedure to treat irreversible decompensation of the corneal endothelium (Al-Yousuf et al., 2004; Tan et al., 2014). To avoid suture-associated complications and high post-operative astigmatism in PKP, a partial-thickness graft replacement, endothelial keratoplasty, has become the standard surgical treatment for corneal endothelial dysfunction in the last decade (Deng et al., 2018; Lee et al., 2009; Tan et al., 2012). Currently, two main surgical approaches, Descemet stripping automated endothelial keratoplasty (DSAEK) (Gorovoy, 2006) and Descemet membrane endothelial keratoplasty (DMEK)(Melles et al., 2002) have been introduced into the clinic. However, even though the benefit of endothelial keratoplasty outweighs PKP in many ways (fewer sutures, reduced graft failure, better tectonic stability, and earlier recovery), it is still limited by the growing demand for donor tissue.

## 5. Proposed tissue engineering solutions

Expanding corneal endothelial cells on an engineered scaffold has been considered an alternative way to solve the increasing demand on cornea donor grafts across the world (Gaum et al., 2012; Mimura et al., 2013). From the perspective of tissue engineering, corneal endothelial cells can be isolated, cultured and expanded *in vitro*, before being seeded onto a constructed scaffold. Engineered tissue equivalent can be delivered into the posterior

surface of the cornea as a transplanting graft to replace diseased cells and underlying abnormal ECM (Mimura et al., 2013).

Numerous materials have been proposed to serve as a tissue engineered substrate to support endothelial cell expansion. De-cellularised native tissue has been suggested as a suitable carrier for corneal endothelial cell growth since it provides native ECM architecture. For example, decellularized Descemet's membrane (Lu et al., 2020), decellularized stroma (An et al., 2020; Bayyoud et al., 2012; Choi et al., 2010), amniotic membrane (Ishino et al., 2004), and lens capsules (Van den Bogerd et al., 2018; Yoeruek et al., 2009). However, the decellularization process could also damage the ECM structure and leave behind the residual exogenous antigen which could potentially provoke a recipient immune response.

Other research has suggested the benefit of using natural polymers to construct a tissue engineered substrate, such as collagen I hydrogel (Levis et al., 2012; Mimura et al., 2004), chitosan (Liang et al., 2011; Wang et al., 2012; Young et al., 2014), and gelatin (Niu et al., 2014; Watanabe et al., 2011). As natural polymers comprise the major component of ECM, not only can they provide essential binding sites for cells, self-assembling collagen structure can also reconstruct three-dimension geometrical features, such as pores and pits, to stimulate cell adhesion, migration and proliferation. The shortcoming is that the mechanical and optical properties still need to be improved(Levis et al., 2012).

Alternatively, synthetic materials such as poly-ε-lysine (pεK) hydrogel (Kennedy et al., 2019), poly(vinyl methyl ether) (PVME) (Teichmann et al., 2013), and Polydimethylsiloxane (PDMS)(Koo et al., 2014; Palchesko et al., 2015) have also been explored. Easily fine-tuned component and surface modification is the benefit of synthetic material. However, lack of native cell binding sites and resistance to biodegradation are the drawbacks. Long-term implications for synthetic material need to be investigated.

## 6. The role of ECM in developing a tissue-engineered corneal endothelium

ECM comprises of a range of self-assembling structural proteins (collagen I, elastin), adhesive proteins (collagen IV, fibronectin, laminin), and glycosaminoglycans (GAG), all of which construct a scaffolding network providing mechanical support for cell adhesion, arrangement and organization (Gasiorowski et al., 2013; Schwarz and Gardel, 2012). As with other cells of the body, growing evidence has shown that biomechanical properties of the ECM play a crucial role in modulating corneal endothelial cell behaviors (Ali et al., 2016).

The interaction between corneal endothelial cells and their native ECM has been of interest for many years (Hsieh and Baum, 1985; Tseng et al., 1981). Corneal endothelial cells arrange in an orderly, compact, cobblestone monolayer cell sheet covering the posterior surface of the cornea. The basal side of the corneal endothelial cells is attached to its own synthesized ECM, an acellular Descemet's membrane (He et al., 2016).

The major components of Descemet's membrane include collagen IV, collagen VIII, laminin, fibronectin, heparan sulfate and nidogens (de Oliveira and Wilson, 2020; Medeiros et al., 2018; Saikia et al., 2018). Collagen VIII is able to form a unique hexagonal lattice stacked together, and it is the primary ECM scaffolding structures in Descemet's membrane (Hansen et al., 2019; Sawada, 1982; Sawada et al., 1990). Descemet's membrane connects to the posterior stroma via an interwoven collagen fibril network (Schlotzer-Schrehardt et al., 2015). On the other hand, the basal side of corneal endothelial cells display dendritic extensions interconnecting with adjacent cells and attach to the posterior surface of Descemet's membrane (He et al., 2016; Levis et al., 2012). This evidence indicates that there is a strong structural interaction between corneal endothelium and ECM. If it were possible to engineer similar a construct to provide a suitable ECM environment, it could be conducive to corneal endothelial cell growth (Figure 1).

Previous research has shown that corneal endothelial cells can perceive biomechanical changes in their surrounding environment, and in response regulate ECM synthesis during physiological and pathological processes (Gruschwitz et al., 2010; Leonard et al., 2019; Wang et al., 2012). Such dynamic bidirectional interaction between corneal endothelial cells and their microenvironment has been referred to as a signaling process of mechanotransduction, where biophysical cues of ECM can be converted into intracellular biochemical signals leading cellular responses (Crowder et al., 2016; Gasiorowski et al., 2013; Humphrey et al., 2014; Iskratsch et al., 2014).(Figure 2A).

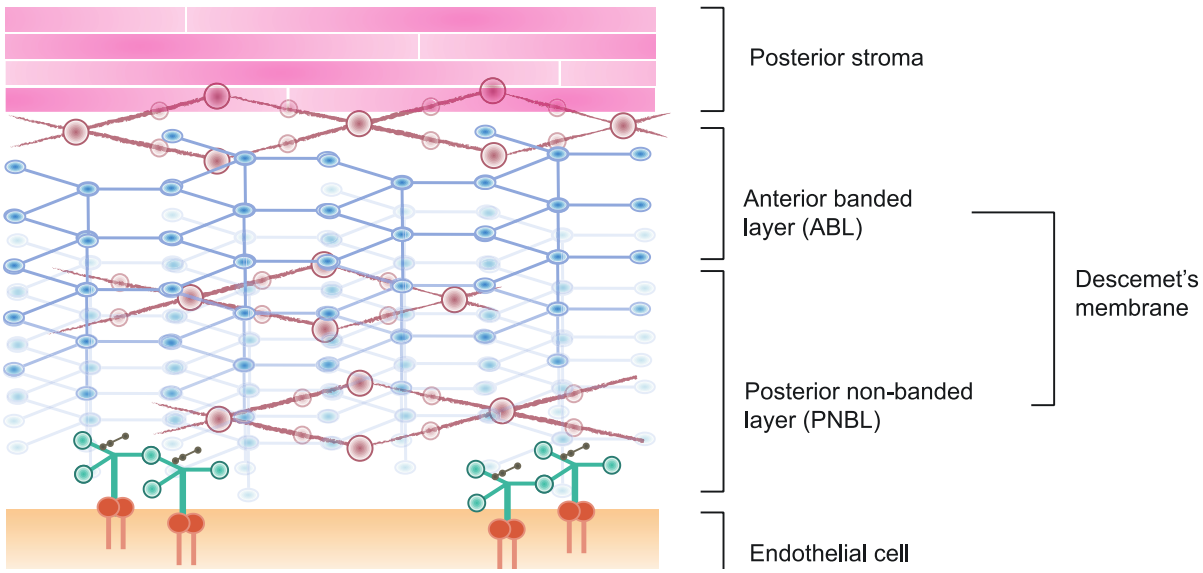


Figure 1. Schematic representation of the ECM structure of Descemet's membrane. The major components of the ECM are collagen IV and collagen VIII, which are both secreted from corneal endothelial cells. The anterior banded layer primarily consists of collagen IV and collagen VIII. Collagen VIII (the primary component of Descemet's membrane) is able to self-assemble into a stacked hexagonal scaffold. After birth, corneal endothelial cells keep secreting collagen IV and depositing it onto the anterior layer to form the posterior non-banded layer. Integrin binding sites connect the cell surface to the extracellular matrix. The extracellular domains of integrin bind to the matrix molecules such as laminin, nidogen, fibronectin and collagen.

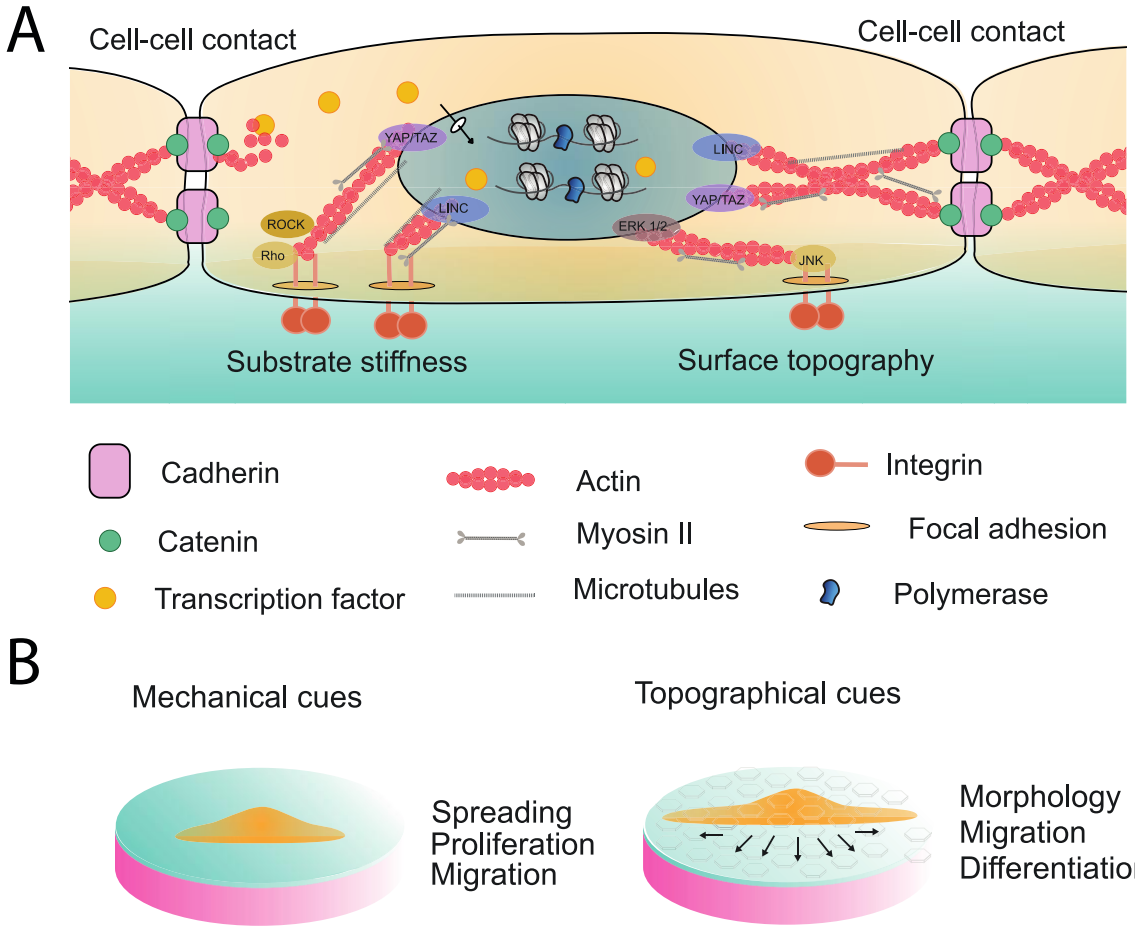


Figure 2. Mechanotransduction (A) Extracellular biomechanical cues can be converted into cellular biochemical signals regulating cell behaviours. Biomechanical cues are derived from the substrate stiffness of ECM, the surface topography of ECM and cell-cell contact. For substrate stiffness, mechanical cues can be sensed by focal adhesion. Cell signals can be transduced into the nucleus by filamentous actin and myosin II through Rho/ROCK – YAP/TAZ pathway. Also, mechanical signals can be transferred into internal mechanical stress and directly affect nucleus deformation by LINC complex (Linker of the Nucleoskeleton and Cytoskeleton). For topographical cues, surface geometry and pattern of ECM stimuli can through JNK-ERK1/2, PI3K pathway affect cell cytoskeletal configuration. For cell-cell contact, adherens junctions connecting adjacent cells can also sense mechanical cues and transduce them into cellular signals through YAP/TAZ pathway. Signals from adherens junctions can also directly transferred into the nucleus through LINC complex. On the other hand, transcription factors dissociated from adherens junctions or filamentous actin can translocate into the nucleus regulating gene expression. (B) From the perspective of tissue engineering, providing suitable ECM for corneal endothelial cells, including topographical and mechanical cues, we can take the advantage to increase cell proliferation, to maintain cell morphology, and to retain cell function during in vitro expansion.

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## 7. Biomechanical properties of ECM in tissue engineered endothelium

To develop a tissue engineered endothelium, the biggest challenge lies in the limited regenerative capacity of corneal endothelial cells. The intrinsic non-dividing nature of endothelial cells hinders the *in vitro* cell culture and expansion. Also, the irreversible transdifferentiation from endothelial to mesenchymal phenotype during *in vitro* cell culture results in a loss of cell-cell contacts, hexagonal appearance and displaying a fibroblastic phenotype. To overcome these challenges, research has turned toward understanding biomechanical properties of the extracellular matrix (ECM). The goal being to identify parameters exploitable by tissue engineers. By providing suitable ECM biomechanical properties for corneal endothelial cells to grow on, it may be possible to increase cell proliferation and to maintain cell phenotype and function. (Figure 2B).

The biomechanical stimulus of ECM comprises two types: topographical and mechanical properties. Topographical features of hexagonal lattice structures and nanoscale pores have been observed on Descemet's membrane (Last et al., 2009; Levy et al., 1996; Sawada, 1982). However, how these topographies exactly modulate corneal endothelial cell behaviors still needs to be studied. With regard to the mechanical features, tissue stiffness is a key biomechanical parameter that can be incorporated into engineering models. Although cornea tissue is considered to be nonlinear, anisotropic, presenting both elastic and viscous characteristics, measuring the mechanical properties of cornea tissue in a linear elastic model can still provide useful information for the design of a tissue equivalent. Recently, scientists tried to estimate the *in vivo* mechanical properties of the cornea by different algorithms. From published results, the estimated *in vivo* Young's modulus (elastic modulus) of cornea is around 0.25 - 0.79 MPa (Lam et al., 2015; Pye, 2020; Qin et al., 2019; Sit et al., 2017) ,while the *ex vivo* measurements of cornea Young's modulus were documented from 0.3 to 3.0 MPa (Jayasuriya et al., 2003), and from 0.8 to 2.2 MPa (Wollensak et al., 2003). Additionally, *ex vivo* investigation of the mechanical properties of Descemet's membrane have also been tested and documented in many studies; however, due to different measurements and tissue preparations, the stiffness of Descemet's membrane vary from 50 ± 17.8 KPa(Last et al., 2009), 1.8 ± 0.8 MPa (Xia et al., 2016) to 2.57 ± 0.37 MPa (Danielsen, 2004). Understanding the mechanical properties of native tissue paves the way for creating a similar biomechanical environment while developing a tissue engineered endothelium. Here, we summaries studies applying biomechanical properties of ECM to the corneal endothelium tissue engineering, including topographical properties and mechanical properties. (Table 1)

### *7.1 Topographical properties of ECM*

Topographical cues refer to the three-dimensional physical configuration of the ECM surface, including shape, geometry, size and organization of the ECM (Chuah et al., 2013). These parameters are determined by the alignment and texture of the ECM, including patterns on fibrils, pores, and pits (Gasiorowski et al., 2013). ECM topography exerts substantial impact on cell morphology, migration, and differentiation to maintain tissue homeostasis (Urbanczyk et al., 2020). In general, topographical features may range from nano-scale to macro-

scale level, and the most commonly used topography patterns for tissue engineering are grooves, pillars and walls. Considering the mean cell area of the human corneal endothelial cell, ranged from 332.3 ± 46.3 $\mu m^2$ to 390.59 ± 149.94 $\mu m^2$ (Abdellah et al., 2019; Carlson et al., 1988; Tananuvat and Khumchoo, 2020; Yunliang et al., 2007), cells are able to perceive topographic features lying on fibrils between nanometer-to-micrometer in size. Those topographical feature size greater than 2 µm might not be able to be sensed by one cell (Gasiorowski et al., 2013; Teixeira et al., 2003; Wilkinson et al., 2002).

Previous research has shown that cell proliferation of primary cultured human corneal endothelial cells can be significantly increased on 1 µm pillar modified tissue culture polystyrene (TCPS) coated with FNC Coating Mix containing fibronectin, collagen I and albumin (Muhammad et al., 2015). Initially, the authors used soft lithography to fabricate nano to microscale topographies on Polydimethylsiloxane (PDMS) with various geometries, including pillars and walls, to mimic native ECM. The results showed that bovine corneal endothelial cells on the pillared surface displayed a higher density of microvilli similar to native tissue and enhanced Na+/K+ ATPase immunofluorescence expression, mRNA upregulation and a higher Na+/K+ ATPase activity (Teo et al., 2012). Their further research indicated that fibronectin coating in combination with 1µm micropillars increased human corneal endothelial cell proliferation, and it also gave rise to the highest Na+/K+ ATPase and ZO-1 gene and protein expression in comparison to unattended PDMS (Koo et al., 2014). Recently, they put effort into using hybrid crosslinked gelatin methacrylate hydrogel (GelMA+) as a carrier, where cells grown on 1 µm pillars of square-array topography displayed higher ZO-1 expression. The corneal endothelial cells on 1 µm pillar of hexagonal-array topography displayed higher cell density and smaller cell size compared to the unpatterned control (Rizwan et al., 2017). On the other hand, another research group showed that human mesenchymal stem cells can successfully differentiate into corneal-endothelial-liked-cells on fabricated PDMS or collagen with hexagonal microtopography. The cells arranged in monolayer with polygonal cell morphology, and the typical gene transcription and the protein expression were also enhanced after 24-day cell culture (Gutermuth et al., 2019).

### *7.2 Mechanical properties of ECM*

Mechanical cues are provided by varying stiffness and strength of ECM and therefore biomaterials. Stiffness usually refers to how a material resists elastic deformation under stress, and it can be quantified by measuring the Young's modulus (elastic modulus). The strength of the material refers to how much stress can be imposed on a material before permanent deformation. The maximum stress can be quantified by measuring the ultimate tensile strength (breaking stress) (Antoine et al., 2014; Chuah et al., 2013). Both parameters are measurements to indicate the resistance of a material to plastic deformation. The elasticity of ECM is dictated by the arranging and disruption of fibre networks, such as intercalated laminin arrangements and short fibril structures (Gasiorowski et al., 2013). Meanwhile, tensile strength of ECM relates to the orientation and density of collagen fibres (Gasiorowski et al., 2013; Roeder et al., 2002; Urbanczyk et al., 2020; Whelan et al., 2019).

The impact of mechanical properties on corneal endothelial cell proliferation has been observed in previous research. Wang et al. blended chitosan and polycaprolactone (PCL) to fabricate a biodegradable material for bovine corneal endothelial cells growth. Even though they did not measure the stiffness of ECM, however, according to their results, the cell proliferation and adhesion increased as the content of PCL increased (with presumed increase in ECM stiffness). As the cells reached confluence, they displayed the typical corneal endothelial cells hexagonal shape and also expressed N-cadherin and tight junction ZO-1. The RNA expression of collagen IV also increased on stiffer ECM (Wang et al., 2012; Young et al., 2014). Furthermore, primary cultured corneal endothelial cells were seeded onto a decellularized thin stroma as a scaffold with similar stiffness to native tissue. The results showed that corneal endothelium can be regenerated with expression of typical endothelial markers, ZO-1, anti-connexin 43 and Na+/K+ ATPase (Choi et al., 2010). The same research team, additionally, fabricated a series of thin gelatin gel (TGG) scaffold with stiffness of ECM ranging from 0.8 ± 0.2 MPa to 5.8 ± 1.2 MPa. The results indicated that compared to softer scaffold, primary cultured human corneal endothelial cells grow better in a higher cell density on scaffold with higher stiffness (Niu et al., 2014). These results provide useful information for the application of manipulating ECM stiffness to promote corneal endothelial cell expansion and proliferation. Another research group conducted a more comprehensive study looking for suitable mechanical properties for *in vitro* endothelial cell culture. An elasticity tuneable PDMS system with various ECM coating was used to mimic the biomechanical properties of native Descemet membrane. The results indicated that PDMS with stiffness at 50 kPa and coating with collagen type IV is conducive to forming a high-cell-density monolayer, maintaining small size hexagonal cell shape, and expressing cell-cell tight junction ZO-1. (Palchesko et al., 2016; Palchesko et al., 2015). Recently, Kennedy et al. fabricated a synthetic poly-ε-lysine hydrogel (pεK), stiffness at 0.11 ± 0.01 MPa, for *in vitro* expansion of porcine corneal endothelial cells. Cell adhesion increased when collagen I, collagen IV and fibronectin were electrostatically bound to the surface of pεK hydrogel (Kennedy et al., 2019). As shown above, modification of the ECM stiffness on tissue engineered materials could be a useful tool to promote cell proliferation and to maintain cell morphology.

### *7.3 The thickness of ECM*

The thickness of the ECM or the thickness of the substrate is another biomechanical factor that we need to take into consideration when engineering a tissue equivalent for transplantation. Cells can sense much farther than we expected. Mullen et al. compared the cell spreading area on a wedge-shaped polyacrylamide gel with various thickness. The results showed that the cell spreading area significantly increased when the substrate thickness was below a critical threshold, even though the elastic modulus of the substrate was unchanged (Mullen et al., 2015). The increase in cell spreading area has much to do with the underlying material beneath the substrate, such as TCPS or glass. Cells might be able to sense the underlaying rigid material instead of the substrate alone, if the thickness of the substrate is lower than the mechanosensing length of the cells (Lin et al., 2010; Mullen et al., 2015; Rudnicki et al., 2013). Therefore, knowing the distance of cell sensing is another important issue in investigating the relationship between mechanical properties and cell behaviours. Different cell type has

different cell sensing distance, and the intrinsic nature of the substrate also governs how far cells can sense. For example, cells might be able to sense the mechanical signals beyond hundreds of microns away on fibrous materials, like collagen gels, but only several tens of microns can be sensed by cells cultured on nonfibrous materials, like polyacrylamide gel (Rudnicki et al., 2013; Sen et al., 2009; Tusan et al., 2018). Apart from the influence of substrate thickness on cell behaviours, we also need to consider the practicality for clinical use. The goal of tissue-engineering a corneal endothelium is to serve as a cell carrier for transplantation. Cells might be damaged or lost during the transplantation, if the substrate is too thin or too fragile. Ideally, the thickness for an engineered corneal endothelium should be as thin as the DMEK graft around 10 to 20 microns, containing only monolayer of endothelial cells and Descemet's membrane (Palchesko et al., 2016). However, considering the difficulty of handling and the mechanical support for cell delivery, a reasonable target thickness for engineered corneal endothelium would be around 100 μm to mimic the DSAEK graft with partial thickness of stroma, endothelial cells, and Descemet's membrane (Kennedy et al., 2019; Levis et al., 2012).

## 8. Conclusion

Biomechanical properties of ECM are closely interacting with corneal endothelial cells, which not only can influence cell behaviours and morphology, but also has impact on disease developing. In Fuchs' endothelial dystrophy, abnormal deposits secreted from dysfunction endothelial cells can affect the stiffness of Descemet membrane, cell synthesis and ECM remodeling. Prolonged or sudden increase in intraocular pressure can damage endothelial cells and alter the ECM biomechanical properties of the cornea. Biomechanical properties, both topographical and mechanical, exert substantial influence on cell behaviours and morphology. Providing favourable biomechanical properties of ECM for corneal endothelial cells can increase cell proliferation, maintain cell phenotype, and develop a tailored tissue-engineered substrate for endothelial cell transplantation. Thus, if developing an endothelium equivalent through tissue engineering method is as structural and functional as native tissue, while easing donor tissue demand and achieving cell-based therapy, there is no reason to not to embrace it as a better choice to treat malfunctioned corneal endothelial cells.

## 9. Method of literature review

Literature review was conducted by searching articles from online database, including PubMed, Web of Science, Google Scholar and Science Direct. The search terms were corneal endothelial cells, tissue engineering, biomechanics, topographical properties, mechanical properties, physical cues, mechanotransduction, and combinations thereof. Searching results were restricted to English publications, including peer-reviewed journal articles, review articles, and book chapters. The searching results were reviewed by titles and abstracts for relevance. Additional articles pertaining to the topic were identified and reviewed from the reference lists of the articles. A cited reference searching was also performed to track specific journal articles and their connection to the topic.

Table 1. Summary of studies applying biomechanical properties in corneal endothelium tissue engineering

| Biomechanical properties | Definition | Cell type | Substrate material | Method | Surface modification | Property features | Reference |
|---|---|---|---|---|---|---|---|
| Topographical properties | 3D configuration and structure of the ECM surface | BCEC | PDMS | Lithography | N/A | 1 µm pillars, 1 µm wells, 250 nm pillars and 250 nm wells | (Teo et al., 2012). |
| | | HCEC-B4G12 | PDMS | Lithography | Fibronectin-collagen I FNC Coating Mix laminin-chondroitin sulphate | 1 µm pillars, 1 µm wells, and 250 nm pillars | (Koo et al., 2014) |
| | | Primary HCEC | TCPS | Lithography | FNC Coating Mix | 1 µm pillars, 1 µm wells, and 250 nm pillars | (Muhammad et al., 2015) |
| | | hMSC | PDMS<br>Collagen | Lithography | N/A | Hexagonal wall with 16.3 µm width and 2.02 µm depth, including 185 nm steps that varied in deep from 20 to 116 nm. | (Gutermuth et al., 2019) |
| | | Primary HCEC | GelMA | Lithography | N/A | 1 µm pillars of square pillars,<br>1 µm pillar of hexagonal pillars,<br>250 nm pillars | (Rizwan et al., 2017). |
| Mechanical properties | Resistance to the deformation under stress | Primary HCEC | Thin human corneal stroma | Chemical decellularization | N/A | Young's modulus 47.6 ± 6.7 MPa<br>Tensile strength 10.2 ± 2.0 MPa | (Choi et al., 2010) |
| | | BCEC | Chitosan and PCL | Cross-linking | N/A | N/A | (Wang et al., 2012; Young et al., 2014). |
| | | Primary HCEC | Thin Gelatin gel (TGG) | Cross-linking | Heparin | Young's modulus 3.5 ± 0.3 MPa<br>Tensile strength 1.4 ± 0.4 MPa | (Niu et al., 2014). |
| | | BCEC | PDMS | Tuneable | Fibronectin, laminin 111, | Young's modulus 5, 50, 130, 830, 1340 or 1720 KPa | (Palchesko et al., |

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|---|---|---|---|---|---|---|---|
| | | | | elastomer system | collagen type I, collagen type IV, a blend of laminin and collagen type IV | | 2016; Palchesko et al., 2015). |
| | | PCEC HCEC-12 | poly-ε-lysine (pεK) hydrogel | Cross-linking | Electrostatical bound: Fibronectin, Collagen I, Collagen IV, Chondroitin sulphate, Laminin, FNC coating mix<br>Covalent binding peptides: RGD, DGEA | Young's modulus 0.11 ± 0.01 MPa<br>Tensile strength 0.04 ± 0.004 MPa | (Kennedy et al., 2019). |

BCEC, Bovine corneal endothelial cell; HCEC, Human corneal endothelial cell; hMSC, Human mesenchymal stem cells; PCEC, Porcine corneal endothelial cells; PDMS, Polydimethylsiloxane; TCPS, tissue culture polystyrene; GelMA, gelatin methacrylate; PCL, Polycaprolactone; RGD, H-Gly-Gly- Arg-Gly-Asp-Gly-Gly-OH; DGEA, H-Asp-Gly-Glu- Ala-OH

## Reference


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