



Electrospun Fibers for Corneal Regeneration

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Abstract

Purpose of review Electrospun fibers have attracted attention as scaffolds with a three-dimensional fibrous structure which can mimic the structure of the extracellular matrix. This review summarizes how electrospun fibers can be utilized for corneal regeneration.

Recent findings Recent studies have demonstrated how electrospun fiber parameters, including diameter, alignment, density, membrane thickness, and mechanical and optical properties determine the availability and efficacy of corneal regeneration. The well-organized three-dimensional electrospun fibers were modified depending on the purpose, such as corneal tissue substituent and therapeutic factor delivery.

Summary Electrospun fibers can be applied as corneal tissue substitutes for direct application and as paracrine therapy to support materials for indirect applications. The developed applications of electrospun fibers may be an alternative to corneal transplantation and amniotic membranes in the future.

Keywords Electrospinning · Electrospun fiber · Cornea regeneration · Corneal substituent · Paracrine therapy

Introduction

The cornea is a transparent, avascular, and multilayer-structured tissue that is one of the most important parts of vision [1]. It consists of five layers, including the epithelium, Bowman's layer, stroma, Descemet's membrane, and endothelium [2]. The corneal tissue is exposed to the external environment; hence, it is susceptible to external physical damage and biological infections. Moreover, corneal injuries from

degenerative, dystrophic, infectious, and expansive corneal diseases can cause vision loss or blindness [3]. Corneal transplantation is the main method used to preserve or improve a patient's sight by increasing corneal clarity. The lack of vascular and lymphatic vessels in the corneal tissue reduces the risk of immune rejection; therefore, corneal transplantation has a higher success rate than those of other tissue or organ transplantations [3, 4]. However, corneal transplantation is dependent on the availability of donated corneal tissues; thus, a lack of donations could be a major limiting factor [5, 6]. Therefore, there is a need to develop and improve treatment methods that are simpler and more suitable for corneal diseases.

One alternative is a tissue engineering approach, which can substitute the whole or part of the corneal tissue [7–10]. The principle of tissue engineering is the fabrication of corneal tissue substitutes that integrate cells and scaffolds in vitro. Another method is promoting regeneration by delivering bioactive molecules through substrates, such as amniotic membranes, to reconstruct damaged ocular surfaces [11, 12]. Various methods, such as hydrogel matrices and decellularized corneal tissues, generate three-dimensional (3D) scaffolds, and substrates in tissue engineering and bioactive molecule delivery approaches, respectively [13–18].

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In particular, electrospun fibers have emerged as promising candidate for corneal regeneration in ophthalmology [8, 19].

Electrospinning is a versatile fabrication technique that generates extracellular-matrix (ECM)-mimicking 3D fibrous structures [20]. The scaffold materials, commonly natural and synthetic polymers, are dissolved in a solvent and ejected through a needle under a high electrostatic charge. This charge affects the surface tension of the polymeric solution, leading to a pull toward a grounded or oppositely charged collector. While the solution moves from the tip to the collector, the solvent evaporates and polymeric micro- or nanofibers are deposited. The morphology of fibers can be adjusted by certain processing parameters including polymer materials, molecular weight, solution concentration, solvent, applied voltage, feeding rate, humidity, and temperature [20]. In addition, randomly oriented fibrous structures are obtained on the flat plate collector, while aligned fibrous structures can be generated using a rotating mandrel collector [21–24]. Consequently, the cytocompatibility, degradation behavior, and optical characteristics of electrospun fibers could be designed for use in corneal regeneration [7, 8].

The electrospinning product is generally a thin membrane. Therefore, electrospun fibers are suitable as epithelial [24–27], corneal stroma [28, 29], and endothelial layers [30, 31] but not for full-thickness tissue-engineered corneal scaffolds. Recently, some researchers have utilized electrospun fibers for cell attachment and proliferation differentiation, as well as a medium for the controlled release of bioactive molecules [8].

This review presents the electrospun fiber parameters in the context of corneal regeneration and provides a comprehensive overview of electrospun fiber as a direct corneal replacement and indirect paracrine therapy. Finally, a future perspective is provided, which discusses future directions of electrospun fiber research.

Electrospun Fiber Parameters

Fiber Diameter and Density

The fiber diameter is the most fundamental parameter for electrospun fibers. The typical diameters range from tens of nanometers to several micrometers [8]. Ideally, electrospun nanofibers are favored to mimic the ECM, especially collagen fibrils, which are 31–34 nm in diameter [32]. Few studies have been able to fabricate a favorable diameter range under 100 nm using polylactic-co-glycolic acid (PLGA) [33], collagen [34], and gelatin [35]. Nanofibers within a desired range of diameters can be achieved by manipulating the distance from the nozzle to the collector, volumetric charge density, elongational viscosity, and relaxation time [36]. The fiber diameter determines the structure density,

while the spacing between the fibers determines the mass transfer, cell penetration, mechanical properties, and optical properties. If nanotopography is a more important factor than cell penetration into the electrospun scaffold, electrospun nanofibers will be chosen. In contrast, if cell penetration is a critical factor, electrospun microfibers can be used.

Membrane Thickness

The thickness of the resulting fibrous membrane is a major limitation of electrospun fiber scaffolds in tissue engineering. The existing fibers between the grounded collector and the deposited fibers inhibit charge movement from the upper layer to the grounded collector. Thus, the accumulated charge repels the newly deposited fibers, and the fibers move to the side with a lower thickness. As a result, while each condition has different limits, the fibers do not increase in thickness after a certain limit.

For clinical application in a general tissue engineering approach, scaffolds are required with volumes ranging from several millimeters to several centimeters or more. The cornea is a thin-layered tissue with a thickness of approximately 500 μm . However, it is still not possible to manufacture a full-thickness corneal scaffold with electrospun fibers, as their thicknesses are generally less than 100 μm [37, 38]. Some trials have attempted to generate volumed electrospun fiber scaffolds using multi-layered fibrous structures [39] and thermally processed polysulfone/polyglycerol sebacate core/shell fibers [40]. These bulky electrospun fiber scaffolds showed potential as full-thickness tissue scaffolds. Nevertheless, the low variability in materials and processes requires additional efforts to control the appropriate properties of corneal tissue scaffolds. Therefore, electrospun fibers are more suitable for generating layers of the cornea, such as the epithelial, [24–27] corneal stroma [29], and endothelial layers [30, 31].

Mechanical Properties

Electrospun fibers should have sufficient mechanical strength to withstand handling and suturing during the implantation process. In addition, after implantation, they should be resistant to physiological forces such as intraocular pressure, eyelid motion, and tear film motion [41]. However, excessive stiffness affects the phenotypes of cells such as corneal epithelial, stromal, endothelial, and limbal-derived stem cells [42–45]. Therefore, the mechanical properties of electrospun fibers are important factors affecting cell and corneal tissue regeneration.

Ideally, scaffolds should have mechanical properties similar to those of the native corneal tissue, with a Young's modulus ranging from approximately 0.3 to 7 MPa and a tensile strength ranging from approximately 3 to 6 MPa

[46–48]. While mechanical strength is affected by the fiber diameter and density, the electrospun fiber material primarily determines the mechanical properties. Generally, natural biopolymers such as collagen, gelatin, and silk are favored because of their good biocompatibility for corneal scaffolds or regeneration [7]. However, electrospun fiber membranes generated by biopolymers have poor mechanical properties to be used in clinical applications. In contrast, synthetic polymers generally have good mechanical strength but have disadvantages such as poor cell attachment and low wetting due to their high hydrophobicity [49]. Combining natural and synthetic polymers and tuning of the fiber diameter and density allows control of the mechanical strength in the required range and compensation for the disadvantages of each material. Synthetic polymers such as polycaprolactone (PCL), polyvinyl alcohol (PVA), polylactic acid (PLA), and PLGA have been blended with natural biopolymers [7, 8]. Carter et al. showed a 10-fold increase in tensile stress for an electrospun fiber combining gelatin with PCL, from 0.11 to 1.23 MPa [21].

Chemical cross-linking, including glutaraldehyde, 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC), genipin, and photo-cross-linking, has also been explored to increase the mechanical strength of electrospun fibers made from natural biopolymers [7]. For example, Zhang et al. reinforced electrospun gelatin nanofibers by glutaraldehyde vapor crosslinking, reporting a tensile strength increase from 1.28 to 12.62 MPa [50]. However, additional chemical cross-linking methods, often cytotoxic chemicals, were not straightforward, as they required further treatment and washing steps.

Optical Properties

The cornea is a transparent tissue with important light transmission properties; therefore, the light transmittance of the electrospun fiber is also important for corneal tissue scaffolds. For use as a corneal tissue scaffold, a visible light spectrum corresponding to a wavelength of 400–780 nm must be transmitted. The transparency of native corneas depends mainly on the fibrillar collagen structure; similarly, the optical properties of electrospun fibers are determined by their material and fibrous structure. In actual corneal tissue, collagen fibrils with a diameter of approximately 30 nm are aligned in line with a spacing of approximately 60 nm [32]. However, synthetic polymer fibers have narrow interfiber spacing due to the electrospun structure, which prevents light transmission, resulting in low transparency.

There have been some efforts to improve the transmittance of electrospun fibers for application as a corneal scaffold. Sharma et al. treated PCL electrospun fibers using helium-oxygen plasma to increase the water wetting property of electrospun fibers, which resulted in 47% visible light transmittance

[51]. Kong et al. improved the transmittance of PLGA fiber mats to approximately 72% by generating holes using laser-perforation combined with compressed collagen hydrogel [52]. Arica et al. fabricated gelatins with methacrylic acid anhydride (GelMA) fibers by electrospinning and embedding poly(2-hydroxymethyl methacrylate) (p (HEMA)) through photopolymerization, resulting in an optical transmittance of 70% at 550 nm [53]. The transmittance of the wet amniotic membrane is 77%, but a transmittance of 80% or more is required for electrospun fiber membranes to be used as a corneal scaffold. If the electrospun fiber is used on the ocular surface outside the visual axis where light passes through or biodegradable fiber material is used, the material's transparency becomes less important and, in some cases, unimportant.

Fiber Alignment

Electrospun fiber alignment influences cell behavior. It is simple to obtain an ordered fiber structure using the electrospinning method. A basic electrospinning collector is a plate that generates a randomly oriented fibrous structure, while aligned fibrous structures can be obtained on a rotating substrate using rotating mandrels as a collector [22, 29, 34, 54]. Electrospun fibers arranged in different patterns were obtained through different collector designs, using the principle that the ejected solution is preferentially deposited where it is grounded or oppositely charged. Two metal parallel plates with air gaps fabricated parallel aligned electrospun fibers vertical to the collector [16]. Fernandez-Perez et al. obtained radially and perpendicularly aligned electrospun fibers using a cup-pin collector and four separate post collectors, respectively [55].

As mentioned above, if a structure similar to the original tissue is provided, the desired natural phenotype of the cells can be promoted. For example, the corneal stroma comprises aligned collagen fibrils and stroma cells reside in an aligned structure [32]. Furthermore, the aligned fiber structure promoted corneal stromal cell alignment, migration, and keratocyte phenotype expression [29, 34, 54]. In another study, keratocytes proliferated more favorably in the aligned composite gelatin-PLA scaffold as opposed to a randomly oriented scaffold composed of the same materials [23]. Therefore, aligned electrospun fibers are ideal for controlling the behavior of corneal cells. However, there is a limitation in increasing the thickness of the aligned electrospun fibers.

Corneal Applications

Direct Application as Corneal Substituents

Direct applications refer to the use of electrospun fibers as corneal substituent scaffolds for corneal cells. Several

types of keratoplasties have been applied in the clinical field, depending on the injuries or medical conditions. First, penetrating keratoplasty (PKP) is applied if the full-thickness cornea is to be replaced. Second, anterior lamellar keratoplasty (ALK) or deep anterior lamellar keratoplasty (DALK) replaces the epithelium, Bowman's layer, and partial or most of the stroma. Finally, Descemet's membrane endothelial keratoplasty (DMEK) and limbal transplant (LT) are keratoplasties that replace relatively thin endothelium/Descemet's membranes and limbus, respectively. Electrospun fibers are commonly thin membranes; thus, they are considered more suitable for DMEK or LT, which require thin layer substitutes, rather than PKP, ALK, or DALK. Recently, the proportion of PKP in keratoplasties has been greatly reduced by increasing LT [56–58]; the utilization of electrospun fiber scaffolds is expected to further increase despite the thickness limitation.

In applications for epithelial regeneration, electrospun fibers allow epithelial cells to form cell sheets. A study with keratocytes as well as corneal epithelial cells showed better adherent ability on gelatin-PLA scaffold [23]. In another research, corneal epithelial cell attachment was the same in two different diameters of PCL whereas adult retina pigmented epithelium (RPE-19) cells preferred the scaffold with a greater fiber diameter (1300 nm) [59]. Hong et al. utilized a PLGA electrospun fiber membrane as an epithelial layer substituent [60]. Conjunctival epithelial cells (CJECs) were cultured on the electrospun fiber membrane *in vitro* for 2 weeks; the resulting epithelial sheets were then transplanted into rabbits whose corneas were burned. This epithelial sheet transplantation reduced the epithelial defect area to $6.1 \pm 1.6\%$ compared to $30.5 \pm 6.3\%$ in the control group after 2 weeks.

Limbal stem cells (LSCs) present in the corneal limbus of the eye play a crucial role differentiating into corneal epithelial cells. For example, limbal epithelial stem cells cultured on a PCL scaffold increased the expression of K3/12 indicating the potential of the cell line to differentiate [61]. Similarly, LSC retained their differentiation ability on a PLA scaffold and expressed specific epithelial transcription factors like $\Delta Np63\alpha$ and SOX17 after 14 days [62]. Nonetheless, the stem cells derived from bone marrow also showed similar therapeutic effects as LSCs for corneal epithelial regeneration [63, 64]. Likewise, mesenchymal stem cells (MSCs) derived from conjunctiva differentiated into corneal epithelial-like cells on a composite polyurethane/silk nanofiber scaffold expressing several epithelial differentiation markers such as cytokeratin-8 (CK-8), CK-12, and desmoglein-1 (DSG-1) [65].

Electrospun fiber scaffolds can substitute the corneal stromal layer. Since the corneal stroma comprises aligned collagen fibrils, studies have mainly used fibers aligned to resemble this structure [29, 54, 55]. A stem cell line from corneal

stroma differentiated into keratocytes on an aligned poly (ester urethane) urea fiber that expressed keratocan markers [29]. Other studies aligned PLA/gelatin electrospun fibers were inserted into the rabbit stroma layer and observed for 32 weeks [66]. The aligned composite scaffold showed good compatibility with the corneal tissue and induced keratocyte ingrowth. In a different study, Aslan et al. observed that aligned PLA fiber showed higher proliferation of stromal keratocytes as opposed to collagen foam scaffold as well as decellularized cornea tissue [67]. Thus, a thin layer of electrospun fibers showed the potential as a substituent to increase host cell function, even without providing a full-thickness scaffold.

Corneal endothelial cells (CEnCs) were cultured on electrospun fiber, and the potential of the fibrous scaffolds as endothelial substitutes was evaluated [30, 31]. Chen et al. blended SF and poly(lactic acid-co-caprolactone) (PLACL) with various ratios of electrospun fiber materials, in which the 25:75 blend ratio of SF and PLACL showed potential as a scaffold for corneal endothelial transplantation, with approximately 70% transmittance and the highest cell proliferation rate [30]. Kruse et al. compared PLGA, PCL, and polymethyl methacrylate (PMMA) electrospun fibers for CEnCs scaffolds, among which PLGA was revealed as a promising candidate for preserving CEnC viability and morphology [31]. Table 1 summarizes the direct applications of electrospun fibers as corneal substitutes.

Previous studies combined hydrogels to supplement the thickness limitation of electrospun fibers [16, 35, 52]. Table 2 summarizes the composites of electrospun fibers and hydrogels for corneal substitutes. Wilson et al. stacked aligned electrospun fibers by inserting spacers in the middle, applying a collagen solution, and gelating the resulting product [16]. Combining hydrogels with multilayered electrospun fibers showed that it is possible to tailor the culture environment and control the cell phenotypes. Kong et al. combined PLGA electrospun fibers with compressed collagen hydrogels [52]. Keratocytes were cultured inside the gel with aligned electrospun fiber, and the CECs were cultured on the gel surface to form a construct similar to that of the native cornea, demonstrating its potential as an engineered corneal tissue to replace the full thickness.

Studies on the electrospun fiber scaffold substituent, which replaces multiple layers including the epithelial, stromal, and endothelial layers, as well as *in vivo* studies, are lacking. However, as *in vitro* studies have verified this application, future development is expected.

Indirect Application as Paracrine Therapy

In an indirect application, electrospun fibers promote corneal recovery by releasing bioactive molecules rather than replacing corneal tissue. Amniotic membranes are currently used

Table 1 Electrospun fibers parameters for direct applications as corneal substituents

Polymer ^a	Fiber diameter (μm)	Membrane thickness (μm)	Young's modulus (MPa)	Tensile strength (MPa)	Transmittance (%)	Fiber alignment	Cell type ^b	Application	Refs.
PCL	0.1	-	-	-	-	Random	LSC	Epithelial regeneration	[86]
PU/SF	0.1–0.6	-	0.1	10.8	-	Random	CJMSC	Epithelial regeneration	[65]
PLGA	-	12–20	-	-	-	Random	CJEC	Epithelial regeneration	[60]
PCL	0.1–0.2	-	-	2.0	47	Random	LEC	Epithelial regeneration	[51]
PU /silk	-	-	0.13	-	-	Random	CJMSC	Epithelial regeneration	[65]
PLA	-	-	-	-	-	Random	BM-MSC & LSC	Epithelial regeneration	[63, 64]
PLA	5–15	190–210	-	-	-	Random	LSC	Epithelial regeneration	[62]
PCL	0.9–0.17	-	-	1.6–1.9	3–28	Random	LEC	Epithelial regeneration	[61]
PCL	0.5 and 3	-	-	-	-	Random	ARPE-19 & HCE-T	Epithelial regeneration	[59]
Gelatin/PLA	0.81–1.0	-	-	3.1–6.8	-	Random and aligned	Keratocytes & CEC	Epithelial regeneration	[23]
PCL/DCP	0.1–0.2	0.1–0.4	9.2	-	-	Random and radial and perpendicular	Keratocyte	Stroma regeneration	[55]
PCL/SF	0.3–0.6	-	0.8–2.6	2.0–5.0	30–80	Random and aligned	Keratocyte	Stroma regeneration	[54]
PCL/gelatin	0.6–2.2	60–75	15–1860	-	-	Random	Keratocyte	Stroma regeneration	[87]
PEUU	0.1–0.2	8–10	-	-	-	Random and aligned	CSSC	Stroma regeneration	[29]
PLA/gelatin	0.7–1.0	-	-	-	-	Aligned	-	Stroma regeneration	[66]
PLA	0.62–0.72	9.5–81	-	-	76–78	Random and aligned	Keratocytes	Stroma regeneration	[67]
PVA/collagen	0.1–0.3	-	-	2.3–3.6	48–54	Random and aligned	Keratocyte & CEC	ALK	[24]
SF/PLACL	0.1–0.6	30–130	-	1.9–9.4	20–80	Random	CEnC	Endothelial regeneration	[30]

Table 1 (continued)

Polymer ^a	Fiber diameter (μm)	Membrane thickness (μm)	Young's modulus (MPa)	Tensile strength (MPa)	Transmittance (%)	Fiber alignment	Cell type ^b	Application	Refs.
PLGA	1.8	109	-	-	15	Random	CEnC	Endothelial regeneration	[31]
PCL	2.3	114	-	-	6	Random	CEnC	Endothelial regeneration	[31]
PMMA	3.0	150	-	-	28	Random	CEnC	Endothelial regeneration	[31]

^aPCL, polycaprolactone; PU, polyurethane; SF, silk fibroin; PLGA, poly(lactic-co-glycolic) acid; PLA, polylactic acid; DCP, decellularized cornea powder; PEUU, poly(ester urethane) urea; PVA, polyvinyl alcohol; PLACL, poly(lactic acid-co-caprolactone); PMMA, polymethyl methacrylate.

^bLSC, limbal stem cell; CJMSC, conjunctiva-derived mesenchymal stem cell; CJEC, conjunctival epithelial cell; LEC, limbal epithelial cell; CSSC, corneal stromal stem cell; CEC, corneal epithelial cell; CEnC, corneal endothelial cell

for corneal regeneration, as they have high biocompatibility and anti-inflammatory properties and contains multiple growth factors that promote corneal regeneration [11, 68]. Nevertheless, the limitations include availability, donor variability, and uncontrolled degradation [68]. Loading bioactive molecules onto a thin fiber scaffold through electrospinning is an alternative method to the use of amniotic membranes.

Bioactive Molecules

Electrospun nanofibers have several advantages as they are used in drug delivery systems. First, a sustained release is possible through mass transfer, such as diffusion, due to the large surface-area-to-volume ratio. Second, they are less affected by the dissolution properties of the drug. Selecting appropriate polymers and solvents for electrospinning evenly distributes the drugs in the electrospun fiber according to the hydrophobicity/hydrophilicity of the drugs. Third, the drug release rate can be controlled by the biodegradation rate of polymers in addition to diffusion. Table 3 summarizes the bioactive molecules used to deliver electrospun fibers for paracrine therapy.

Vitamin C [69], epigallocatechin gallate (EGCC) [70], moxifloxacin, and pirfenidone [71, 72] have been incorporated into electrospun fibers for stroma regeneration, antiangiogenesis, antibiotic, and anti-scarring, respectively. To treat fungal growth, Göttel et al. fractionated an amphotericin B-loaded Gellan gum/pullulan composite nanofiber as an alternative for eye drops for the treatment of keratomycosis [73]. In other studies, Gangadeep et al. and Mehta et al. loaded timolol maleate onto their respective hybrid fibers to overcome the necessity for multiple administrations of eye drops for the treatment of glaucoma [74, 75]. However, the release rates of bioactive molecules were much faster

than the degradation rate of electrospun fibers. Thus, further improvements are required to retain the long-term sustained release of bioactive molecules.

Cellular Secretomes

The predominant role is of the secretion of regulatory and trophic factors, such as growth factors, cytokines, and chemokines to support and regulate surrounding cells has recently been emphasized [21, 76–80]. The secretomes of bone marrow-derived mesenchymal stem cells (BMSCs) [77], adipose-derived stem cells (ASCs) [78], corneal stromal stem cells (CSSCs) [79], and LSCs [80] were characterized, and the effects on corneal regeneration were evaluated. One method for efficient secretome delivery is to implant stem cells by culturing them on electrospun fiber scaffolds. Transplanted stem cells with fibrous scaffolds can sustainably provide a secretome in response to environmental changes. For example, MSCs and LSCs co-cultured on the polyamide 6/12 scaffold improved the healing process of injured ocular surface while suppressing inflammatory reactions [81]. We summarize research on the use of electrospun fibers to provide cellular secretomes for corneal regeneration in Table 4.

Fiber diameter, mechanical properties, and fiber alignment are important parameters in cellular secretome applications. Moreover, the mechanical properties and fiber alignment of substrates affect cell behavior, including proliferation, migration, and differentiation [82–84]. Therefore, changes in cellular behavior cause differences in the secretome amount and composition (Table 5).

Carter et al. cultured BMSCs on PCL/gelatin electrospun fibers and co-cultured them with corneal fibroblasts [21]. The co-culture system improved fibroblast proliferation and

Table 2 Composites of electrospun fibers and hydrogels for direct applications as corneal substitutes

Polymer	Hydrogel material	Fiber diameter (μm)	Total thickness (μm)	Young's modulus (MPa)	Tensile strength (MPa)	Transmittance (%)	Fiber alignment	Cell type	Application	Refs.
PLA	Collagen	0.5–3.0	-	0.003	-	-	Aligned	Keratocyte	Stroma regeneration	[16]
Gelatin	Alginate	0.06–0.13	400–600	0.98	0.50	30–60	Random and aligned	-	PKP	[35]
PLGA	Collagen	0.5–2.0	150–200	-	3–5	72	Aligned	CEC & Keratocyte	PKP	[52]

scratch closure compared to the BMSC secretome solution-treated group. Enhanced corneal epithelial wound healing and stromal transparency were also observed after trimming the electrospun fiber sheets into a donut shape and placing them on alkali-burned rabbit corneas *ex vivo*. The research provided a generalized vehicle concept with sufficient mechanical and handling properties placed and secured onto the ocular surface or implanted subconjunctivally.

Sanie-Jahromi et al. evaluated the effect of LSCs on PCL and PCL/gelatin electrospun nanofibers in corneal epithelial regeneration in an animal model [85]. The PCL electrospun fibers showed higher LSC viability, induction of epithelial morphology, and proliferation compared to the PCL/gelatin blended fibers. Although more research is needed regarding material and cell selection, electrospun fibers with stem cells are promising for cell therapy of corneal diseases.

Conclusions

As an increasing number of people are experiencing corneal disease, there is an urgent need for improved therapeutics. Corneal transplant and amniotic membrane grafting are currently the major methods used in ophthalmology to regenerate damaged corneas or promote wound healing. However, rather than relying solely on natural materials, it is necessary to develop engineered materials that can induce corneal regeneration that allow more efficient utilization in a wider range of applications. Moreover, the importance of treatment methods that induce healing or replacement of partially damaged corneal layers is steadily increasing.

The electrospinning process provides a large scope for customizing the nanofibrous scaffold by manipulating a wide range of parameters according to the research objective. Although there is a limitation in the overall thickness, studies have demonstrated a tissue-engineered-cornea scaffold that can replace full-thickness cornea. In addition, research on the replacement of certain layers, such as the epithelium, stroma, and endothelial layers, has confirmed various applications as a layered scaffold that avoid the disadvantages of thin electrospun fiber membranes.

For electrospun fibers to replace all or part of the cornea, conditions such as cornea-mimetic fiber structure and mechanical and optical properties must be satisfied. However, no electrospun fiber yet satisfies all requirements; therefore, more intensive studies and development efforts are required. Nonetheless, indirect applications using paracrine therapy, which require relatively few conditions for electrospun fibers, are expected to be applied in clinical practice soon. The paracrine therapy method of promoting or enhancing spontaneous corneal regeneration can be utilized by loading drugs into electrospun fibers or by culturing and implanting stem cells, which secrete therapeutic factors, on

Table 3 The parameters of electrospun fibers for paracrine therapy using bioactive molecules

Polymer ^a	Fiber diameter (μm)	Young's modulus (MPa)	Tensile strength (MPa)	Fiber alignment	Bioactive molecule ^b	Degradation rate	Target	Refs.
PUU	0.2–0.7	6.6	2.2	Aligned	Vitamin C	105 days ^c	Stroma regeneration	[69]
SF	0.2–0.7	-	-	Random	EGCC	2 days ^d	Anti-angiogenesis	[70]
PVP (core) and PLGA (shell)	0.4–0.9	-	-	Random	Moxifloxacin and pirfenidone	< 2 hours ^d	Antibiotic & anti-scarring	[71, 72]

^aPUU, poly(urethane-urea); PVP, polyvinyl pyrrolidone^bEGCC, epigallocatechin gallate^cPeriod during which the electrospun fiber mass decreased by 30%^dPeriod during which 80% of the bioactive molecule was released**Table 4** The electrospun fiber parameters for paracrine therapy using cellular secretomes

Polymer	Fiber diameter (μm)	Young's modulus (MPa)	Tensile strength (MPa)	Fiber Alignment	Cell type ^a	Degradation rate ^b (Days)	Refs.
PLGA	3.0–4.0	-	-	Ring shape collector	LSC	-	In vitro cell culture and rabbit cornea organ culture [33, 88]
PCL/gelatin	-	1.2	1.2	Random	BMSC	-	In vitro cell culture and rabbit cornea organ culture [21]
PLGA and collagen	0.5–1.0 (PLGA) 0.2–0.3 (collagen)	1.0–1.6	1.0–3.5	Random	EnSC	70–84	In vitro cell culture [89]
PCL/gelatin	0.9–1.0	-	2.0–2.5	Random	LSC	-	In vitro cell culture and in vivo alkali burned rabbit [85]
Polyamide	0.3–0.6	-	-	Random	LSC and BMSC	-	In vitro cell culture and in vivo epithelial damaged mouse [81]

^aPLGA, poly(lactic-co-glycolic) acid; PCL, polycaprolactone; LSC, limbal stem cell; BMSC, bone marrow-derived mesenchymal stem cell; EnSC, endometrial stem cell^bThe period during which the electrospun fiber mass decreased by 30%**Table 5** The importance of electrospun fiber parameters for each application

	Direct application	Indirect application (bioactive molecules)	Indirect application (cellular secretomes)
Fiber diameter	⊙	⊙	⊙
Membrane thickness	⊙	△	△
Mechanical property	⊙	△	⊙
Optical property	⊙	-	-
Fiber alignment	⊙	-	⊙
Cell type	⊙	-	⊙
Degradation rate	△	⊙	△

⊙, important; △, intermediate; -, nonessential

electrospun fibers. In vitro experiments have demonstrated the direct and indirect positive effects of electrospun fibers on corneal cell differentiation or regeneration, showing their high potential for corneal regeneration applications. Furthermore, in vivo experiments have evaluated the biocompatibility and bioavailability of electrospun fibers. The verification of the efficacy of direct and indirect applications of electrospun fibers in in vivo experiments may allow their successful utilization as a modality to foster corneal wound healing.

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Declarations

Conflicts of Interest The authors declare no competing interests.

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