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KEYNOTE (GREEN)

Ocular application of electrospun materials for drug delivery and cellular therapies

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The constraints of delivering conventional drugs, biologics and cell-based therapeutics to target ocular sites necessitate the fabrication of novel drug delivery systems to treat diverse ocular diseases. Conventional ocular drug delivery approaches are prone to low bioavailability, poor penetration and degradation of therapeutics, including cell-based therapies, leading to the need for frequent topical applications or intraocular injections. However, owing to their exceptional structural properties, nanofibrous and microfibrous electrospun materials have gained significant interest in ocular drug delivery and biomaterial applications. This review covers the recent developments of electrospun fibers for the delivery of drugs, biologics, cells, growth factors and tissue regeneration in treating ocular diseases. The insights from this review can provide a thorough understanding of the selection of materials for the fabrication of nano- and/or micro-fibrous systems for ocular applications, with a particular interest in achieving controlled drug release and cell therapy. A detailed modality for fabricating different types of nano- and micro-fibers produced from electrospinning and factors influencing generation are also discussed.

Keywords: ocular drug delivery systems; electrospinning; anterior segment; protein delivery; cellular therapies

Introduction

Vision is one of five primary senses, and any minor disturbance to vision can drastically affect quality of life. Quality of life is one of the constructs related to chronic diseases that govern a person's ability to complete activities related to daily life and their social, emotional and economic well-being.[1] Globally, 2.2 billion people are affected by diseases that can affect vision and, as per the world report on Vision 2020, nearly 1 billion of these cases of vision impairment are preventable or treatable. Hence, the problem could be directed toward two issues: first and foremost the unavailability of medical resources; and, second, suboptimal treatment using current formulation approaches.[2] Most anterior segment eye disorders are treated via conventional topical eyedrops, and posterior segment eye disorders are mainly treated via intravitreal injections (IVTs) of therapeutic agents. Although these routes of administration offer various benefits, they are often limited by many drawbacks and limitations, such as low ocular bioavailability, high invasiveness (applicable for IVT), frequent administration, poor patient adherence and compliance.[3,4] Hence, novel approaches in formulating drug delivery systems combined with alternative routes of administration to IVT and eyedrops could potentially offer improved benefits to patients and ophthalmologists. In this regard, periocular routes, such as subconjunctival, transscleral and intracameral injections,

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can potentially overcome some of the above limitations and could offer higher ocular bioavailability, along with minimally invasive administration strategies.[5]

Electrospinning (ES) is one of several novel drug delivery strategies and offers several benefits. For instance, ES can produce a nanofibrous mesh or mesh-like network of nanofibrous scaffolds that can serve as a drug carrier and release the drug in a controlled manner for an extended duration of time. Another technique known as electrospraying can lead to the formation of particle-based delivery systems with some unique features that are not otherwise feasible via conventional particle fabrication methodologies. These nanofiber or nanoparticle platforms could be used as a drug reservoir when administered via the periocular routes. Furthermore, ES or electrospraying offers significant tailorability that enables loading and release of a wide range of therapeutics via periocular routes that can potentially overcome the limitations of conventional formulations and significantly offer higher ocular bioavailability along with minimally invasive administration strategies.[5]

Electrospun matrices and materials offer various benefits for developing novel ocular therapeutics for different periocular routes. The nanofibrous matrix offers a very high surface area, which is the governing factor for drug release and degradation. Furthermore, control of the surface area aids in modulating these properties.[6] The highly porous structure of eletrospun implants is known not to affect tissue respiration and gaseous exchange, which is one of the crucial factors when designing implants for corneal application.[7–10] The nonwoven loose nanofibrous matrix architecturally resembles the extracellular matrix (ECM; see Glossary for list of abbreviations), and this loose bonding of fibers is beneficial for tissue ingrowth and cellular migration along with promoting good nutrition within the fibrous matrix.[11,12] Considerable research has been directed toward the development of episcleral, subconjunctival and topical drug delivery systems, and electrospun materials are frontrunners in the field. In this review, we aim to discuss the importance of novel ES techniques and the opportunities that they can offer for the development of innovative ocular drug delivery systems. Furthermore, the various limitations of electrospun materials and strategies to overcome those limitations are also discussed to successfully develop novel ocular therapeutics.

Ocular tissues and disorders

The eye is often described as an organ made up of various layers. One of the interesting facts about these layers is that fibrillar proteins such as collagen and elastin make up the majority of these layers, followed by different types of cells and ECM. Membranes such as the sclera, cornea, inner limiting membrane and basement membrane are primarily made up of collagen, which exists in fibrillary structures. The primary function of these collagen fibrils is to provide tissue integrity and a fibrous basement for the attachment of different cells. Although collagen I is a major protein in this structure, the properties of different layers are dictated by the orientation of collagen along with other subsidiary materials (i.e., the homogenous orientation of collagen leads to the highly transparent nature of the cornea with enhanced barrier properties; however, the random orientation of collagen fibers in the sclera leads to opaque and relatively permeable tissue, which is needed to control the amount of light entering the eye and maintain the movement of biomolecules across the tissue). Hence, in this section, we discuss the anatomy of the eye along with highlighting its fibrous nature.

The eye is a very distinct organ in terms of its anatomical features. It consists of three different layers that work in synchronicity to achieve vision (Figure 1). The outermost layer of the eye is the sclera and cornea, which act as a protective layer. The cornea and sclera are made up of collagen fibers with varying fiber orientation, giving a distinct transparent nature to the cornea and an opaque white color to the sclera. Anatomically, the cornea consists of four layers: namely the corneal epithelium, Bowman's membrane, corneal stroma and endothelium. The corneal stroma accounts for 80% of the total cornea and is mainly composed of a highly organized, parallel arrangement of collagen fibers, which also imparts a transparent look to the cornea. A transparent nature is imperative for the function of the cornea. Furthermore, the cornea is continuous with the sclera, where the limbus is the meeting point for both. Similar to the cornea, the sclera also consists of four layers: the episclera, scleral stroma, lamina fusca and endothelium. The scleral stroma consists of 80% of sclera and is made up of Type I (90%) and Type III (<5%) collagen fibers and proteoglycans. The randomly arranged organization of Type I collagen in the sclera is responsible for its opaque nature.[13]

The middle layer of the eye consists of the choroid primarily functioning to supply blood and nutrients to other organoids within the eye along with the ciliary body, which controls the shape of the lens and produces aqueous humor. The choroid is made up of blood vessels, melanocytes, fibroblasts and immune cells along with supporting collagenous and elastic connective tissue. The secondary functions of the choroid are light absorption, thermoregulation, heat dissipation and modulation of intraocular pressure (IOP) via vasomotor control of blood flow.[14] The innermost layer consists of the retina, which contains rod and cone cells along with other secondary cells and retinal pigment epithelial cells. The retina responds to light and makes vision possible. The organization of photoreceptor cells, retinal ganglion cells and other retinal cell types enables the conversion of light signals into neuronal impulses.[15]

Based upon the location of the lens, human eyes can be divided into two segments: the anterior segment and posterior segment. The anterior segment consists of the cornea, iris, lens, aqueous humor and ciliary muscles, which are filled with aqueous humor. The posterior segment consists of the sclera, choroid and Bruch's membrane; and the innermost layer consists of the retina and is filled with vitreous humor.[15]

The most noteworthy feature of ocular anatomy is that the ocular layers are made up of nanofibrous layers of collagen along with different proteins and macromolecules. For instance, the sclera and cornea are primarily composed of collagen fibrils. In addition, the inner limiting membrane, Bruch's membrane and the vitreous humor also consist of collagen fibrils, which provide mechanical stability. Bruch's membrane, for example, acts as the basement for retinal pigmented epithelial cells and is crucial for the accumulation of lipids during the pathogenesis of age-related macular degeneration (AMD). The corneal epithelium is attached to the corneal stroma, which is composed of collagenous fibrils.

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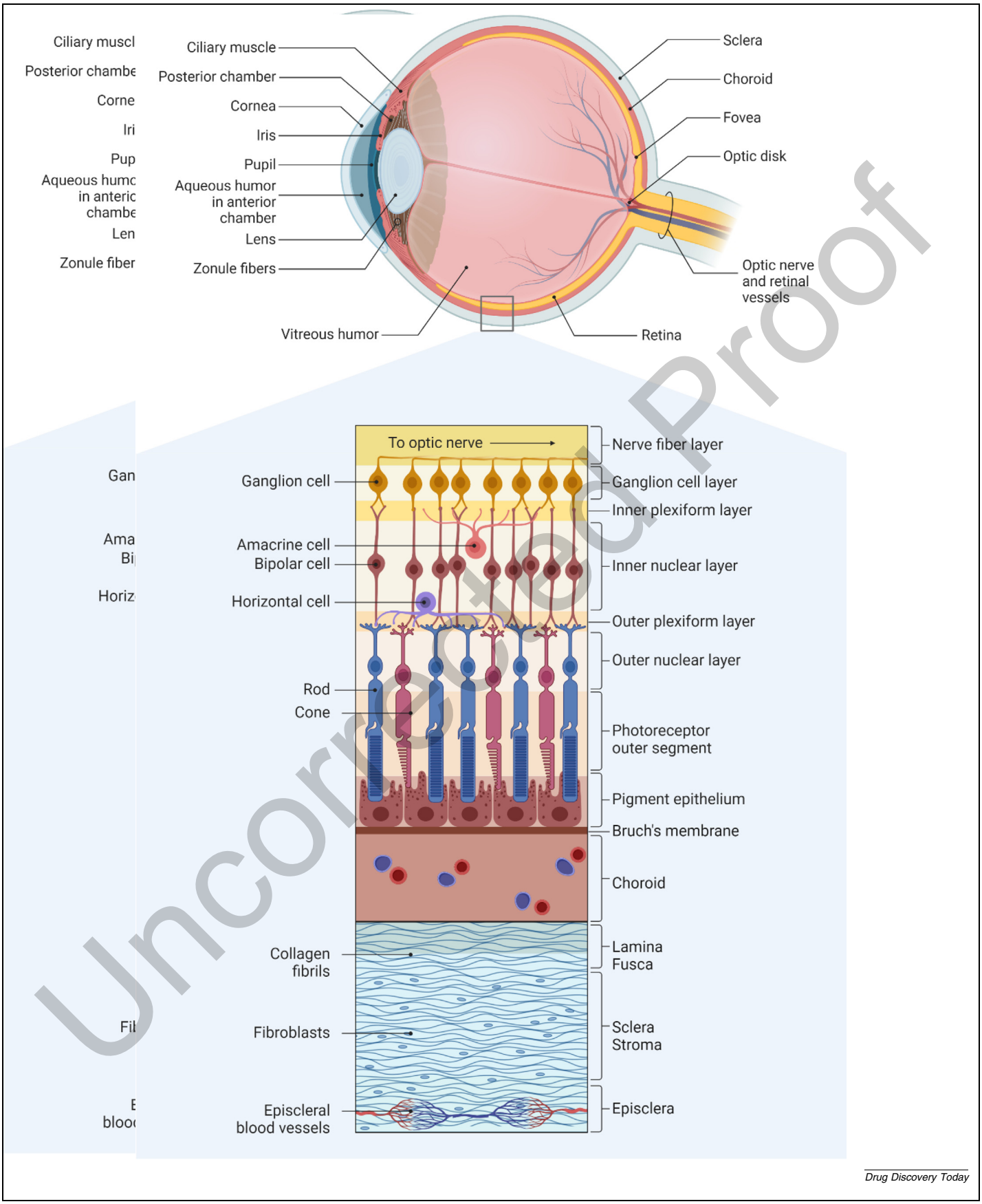

FIGURE 1

The anatomy of the eye.

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Furthermore, these membranes, along with the associated cells and their tight junctions, act as barriers for the movement of fluid and molecules across them. Diseases such as AMD, diabetic retinopathy (DR) and diabetic macular edema (DME) are often associated with retinal angiogenesis and vascular leakage, leading to the loss of barrier properties of retinal membranes along with loss of cells. Cellular therapies aim to promote the repair of the ocular structure lost by disease through cell replacement or paracrine factors. Some cells delivered into the eye will engraft and replace damaged cells, whereas other cells will provide soluble factors to promote repair or modulate tissue inflammation. The mechanisms of action for eye cell therapies are complex and depend on the characteristics of the cell therapy product. It is important to highlight that the basement membrane plays a crucial part in cell viability and function. Hence, the application of nanofibrous materials for tissue regeneration as well as drug delivery is a potential alternative for developing new therapies for eye diseases.

Ocular disorders can be vision threatening or lead to vision impairment, such as AMD, cataracts, corneal injury, DR, glaucoma and refractive errors. The other common ocular disorders that do not cause vision impairment are blepharitis, chelation and hordeolum, conjunctivitis, dry eye, pterygium and pinguecula and subconjunctival hemorrhage.[2] The most common clinical management strategies for anterior segment disorders involve the topical application of the desired drug in the form of a topical formulation – preferably an eyedrop or suspension. For instance, diseases of the anterior segment of the eye are treated with eyedrops; however, recent research has also focused on the development of drug-eluting contact lenses, implants, hydrogels and ocular patches. However, in certain cases of posterior segment diseases of the eye, intravitreal or periocular injections become necessary. Hence, recent developments in the management of posterior segment diseases often focus on intravitreal or periocular long-acting implants to minimize the frequency of administration. The clinical management for some of the common ocular disorders and routes of administration is presented in Table 1.

Traumatic corneal injury and corneal opacification are often treated by corneal transplantation. Tissue damage following corneal scarring results from injuries, such as trauma, surgery or corneal infection. Corneal injury or tissue damage involves injury to the epithelial basement membrane along with defective keratocytes. Ocular disorders associated with tissue scarring or altered wound healing are treated with corneal tissue transplantation. However, the limited number of donors and strict storage requirements of donated corneas for tissue implantation have led to a significant increase in the price for such surgeries.

Opportunities of electrospun drug delivery systems for ocular application

ES is one of several novel drug delivery strategies that offers several benefits. For instance, ES can produce a nanofibrous mesh or mesh-like network of nanofibrous scaffolds that can serve as a drug carrier and release the drug in a controlled manner for an extended duration of time. Another technique known as electrospraying can lead to the formation of particle-based delivery systems with some unique features that are not otherwise feasible via conventional particle fabrication methodologies. These nanofiber and nanoparticle platforms could be used as a drug reservoir when administered via the periocular routes. Furthermore, ES and electrospraying offer significant tailorability enabling loading and release of a wide range of therapeutics via periocular routes that can potentially overcome the limitations of conventional formulations and significantly offer higher ocu-

TABLE 1

Ocular complications and their current clinical management.

Ocular complication	Segment of eye	Clinically prescribed agents	Route of administration	Dosage forms	Refs
Glaucoma	Anterior/ posterior	Cholinergic and adrenoceptor agonist to carbonic anhydrase inhibitors	Topical	Eyedrops	[26]
Cataract	Anterior	Surgical replacement of lens and anti-inflammatory agents	Topical	Eyedrops	
Trachoma	Anterior	Tetracycline, erythromycin, macrolides and rifampin, sulfonamides	Topical	Eyedrops	
Diabetic retinopathy and macular edema	Posterior	Triamcinolone acetonide, dexamethasone and fluocinolone anti-VEGF agents such as ranibizumab (Lucentis®) and aflibercept (Eylea®)	Intravitreal	Intravitreal injection/ intravitreal implants	
Age-related macular degeneration	Posterior	Photodynamic laser therapy, verteporfin and anti-VEGF agents such as ranibizumab (Lucentis®) and aflibercept (Eylea®)	Intravitreal	Intravitreal solution injection	
Uveitis	Anterior	Anti-inflammatory agents such as prednisolone acetate, betamethasone, dexamethasone sodium phosphate, fluorometholone, loteprednol, rimexolone and mydriatics or cycloplegics like atropine, homatropine, cyclopentolate	Topical	Solution, suspension, ointments	[27]
Bacterial or fungal keratitis	Anterior	Topical treatment with antibiotics (ofloxacin, tobramycin), collagenase and steroid drugs and topical and oral antifungal agents such as voriconazole	Topical	Solution, emulsion	[28]
Dry eye disease	Anterior	Topical treatment with artificial tears and ocular lubricants	Topical	Solution, suspension, emulsion	[29]

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lar bioavailability along with minimally invasive administration strategies.[5]

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ECM and biomaterial scaffolds have also been proposed as potential therapeutic options for retinal pathologies to serve as carriers to enable the delivery of stem and progenitor cell populations. Scaffolds are preferred over direct injection of cell suspensions, because cells can be delivered in a structurally comparable formation to the retina. Although initial studies have found that stem and progenitor cells can be delivered via bolus injection and are well tolerated, they also indicate a lack of donor cell survival and neural integration into the host retina.[18] Biomaterial scaffolds and ECM can have natural and synthetic material compositions, with some utilizing a hybrid of both.[19,20] This paper discusses the application of ES in obtaining highly porous 3D scaffolds that can mimic the ECM.[21]

Electrospinning process

As discussed, fibrous materials offer the best substitutes for ocular membranes for regeneration and drug delivery. There are various methods for the fabrication of nanofibrous materials, such as template melt extrusion, melt blowing, flash spinning, bicomponent spinning and molecular self-assembly. Nevertheless, ES techniques stand out as the most attractive and promising method of nanofiber fabrication owing to their scalability and ease of application.[22]

ES can be defined as an electrohydrodynamic process in which a liquid droplet is electrified to form a jet, followed by stretching and elongation to generate fibers – the typical laboratory ES setup is conceptually simple. It consists of a high-voltage power supply, a syringe pump, a spinneret that is a hypodermic needle with a blunt tip and a conductive collector. During the process of ES, the uniform and continuous flow of the solution from a spinneret is ensured using a syringe pump, and then the applied voltage is applied on the tip of the spinneret, which can be adjusted externally. The current from the voltage supply is transferred to the solution by the spinneret needles, which causes a spherical droplet to deform into a Taylor cone and form ultrafine nanofibers at a critical voltage. This critical voltage is a characteristic property for all different polymers and depends upon different parameters, such as the concentration of polymer solutions and distance from the collector.[23]

The typical setup for the ES process is illustrated in Figure 2, and the process parameters affecting ES and their effect on fiber morphology are listed in Table 2. The morphology and orientation of electrospun implants can be modified by changing the equipment and the process parameters for ES. The spinneret needle governs the diameter of electrospun fibers. Furthermore, the modification of the spinning needles helps in the modification of the core–shell structure of fibers; for instance, by using biaxial or multiaxial needles nanofibers with different polymeric cores and shells could be manufactured. Furthermore, there has been development in the needle-free ES process. The ES collector plays an important part in governing the orientation of electrospun fibers. For example, flat surface collectors are often used for the fabrication of random coil nanofibers, whereas rotating drum collectors are used for the fabrication of linearly oriented fibers. Rotating mandrels and rods are used for the preparation of cylindrical implants that are often used for the fabrication of container-type implants for drug delivery applications.

Significance of different electrospinning parameters in the development of implants for ocular applications

Each stage of the ES procedure involves specific and crucial process parameters that dictate the efficacy of the resultant electrospun fibers. The morphology and orientation fiber network in electrospun products are determined by various steps, including the selection of the polymers for ES, the different ES process parameters and the selection of the needle and collector. The polymer material is a crucial factor in the processes of drug release and degradation of ES implants. The determination of implant biocompatibility also holds significant importance in cellular therapies. The subsequent section delineates several pivotal parameters that are imperative for the advancement of ES implants intended for ocular therapeutic applications.

Significance of polymer selection for the fabrication of ocular therapeutics by electrospinning

Drug delivery and cell treatment depend on polymer choice. In electrospun delivery methods, polymer selection affects implant qualities such as surface area, contact angle, hydrophobicity, degradation and tensile strength. Linear polymers have traditionally been used for ES nanofibrous drug delivery systems, whereas branched polymers are chosen for electrospraying nanoparticulate drug delivery systems.[22]

The process of ES results in a significant augmentation of the surface area, thereby promoting polymer degradation and drug release.[24] Hence, the selection of polymers has a crucial role in regulating the release of drugs and the degradation of formed implants. Hydrophilic polymers, including PVA, PVP, gelatin and cellulose, are frequently employed materials for the faster release and degradation. Polymers, namely PLGA, PCL and PGS, are utilized in the production of drug delivery systems that aim for a gradual release and degradation of the drug.[25–27]

Ocular tissue regeneration frequently relies on an engineered active support scaffold that will allow cells to adhere, proliferate and repair or regenerate the damaged tissue. This is strongly dependent on using tissue-equivalent materials, which are based on the inherent structural properties of the tissue. The engineered scaffold approach is often a preferred method for ocular

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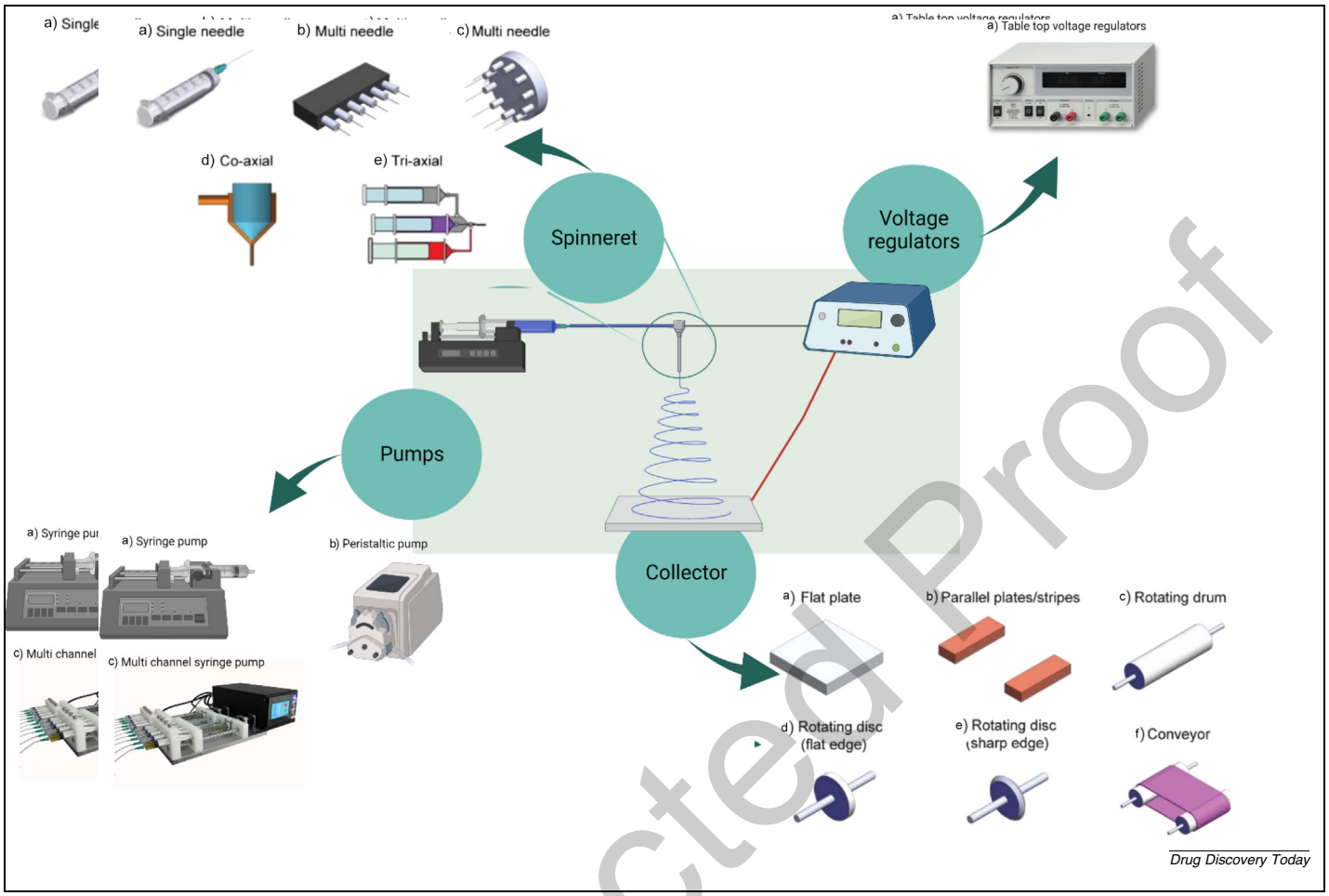

FIGURE 2
Schematic representation of the electrospinning process, various components and process parameters.

tissue regeneration therapies, because it provides a biocompatible environment for the proper maintenance of tissue and cell morphology while preserving cell function.[28] Furthermore, the desired properties for the delivery system for cellular therapy demand very high biocompatibility to enhance cell survival and high surface area to ensure cellular adhesion and differentiation. Other properties that could affect the tissue regeneration process are mechanical strength, porosity, morphology and architecture. Furthermore, a cell therapy delivery system prefers to have a hydrophilic, polar and charge-rich surface to mimic the ECM of the surrounding tissue.[29,30] The development of a scaffold with compatible biological, chemical and physiochemical properties is a technical challenge. The polymeric material should be biocompatible with cells and the host tissue while providing a supportive environment for ocular repair. Furthermore, the material to be used should not trigger an immune reaction.[28]

The polymeric materials that have been found to be suitable for tissue regeneration are natural polymers, such as collagen, gelatin, chitosan and laminin, in addition to synthetic polymers such as PCL, PLGA, PLA, PDLLA and PLLA. These polymers have been approved by the FDA for use in humans. The blending of different polymers is often performed to create a scaffold with the desired properties.[31] Table 3 shows that most of the polymers used for ocular cell therapy have similar properties. Gelatin and collagen have been widely used in ocular tissue regeneration of corneal tissue because they mimic the collagenous nature of the cornea (Table 3). Polymers such as silk fibroin and acid or alkali hydrolyzed gelatin have also been used for the fabrication of scaffolds for corneal tissue regeneration.[32–34]

Significance of the electrospinning method

The selection of ES dictates the morphology and orientation of electrospun implants. Two important selection criteria include: (i) the selection of the spinning needle; and (ii) the selection of the collector. The selection of ES needles is an important criterion for controlling the diameter of fibers. Furthermore, multiaxial needles are used for the fabrication of core–shell fibers. Core–shell fibers are often fabricated to control the initial burst-release of the drug, where the shell acts as a drug-release-controlling membrane. The core–shell fiber is also used for the delivery of more than one medicinal agent where one drug is loaded inside the core and a second drug is loaded in the shell. This is done to ensure the polymer–drug compatibility as well as the sustained release of the drug.[35]

The selection of a collector is particularly important in deciding the orientation of fibers. Horizontal and vertical flat surface

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TABLE 2

Process parameters of the electrospinning process and their effect on the electrospinning process.

Parameter	Effect on electrospinning process
Applied voltage	Increase in applied voltage leads to formation of thinner fibers. Beyond threshold voltage the formation of beaded fibers is observed.
Solution flow rate	Critical parameter for formation of uniformed unbeaded nanofibers. Higher flow rates lead to thicker fiber diameters and can lead to beading
Needle diameter	Low needle diameter leads to thinner fibers and uniform fibers.
Needle to collector distance	Higher needle to collector distance leads to thinner fibers with uniform shape. Lower distance leads to thicker and nonuniform fibers.
Polymer concentration and solution viscosity	Low concentration or viscosity leads to noncontinuous spinning and fragment formation leading to beaded fibers. Higher concentration leads to better chain entanglement and uniform fibers. Beyond critical concentration fast drying is observed leading to formation on nonuniform fibers.
Solution conductivity	Solution conductivity is crucial for the Taylor cone formation. Higher conductivity leads to thinner fibers.
Solvent properties	Solvent with moderate boiling points preferred. Higher boiling point leads to incomplete drying of fibers and nonuniform morphology. Reduced boiling point will lead to blockage of needles.
Environmental conditions	Humidity change can lead to change in fiber morphology. Higher morphology yields porous fibers. Increase in temperature leads to mean decrease in the fiber diameter.

collectors are often used for the preparation of random coiled fibers in which the fibers are oriented in a random fashion. Rotating drum collectors are used for the fabrication of uniformly oriented fibers.[36] Fiber orientation has one of the most important roles in tissue adhesion and cell differentiation.[31,37] Furthermore, the application of nanofiber fragments is also used for enhancing cell adhesion and improving cell viability in cellular therapeutics. Rotating rod-type collectors can be used for the fabrication of hollow tubes, which could be further used for the preparation of microcontainer-type implants. The fibrous matrix of these containers can help control the release of molecules by acting as a diffusion-limiting membrane.[38]

Significance of electrospun implant type and dimensions

Implants are limited by the dimensions for their ocular application, because the eye has a limited volume of 7 µl, and the preferred syringe size for intravitreal and periocular administration is 25G to 27G, limiting the dimensions of the implant that can be administered inside the eye. However, implants that are used on the surface of the eye, such as contact lenses and ocular bandages, are not restricted in terms of dimension but, because corneal respiration is often affected by the air permeability of such implants, the material properties of these implants have an important role, and electrospun materials are known to have great air permeation.[8,9]

Strategies for enhancing the drug delivery properties of electrospun drug delivery implants and devices

Some of the major drawbacks of electrospun drug delivery systems are poor control over drug release and high burst-release of drugs, loading of high molecular weight biomolecules, lack of optimum ocular biocompatibility and poor correlation of drug release and implant degradation timelines. Hence, the key areas for improvement of nanofibrous drug delivery systems include control of high burst-release and drug-release kinetics and enhancement of biocompatibility and tissue adhesiveness. In addition, the optimization of ES process parameters and the careful selection of fabrication materials could aid in the development of novel electrospun drug delivery systems for ocular applications. However, different formulation strategies have been employed to troubleshoot the stated drawbacks above.

Control over high burst-release

The drug distribution in the nanofibrous matrix is often not controlled during the ES process and, hence, the drug is not uniformly distributed on the inner core or shell of the nanofibers. In addition, the surface drug content, along with the high surface area and enhanced contact angle, leads to high burst-release from the nanofibrous matrix compared with traditional preformed implants.

Multiple strategies have been employed to control burst-release from nanofibrous matrices. Core–shell-structured nanofiber implants are a very useful methodology for the control of drug release that often requires low drug loading. ES is performed using coaxial needles that contain concentric needle alignment to form bilayer nanofibers, where the inner layer is often loaded with drugs and the outer envelope acts as a barrier membrane.[39]

Chemical crosslinking of the nanofibrous matrix is also used for controlling the pore dimension and drug release from the matrix. Glutaraldehyde is a commonly used crosslinking agent in the fabrication of electrospun implants. Other crosslinking agents, such as genipin, formaldehyde, EDC/NHS and thermal treatment, have also been used for crosslinking gelatin-based electrospun materials.[40] Different examples of crosslinking and its effect on drug release have been discussed in the review.[40]

Electrospun drug delivery systems often suffer from high burst-release of the drug due to the very high surface area of the nanofibrous matrix. Layer-by-layer ES is often used for controlling drug release and introduces different properties, such as mucoadhesiveness and tensile strength. The drug-loaded electrospun layer is coated with a layer of blank polymers to provide barrier function. This layer is often made with different polymers to enhance the properties of the matrix, such as mucoadhesiveness, tensile properties and mechanical strength.[41] This strategy can offer control of the burst-release of medical agents from the electrospun matrix. Nanoparticle-loaded nanofibers have also been prepared to offer control of the drug-release kinetics

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TABLE 3

Recent developments in electrospun material in ocular tissue regeneration.

Author	Polymer used	Cells	Refs
Behtaj *et al.*	PCL/PLLA, PCL/PLGA, PCL/PGS blends and PCL scaffold	RPC cells	39
Bakhshandeh *et al.*	PCl	HUVECs	40
Sahi *et al.*	Acid and alkaline hydrolyzed gelatin and silk fibroin		41
Jafari *et al.*	PCL, PGS, and Poly(1, 8- octanediol- co citrate)	RPC cells	42
Kim *et al.*	PCL and collagen	Corneal epithelium	43
Wu *et al.*	Type I collagen and PVA	Corneal epithelium	44
Salehi *et al.*	PGS and PCL	HCEC and HCjEC cells	45
Foroshani *et al.*	Gelatin glycosaminoglycan matrix and fibrin	Human corneal fibroblast	46
Ruiter *et al.*	PLA and PDEGMA peptide blend	Human corneal stromal cells	47
Fernandez-Perez	Extracellular matrix modified poly(e- caprolactone)	Corneal stromal cells	48
Moghanizadeh-Ashkezari *et al.*	PUU and PGPL copolymer nanocomposite	Stromal keratocyte cells	49
Shahmoradi *et al.*	Poly(caprolactone)	Human retinal pigmented epithelium (ARPE- 19)	50
Kruse *et al.*	PMMA, PLGA, and PCL	HCEC-12	51
Chan *et al.*	Pectin- polyhydroxybutyrate (pec- PHB)	Human retinal pigmented epithelium (ARPE- 19)	52

and sustain the release of drug molecules. The drug is loaded in the nanoparticulate system, and these nanoparticles are then embedded in the nanofibrous matrix during the ES process. The overall drug loading of the system decreases drastically and, hence, it is suited for highly active and low-dose molecules.[42]

Loading of high molecular weight biomolecules

High molecular weight biomolecules such as DNA, RNA and proteins are often used for the treatment of ocular lesions. However, the formulation development of biomolecules is often challenging owing to their stability issues. The process of ES requires the application of high voltage for the formation of nanofibers.[43,44] Several reports suggest that protein can be loaded directly in the nanofibrous matrix during ES without alteration of structure and loss of activity, as mentioned in Table 4..[44] Angkawinitwong *et al*. reported the fabrication of a bevacizumab-loaded PCL nanofibrous matrix. The core–shell structure ES was performed with the inner core of bevacizumab in Trizma® buffer and the PCL outer shell. It was observed that up to 60% of bevacizumab was released from the matrix in 60 days, maintaining bioactivity. Different reports of enzyme loading in the electrospun matrix have been published, suggesting that blending of polymer and protein solution under suitable solvent conditions could be used for ES purposes.[45,46]

Ultrafiltration can also be used to passively load biomacromolecules in an electrospun matrix. The protein solution is filtered by the application of negative or positive pressure to load the protein particles in the nanofibrous matrix. The protein and DNA molecules become entangled in the fibrous meshwork of the matrix, and the physical interaction of the fibrous matrix and the drug often governs the release.[47] Similar strategies have been used for the loading of nanoparticulate systems in nanofibrous matrices. The loading of silver nanoparticles (AgNPs) was performed in the PLA nanofibrous matrix similarly by Yang and co-workers.

The preparation of nanoporous containers and devices using ES has also been explored to deliver macromolecules. ES offers the fabrication of a highly tunable matrix that could be modified for the preparation of containers and pockets that could be used to encapsulate the drug. Furthermore, the ES setup could be modified to create seamless containers, and crosslinking could be useful to control the pore dimensions governing drug release. In a similar fashion, hollow intravitreal implants loaded with the anti vascular endothelial growth factor (VEGF) agent bevacizumab were fabricated with further salt addition and a high temperature and were used for manufacturing implants.[45,48]

Improving tissue biocompatibility and implant degradation

Long-chain polymers such as PCL, PLA and PLGA have been widely used for ES purposes. One of the major drawbacks of these polymers is slow degradation and biocompatibility issues owing to formation of acidic degradation products.[49] The ES process drastically increases the surface area, improving the solvent contact angle and maximizing the degradation of implants; however, this can also lead to an unwanted increase in burst-release. Hence, different approaches of polymer blending and fabrication have been used to improve tissue biocompatibility and degradation.[50]

Polymer blending of hydrophilic biocompatible polymers such as PVA, PVP and gelatin is one of the most widely used strategies to improve tissue biocompatibility and degradation. Various copolymer conjugates of PEG, such as PLGA-PEG, PCL-PEG and PLA-PEG, have also been tested for improving the fabrication characteristics of hydrophobic polymers by improving the surface tension of the matrix. Zhang *et al*. reported the comparison of PLGA and PLGA-PEG electrospun fibers with a drug loading of amoxicillin. The results suggested that the PEGylated nanofibers were more hemo- and cyto-compatible, along with a minimal effect on morphology and drug release kinetics.[51] However, the increased surface area due to the ES process leads to faster degradation. In certain cases, the addition of salts and hydrophilic salt-forming agents has also been attempted to improve the porosity of electrospun materials fabricated using hydrophobic polymers with a limited degradation profile. These salts tend to dissolve faster than the polymer matrix, hence creating pores and increasing the surface area, accelerating degradation.[52]

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TABLE 4

Recent developments in electrospun materials in ocular drug delivery.

Anterior/posterior segment delivery	Polymer(s) used	Therapeutic tested	Proposed clinical application	Refs
Anterior segment	Poly lactic acid	Cyclosporine	Alkali-injured cornea	54
Anterior segment	Chitosan, PVA and Eudragit® RL100	Ofloxacin	Microbial keratitis	33
Anterior segment	PVA and gelatin	Propolis	Microbial keratitis	67
Anterior segment	PLGA and PVP	Pirfenidone and moxifloxacin	Corneal abrasion	68
Anterior segment	PLC/PEG	Besifloxacin hydrochloride	Bacterial keratitis	69
Posterior segment	PCL	Fluocinolone acetonide	Retinal inflammation	70
Anterior segment	PCL	Dexamethasone	Ocular inflammation	71
Anterior segment	PAMAM dendrimers and PEO	Brimonidine tartrate	Glaucoma	72
Anterior segment	Pullulan/Gellan	Fluorescein		73
Anterior segment	Sodium hyaluronate and PVP	Ferulic acid and ε-polylysine	Corneal infections	74
Anterior segment	Chitosan PVA and PVP	Ofloxacin	Corneal infections	
Anterior segment	Chitosan PVA and PVP	Azithromycin	Corneal infections	75
Anterior segment	PVP	Azithromycin-loaded poly(lactic-co-glycolic acid) copolymer/Pluronic NPs	Corneal infections	76
Anterior segment	Silk fibroin	Epigallocatechin gallate	Corneal regeneration	77
Anterior segment	PEG-PPG-PEG	Azithromycin	Corneal infections	78
Anterior segment	Chitosan, Eudragit S100 and Zein	Triamcinolone acetonide	Glaucoma	51
Posterior segment	Poly(caprolactone)	Bevacizumab	AMD	79
Posterior segment	PEG/PCL)	Pigmented-epithelium-derived factor	Retinal regeneration	79
Anterior segment	Polyvinyl Alcohol and hydroxypropyl-β-cyclodextrin	Voriconazole	Corneal infections	80
Anterior segment	PLA (PLA)/(PVA)	Dexamethasone	Ocular inflammation	85
Anterior segment	PLGA and PEG	Dorzolamide	Glaucoma	86
Anterior segment	PVA, acrylic resin, PVP	Voriconazole	Keratomycosis	87
Anterior segment	PCL and poly(butylene succinate)	Ofloxacin	Ocular infections	88

Hydrophobic polymers such as PCL, PLLA, PLA and PLGA are often the polymers of choice for sustaining the release of hydrophobic drugs; however, owing to their limited contact angle and acidic microenvironment, they can trigger foreign-body reactions after implantation.[49] Hence, coaxial ES with biocompatible polymers such as chitosan and gelatin has also been used to increase the biocompatibility of electrospun matrices. The inner core of the hydrophobic polymer can be coated with the outer core of the hydrophilic polymer to enhance the material's surface characteristics.[53]

Applications of electrospun materials for ocular therapeutics

Electrospun materials for ocular tissue repair

The human retina is an intricate assembly of specialized cells that form the neural retina and the complementary blood–brain barrier.[54] The neural retina comprises light-absorbing photoreceptors, an inner layer of bipolar neurons and retinal ganglion cells. Here, visible light is converted into electrochemical signals transmitted by retinal ganglion cell axons through the optic nerve and to the brain visual cortex, where it is interpreted as vision. A specialized vascular network that caters to nutrient requirements also supports the neural retina. The retina also contains a specialized polarized epithelial barrier called the retinal pigment epithelium (RPE), which lines and nourishes the photoreceptors and regulates nutrient flow to the outer retina while minimizing visual obstruction. The RPE together with the vascular endothelium forms the retinal blood–brain barrier. Therefore, cell replacement strategies for a highly organized tissue such as the retina require special attention.[28]

Owing to its surgical accessibility, small size and smaller requirement for therapeutic cells compared with other tissues and organs, cell replacement strategies are a favorable option for treating retinal degenerative diseases. In fact, many studies have been carried out in the past for treating AMD and retinopathy (RP). Most cell replacement strategies have involved the delivery of healthy RPE and PCs into the subretinal space.[55] However, cell transplantation or bolus injection of cell suspension into the subretinal space often leads to poor cell engraftment, cell loss, immune reaction, compromised vision, damaged retina and, over the long term, subretinal gliosis.[56,57] Therefore, to maximize clinical outcomes, a preformed monolayer of therapeutic cells on supportive electrospun substrate materials has been investigated by a number of researchers (Table 2).

Corneal scarring, keratoconus, Fuch's dystrophy, corneal thinning, corneal swelling and ulcers are complications associated with corneal and vision impairment. The currently available treatment method for these diseases is corneal transplantation, and these treatment methods significantly depend on the donor.[58] Treatment of these diseases relies on parameters such as donor eyes and matching of the DNA, making it a limited option, and storage of tissues is also a crucial parameter for their vitality, which makes the current approach a very expensive treatment option. Moreover, regeneration of the cornea and scleral stroma is challenging owing to its mechanical strength and

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structural complexity. ES provides a 3D scaffold of polymers with varying mechanical and structural properties. In such cases, the polymeric ECM would provide a replaceable matrix for tissue repair, in this case the cornea. Ashkezari *et al.* prepared biodegradable electrospun nanofiber scaffolds of polyurethane urea in HFIP with a nanofiber diameter of 414 ± 275 nm with aligned orientation.[59] Fiber alignment is an important parameter for the proliferation of corneal epithelial cells.[60] Tensile strength and contact angle have an important role in the proliferation of various types of cells. Loading of cell proliferation factors such as vitamin C or zinc has been shown to enhance the proliferation of keratocytes by enhancing the secretion of Type I collagen by procollagen by keratocytes.[61]

Similarly, corneal wound healing and repair of corneal epithelium, endothelium and stromal cells are crucial for the restoration of corneal transparency, preventing retinal damage and vision loss. Different electrospun matrices have been developed as carriers of the human corneal epithelium to enable cell delivery into injured corneal tissue. Recent literature pertaining to the development of such novel systems for ocular tissue regeneration is listed in Table 3.

One of the major challenges in developing products for corneal tissue engineering is achieving the optical and mechanical properties of the native cornea to maintain corneal transparency.[37] A recent study investigated the fabrication of a PGS and PCL e-based nanofibrous matrix for a bio- and immune-compatible system for corneal repair. The ES fabrication was carried out by mixing different proportions of PGS and PCL in a chloroform and ethanol mixture and carrying out the ES process. The 1:1 PGS:PCL mixture was found to exhibit a fiber diameter of 258 ± 80 nm. The parallel oriented PGS–PCL matrix showed increased proliferation of human corneal endothelial cells (HCECs) and human conjunctival epithelial cells (HCjECs) in the MTT assay for up to 7 days, in combination with negligible immunogenicity.[37] Another study by Yan *et al.* showed that the orientation of electrospun fibers had a significant effect on the proliferation of keratocytes and corneal epithelial cells.[60] This could be associated with the effect of fiber alignment on the tensile strength, mechanical properties and wetting angle of scaffolds. Randomly oriented scaffolds lead to better cell adhesion than aligned scaffolds owing to the rough surface. In conclusion, different cells respond differently in terms of proliferation, cell adhesion and orientation. By changing the collector shape (flat, round) and orientation of needles, fiber alignments could be arranged to match the native tissue constructs (Figure 3).[62]

Furthermore, another study reported the fabrication of PLA nanofibers using a ternary solvent mixture of chloroform dichloroethane and ethyl acetate loaded with up to 2.5% cyclosporine, which is an immunosuppressive agent for the management of alkali-injured corneas. Alkali injuries are often associated with severe inflammatory responses in the eye, leading to infiltration of T cells, expression of VEGF and corneal neoangiogenesis. The *in vivo* studies suggested that cyclosporine-loaded nanofiber implants led to a decrease in the infiltration of $CD3^+$ cells in the corneas and a decrease in VEGF expression. Other inflammatory genetic markers include interleukin (IL)1β, IL8, matrix metalloproteinase (MMP)9 and interferon (IFN)γ that were also found to be downregulated when compared with topical eyedrops, which could be possibly due to sustained delivery of cyclosporine over a longer duration.[63]

Nanofibrous mesh could be highly effective in lowering IOP owing to the sustained release of IOP-lowering drugs compared with eyedrops.[64] Nanofibrous mesh (inserts) could enhance patient compliance, reduce the frequency of administration and maintain the therapeutic concentration for a prolonged period of time. A Eudragit® RL100 nanofibrous insert of timolol maleate led to sustained release for 3 days and an *in vivo* IOP-lowering effect was observed for 6 days in equine eyes.

Crosslinking of the nanofibrous matrix offers control over various properties of the matrix, such as swelling, biodegradation and fiber morphology, including tailored drug delivery. Chou *et al.* studied the role of solvent in the crosslinking efficiency of gelatin-based nanofibers, which is particularly useful for ocular applications. It was observed that increasing the content of water in the crosslinking reaction solvent binary mixture of ethanol and water had a profound effect on the crosslinking index, where up to a 50% increase in crosslinking was observed with a 20% increase in water in the solvent mixture. The higher crosslinking leads to slower degradation, as evident from elevation in shrinkage temperature and a lower reduction in matrix mass upon MMP9 treatment.[65]

Control over the pore size of the nanofibrous matrix could be better exploited for post-fabrication drug loading of nanoparticles. In one instance, Yan *et al.* reported the application of ultrafiltration for the loading of AgNPs in PLA nanofibrous matrix cellulose nanofibrils as a filtration aid to assist in the loading of AgNPs (Figure 4). The addition of AgNPs led to enhanced antibacterial properties of the matrix, leading to better HCEC attachment and proliferation.[66]

A recent report utilized the fabrication of gelatin glycosaminoglycan electrospun matrix mixed with fibrin as a carrier of human corneal fibroblasts. The components of the matrix resemble the ECM to promote cell adhesion and tissue repair along with being biodegradable in nature. The fabrication of the nanofibrous matrix involved the preparation of gelatin and chondroitin sulfate solution, which was electrospun under suitable conditions. Furthermore, fibrin was loaded into the matrix following an EDC-based crosslinking reaction. The combined matrix offered a significant increase in degradation time and enhanced cell viability of corneal fibroblasts, with an increase in HCFC proliferation and attachment over 5 days, over the fibrin-based scaffold.[33]

The electrospun matrix could be used as a base for further fabrication and modification that could lead to functional matrices that allow optimum and physiologically relevant biological functions such as signal transduction, ECM interaction, cell–cell adhesion and cell migration. An example of such an approach is the use of a PLGA-based electrospun matrix using a PEGDA-based micro-stereolithography setup to obtain PLGA-based electrospun pockets. The horseshoe shape of limbus palisades was replicated using combinations of these experiments. Furthermore, these scaffolds were used to cultivate corneal epithelial cells.[67]

Electrospun scaffolds and extracellular matrix for retinal regeneration. The polymer solution parameters (such as concentration of

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polymer, viscosity and conductivity) and ES rig parameters (such as voltage, distance of needle to collector and flow rate) can be used to produce the final scaffold product for a desired clinical indication.[68] For example, the pore size of electrospun scaffolds can be altered to be more porous (to allow cellular invasion within the scaffold) or less porous (to serve as an ECM and maintain a 2D construct) based upon the downstream application of the material.[69]

Usually, polymers are electrospun using a rotating drum collector. This facilitates the collection of a large quantity of randomized fibers, distributed into a sheet collected on the rotating drum. The sheet can then be removed and cut to size for downstream applications.[70] Randomized fibers are useful for most purposes where fiber alignment and orientation are not paramount, such as for the delivery of sheets of RPE or induced pluripotent stem cells (iPSCs).[71] However, for cell types such as retinal ganglion cells (RGCs), which have a specific alignment and polarization, randomized fibers can result in improper growth and guidance, with randomized growth and connectivity of RGC axons. In this instance, it is possible to use a radial collector, which results in aligned fibers, resulting in outgrowth of RGC axons in alignment with the fibers. This formation is more akin to the optic nerve head, where RGC axons extend from the retina toward the optic nerve.[72]

Electrospun scaffolds for the delivery of cell therapy. Several groups have applied electrospun scaffolds for the delivery of reti-

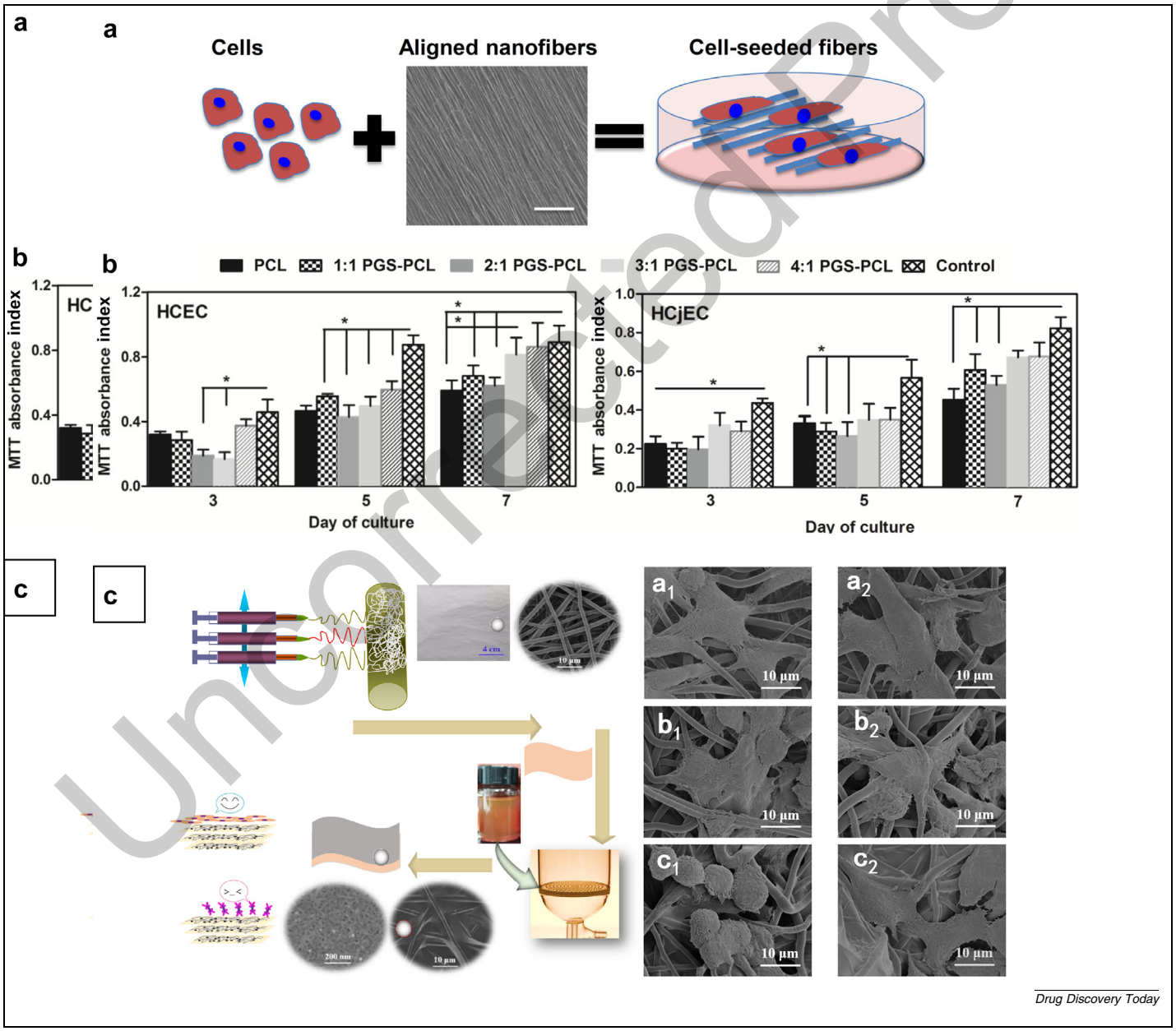

FIGURE 3

(a) Cell adhesion on the aligned PGS PCL fibers. **(b)** Role of polymer composition in the change in cell adhesion as observed and reported by Salehi and colleagues.[37] **(c)** Various ratios of PCL and PGS have a profound effect on cell viability. Electrospun matrix of PLA polymer for delivery of AgNPs.[66]

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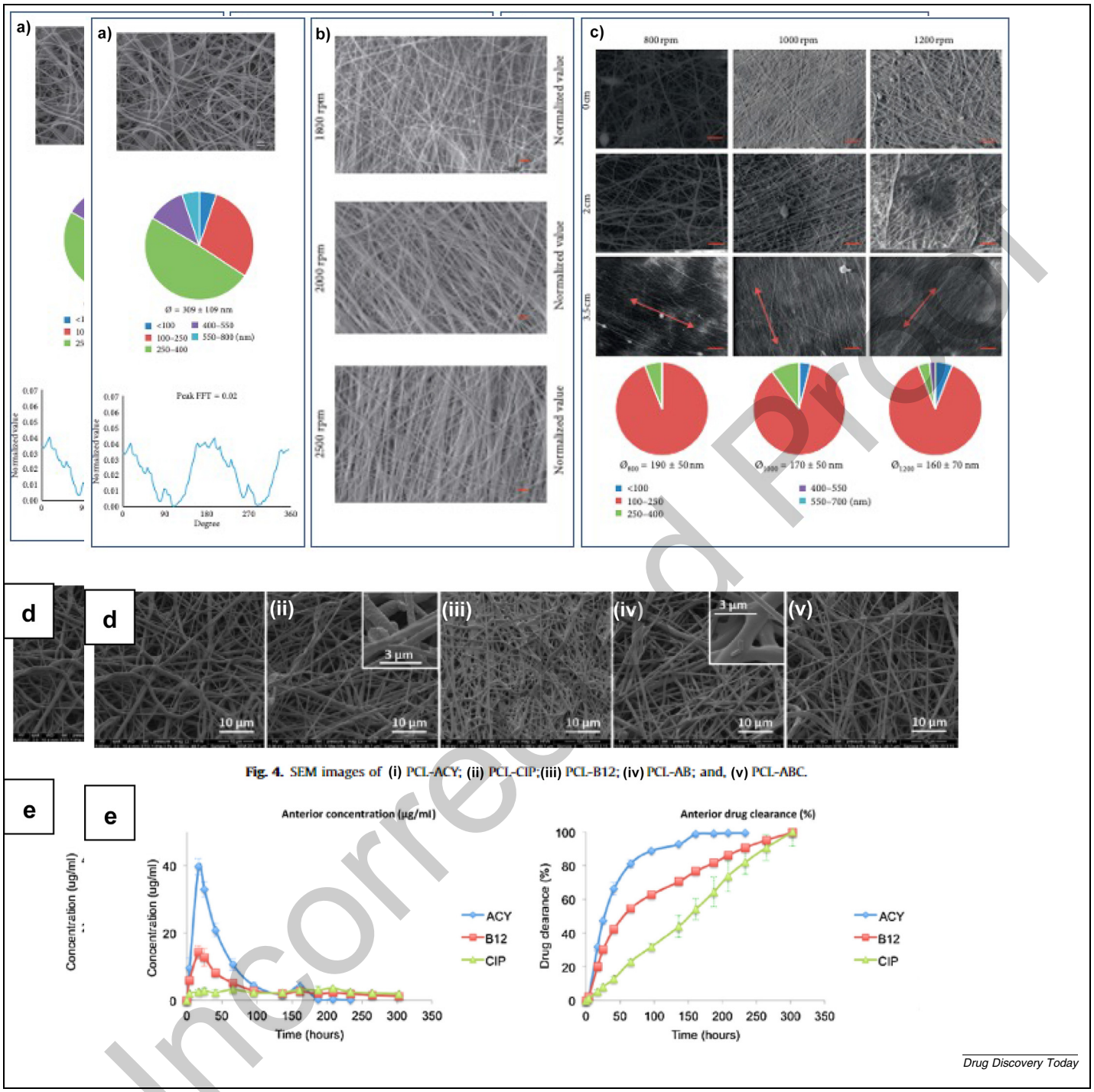

Fig. 4. SEM images of (i) PCL-ACY; (ii) PCL-CIP;(iii) PCL-B12; (iv) PCL-AB; and, (v) PCL-ABC.

FIGURE 4

Cs/PEO nanofibers produced using **(a)** a static collector, **(b)** a rotating drum collector at varying rpm from 1000, 2000 and 2500 rpm and **(c)** a rotating disk collector (of varying diameters 0, 2 and 3.5 cm) at 800, 1000 and 1200 rpm.[62] **(d)** SEM image of drug-loaded fibers of acyclovir, ciprofloxacin and cyanocobalamin and **(e)** *in vitro* drug release profile for triple drug-loaded nanofibers used for the management of viral infections in the anterior segment of the eye.[79]

nal cell types. One of the more common cell types investigated has been the RPE, derived from either iPSCs or human embryonic stem cells (hESCs). There are reports of using 10% (w/v) PLCL polymer to generate biodegradable PLCL electrospun membranes. These scaffolds were then further treated with dielectric barrier discharge (DBD) plasma treatment to increase surface wettability before finally coating with collagen IV protein to facilitate the adherence of hESC–RPE cells. After 42 days, hESC–RPE cells grown on these scaffolds formed confluent RPE monolayers with typical hexagonal RPE cell morphology and abundant pigmentation, indicating a functionally relevant cell monolayer. Further analysis of the gene expression profile revealed the

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expression of mature RPE markers such as BEST, RPE 65 and TYR, with a corresponding lack of pluripotency marker expression OCT3/4. Furthermore, immunostaining revealed MITF and bestrophin staining, as well as the tight junction marker ZO-1. Seeded hESC–RPE cells were also able to phagocytose and internalize photoreceptor outer segments (POS). This porous biodegradable electrospun scaffold has been proposed by the authors as a potential tissue engineering construct for retinal regeneration purposes.[73] Similarly, an RPE patch based on a human vitronectin-coated polyester membrane has been used to deliver hESC–RPE cells as a cell therapy for age-related macular degeneration.[74]

Electrospun radial scaffolds have also been used for the seeding and functionality of RGCs and to align these cells in a formation more akin to the native optic nerve head. For this study, PLA at 6.6% (w/v) was used to generate electrospun scaffolds before coating with laminin to facilitate the adhesion of RGCs. RGCs showed 50% increased survival when cultured on PLA scaffolds compared with tissue culture plastic coated with poly-D-lysine and laminin, while also maintaining electrophysiological properties (RGCs seeded on scaffolds maintained the ability to be electrically excitable, as evidenced by multiple action potentials in response to electrical stimuli). Furthermore, RGCs seeded on the radial electrospun scaffolds mimicked the axonal orientation of the nerve fiber layer of the native retina and 81% of neurites aligned radially. There was also no significant difference in neurite orientation when compared with the neurite orientation of a retinal explant, indicating that seeded RGCs were successfully able to mimic the native retinal architecture. They were also able to successfully grow into retinal explants and follow the existing patterning of radial neurite tracks. The authors proposed that this scaffold could be used as an RGC transplantation device based on the success of *ex vivo* retina integration.[75]

Finally, photoreceptors also serve as viable candidates for cell delivery on electrospun scaffolds. A recent study utilized a PDMS scaffold as a base material to serve as a scaffold for human pluripotent stem cell photoreceptor (hPSC-PR) seeding. These scaffolds were treated with oxygen plasma, followed by laminin coating. Immunostaining revealed VGLUT1 terminal staining and extension of the PR axons into the PDMS scaffold. Seeded scaffolds were cultured for up to 3 months and continued to express the tdTomato differentiation marker RCVRN for PRs. They also expressed the rod marker NR2E3, indicating that seeded PRs were mature. Despite the usefulness of PDMS as a scaffold for hPSC-PR culture, it is nonbiodegradable; therefore, the biodegradable polymer PGS was investigated as an alternative. PGS was found to behave similarly to PDMS under the same coating conditions (oxygen-plasma treatment followed by laminin) prior to cell seeding, with no significant difference between cell seeding. PGS scaffolds also showed uniform distribution of PR cells and extension of processes throughout the PGS material. The results suggest that either PDMS or PGS scaffolds could be used as a delivery device for hPSC-PRs with a higher long-term viability than bolus delivery of the same cell type in cell transplantation models.[76]

In summary, several cell types can be delivered using electrospun scaffolds of different materials to provide the ideal conditions for retinal cell therapies. The type of cell that needs to be delivered primarily (either PR, RPE or RGCs) will dictate the material used, as well as the biological ECM. All three cell types can potentially be delivered successfully into preclinical models using the scaffolds and conditions outlined in this review, outlining the potential for electrospun scaffolds for the treatment of retinal pathologies.

Electrospun materials for ocular drug delivery

Different types of formulations for ocular drug delivery have been researched in previous decades.[77] Most ocular formulations can be divided based upon the segment of the eye where they are proposed to deliver drug. Formulations for the anterior segment of the eye typically include topical eyedrops, ointments and emulsions, nanoparticulate drug delivery systems, drug-loaded contact lenses and ocular implants and patches. Drug delivery for the posterior segment of the eye is often attempted with intravitreal or periocular administration of nanoparticle systems, *in situ* forming implants, and solid implant hydrogels.[78]

Electrospun materials have been widely used for therapeutic applications in the anterior segment of the eye. Different diseases, such as infection, inflammation and aging and neurodegeneration, have been targeted for management using electrospun drug delivery systems.[38,51,79] A considerable amount of research has been carried out in the field of novel electrospun materials for anterior and posterior segment drug delivery. The recent research for the development and fabrication of such research is shown in Table 3. Furthermore, various recent innovative formulation strategies offer promising benefits over traditional electrospun systems and have been discussed in detail. Table 5.

Most ocular disorders involve multifactorial disease progression along with complex pathogenesis; hence, a suitable drug delivery system should be able to encapsulate multiple drugs at a time while allowing controlled and sustained release. In one case, Shekh *et al.* reported the fabrication of a PCL-based nanofibrous matrix loaded with acyclovir, ciprofloxacin and cyanocobalamin for the management of cytomegalovirus infections. Often, antiviral therapy demands multidrug administration. The PCL/PVA-based matrix was found to show sustained release of all three drugs for up to 300 h in the PK-eye model with up to 39.7 ± 2.4 μg/ml (17 h), 14.3 ± 1.9 μg/ml (17 h) and 3.6 ± 0.12 μg/ml (208 h) for acyclovir, cyanocobalamin and ciprofloxacin, respectively (Figure 5). The difference in the release could be attributed to the difference in hydrophilicity of the drug, with the hydrophilic drug showing faster release followed by slower release of the hydrophobic drug (CIP).[79]

ES also offers a great tool for surface coating and surface modification of medical devices such as implants and contact lenses. Mehta *et al.* developed a novel PVP–PNIPAM-based contact lens coating by electrohydrodynamic (EHD) engineering along with permeation enhancers such as EDTA, borneol and benzalkonium chloride in a varying concentration range. The in-house ES setup contained the modified base electrode that controlled the voltage over the lens surface and allowed uniform coating of the lens. The presence of borneol led to enhanced release of timolol from the fibrous matrix and was also found to be biocompatible in the bovine corneal opacity and permeability test (BCOP).[80]

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KEYNOTE (GREEN)

TABLE 5

Electrospinning and loading of various protein molecules reproduced from Stojanov *et al.*[44]

Protein	Nanofiber material	Purpose	Refs
Insulin	Polyvinyl alcohol/sodium alginate	Diabetes treatment (transmucosal delivery)	Sharma et al., 2013
	Chitosan/PEO	Diabetes treatment (transbuccal delivery)	Lancina et al., 2017
	Fish sarcoplasmic proteins	Diabetes treatment (oral delivery)	Stephansen et al., 2015
Peroxidase and alkaline phosphatase	Eudragit® L100	Simulating oral enzyme delivery	Frizzell et al., 2017
PDGF-BB	PEO/PCL	Bone tissue regeneration	Briggs and Arinzeh, 2014
Growth hormone	Eudragit® L100/chitosan	Oral mucositis treatment	Choi et al., 2016
EGF	Silk/PEO	Chronic nonhealing wounds treatment	Schneider et al., 2009
Glial-cell-derived neurotrophic factor	Polycaprolactone-co-ethyl ethylene phosphate	Nerve regeneration	Chew et al., 2007
Nerve growth factor	Polycaprolactone-co-ethyl ethylene phosphate	Nerve regeneration	Chew et al., 2005
Nerve growth factor + monosialoganglioside	PLCL/silk fibroin	Simulating cell proliferation and differentiation	Sun et al., 2016
Vascular endothelial growth factor	Polyethylene carbonate-ε-caprolactone	Simulating cell proliferation and adherence	Zhang et al., 2012
Lysozyme	Poly (DL-lactide)/methylcellulose	Simulating enzyme release	Yang et al., 2008
Lipase from *Candida rugosa*	PVA	Biocatalysis	Wang and Hsieh, 2008
Bovine serum albumin	PEO	Biosensing (pH)	Kowalczyk et al., 2008

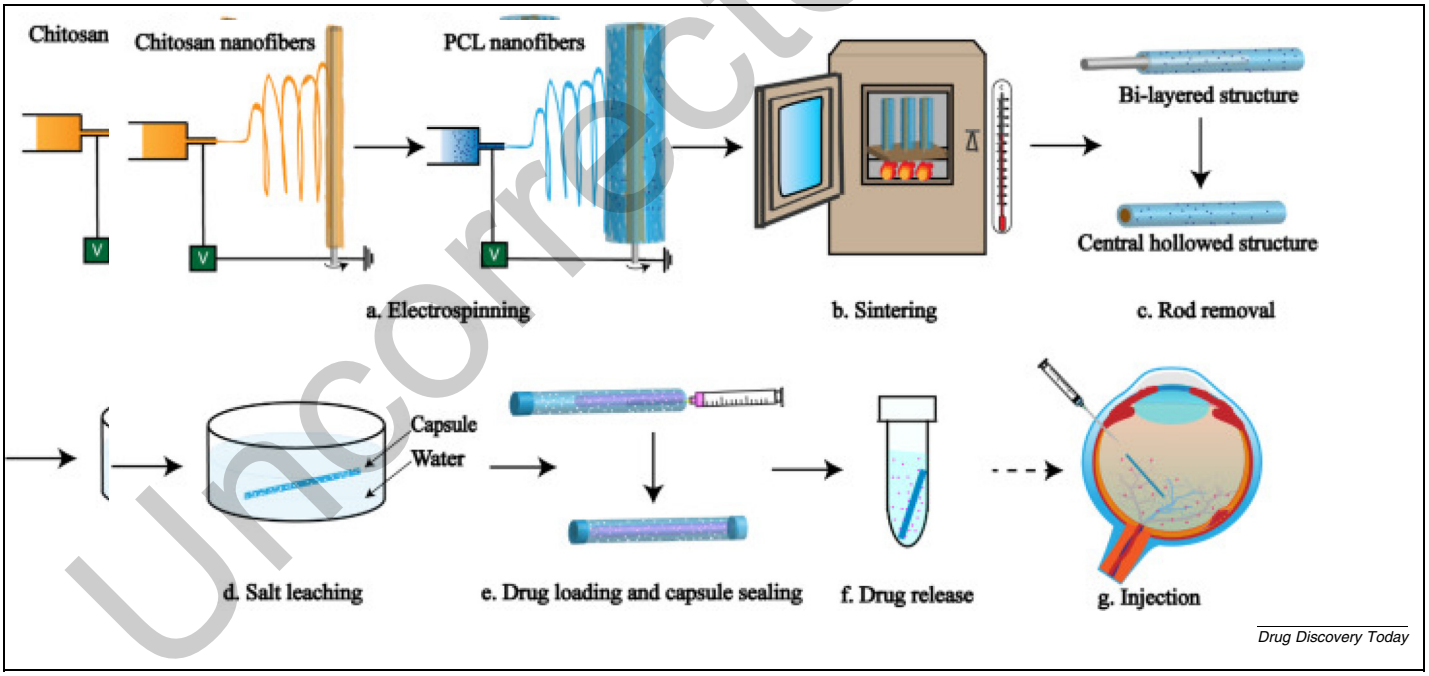

FIGURE 5

Fabrication of novel electrospun intravitreal implants loaded with anti-VEGF protein by Jiang and co-workers.[48]

Electrospun matrices also offer an attractive solution for the fabrication of medical devices for the long-term delivery of biologics. Unlike the solvent-cast material, the electrospun matrix is highly malleable, flexible and amendable by different techniques. Jiang *et al.* reported the fabrication of a hollow intravitreal medical device loaded with the anti-VEGF agent bevacizumab. The fabrication of the capsule involved the bilayer ES of chitosan and PCL matrix loaded with HEPES salts followed

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by heat-based sintering of the fibrous matrix for crosslinking of the matrix, offering control of drug release. The addition of salt was performed to control the pore formation of the capsular matrix that could be used as a release modifier. The bilayer matrix of the capsule offered control over the release of bevacizumab for up to 9 months, which is highly desirable. However, the biodegradation of the matrix was very slow, wherein no significant difference in the morphology of the capsule was observed over the course of 9 months in terms of the fiber morphology and thickness of the capsule (80–90 μm).[48]

Concluding remarks

Owing to the fibrous structure of different layers of the eye, nanofibrous materials could be suitable platforms for anterior drug delivery systems, offering great biocompatibility, sustained release of drug, optimum implant degradation profile and great tuneability of all these properties. However, more research needs to be carried out on the development of electrospun materials and devices for posterior segment drug delivery. One of the major drawbacks of preformed implants is the delayed degradation profile, which is often the result of slow-degradation materials and limited surface area. The electrospun matrix could be a game changer for such systems, because it drastically increases the surface area and improves the contact angle, hence improving the degradation kinetics. This demands the development of a novel ES apparatus for the generation of implants of different shapes, as well as research on post-ES modification of the matrix for the generation of new implants. Because most of the drugs delivered to the posterior segment of the eye are often biological, the effect of ES processes on the stability and structure of protein and DNA molecules as well as cellular biocompatibility should also be studied to ensure no modification of function postdelivery. Furthermore, the application of electrospun materials in cellular therapies should be studied in detail.

Conflict of interest

All authors declare that they have no conflicts of interest.

Data availability

No data was used for the research described in the article.

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Glossary

IVT:: Intravitreal injections
ES:: Electrospinning
PGS:: Polyglycerol sebacate
PLLA:: Poly-L-lactic acid
PCL:: Polycaprolactone
PLGA:: Poly(lactide-co-glycolide)
PLA:: Polylactic acid
PDLLA:: Poly(D,L-lactic acid)
P(LA-co-CL):: Poly(L-lactide-co-ε-caprolactone)
PLCL:: Poly(L-lactide-co-caprolactone)
PET:: Polyethylene tetrephthalate
PEGDA:: Polyethylene glycol diarcylate
PDMS:: Polydimethylsiloxane
PVA:: Polyvinyl alcohol
PNIPAM:: Poly(N-isopropylacrylamide)
EDC/NHS:: 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxy succinimide
AMD:: Age-related macular degeneration
DR:: Diabetic retinopathy
DME:: Diabetic macular edema
RP:: Retinopathy
ECM:: Extracellular matrix
HFIP:: 1,1,1,3,3,3-Hexafluoroisopropanol
HCECs:: Human corneal endothelial cells
HCjECs:: Human conjunctival epithelial cells
hESCs:: Human embryonic stem cells
hPSC-PR:: Human pluripotent stem cell photoreceptor
iPSCs:: Induced pluripotent stem cells
MTT:: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
VEGF:: Vascular endothelial growth factor
IL1b:: Interleukin 1b
IL8:: Interleukin-8
MMP9:: Matrix metallopeptidase 9
IFNc:: Interferon gamma
IOP:: Intraocular pressure
AgNPs:: Silver nanoparticles
ILM:: Inner limiting membrane
RPE:: Retinal pigment epithelium
RGC:: Retinal ganglion cell
EDC:: 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
HEPES:: N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid

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