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Teaser Electrospun polymeric fibrous scaffolds with excellent physicochemical and biological properties offer great flexibility to encapsulate and release various drugs for a long term, with great potential to repair damaged or diseased tissues.

Electrospun polymeric micro/nanofibrous scaffolds for long-term drug release and their biomedical applications

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Electrospun polymeric micro/nanofibrous scaffolds have been investigated extensively as drug delivery platforms capable of controlled and sustained release of therapeutic agents *in situ*. Such scaffolds exhibit excellent physicochemical and biological properties and can encapsulate and release various drugs in a controlled fashion. This article reviews recent advances in the design and manufacture of electrospun scaffolds for long-term drug release, placing particular emphasis on polymer selection, types of incorporated drugs and the latest drug-loading techniques. Finally, applications of such devices in traumatic or disease states requiring effective and sustained drug action are discussed and critically appraised in their biomedical context.

Introduction

Q3 Current ways of maintaining therapeutic levels of medications within the bloodstream are limited to repeated administration of drugs either via the oral or parenteral route. This is inconvenient and, more importantly, puts patients at risk of accidental or intentional overdoses [1,2]. For example, opioid analgesics are commonly administered daily for several weeks or longer to treat patients suffering from severe pain (e.g., post-operative or chronic cancer-related pain) [3,4]. Although the use of opioid analgesics for severe pain is justified, frequent administration poses a potential risk of overdose, abuse or addiction, which can significantly limit its therapeutic efficiency. To improve this, it is crucial to develop a delivery system that, once administered, can continue to release drugs in a controlled and sustained manner to achieve safe delivery and Q4 maintenance of therapeutically appropriate drug levels long term.

Polymeric micro/nanoparticle or micro/nanofibrous scaffolds have been investigated extensively as carrier vehicles for delivery of therapeutic agents. These scaffolds can deliver drugs to a specific predetermined site while avoiding systemic distribution of their cargo. This renders such

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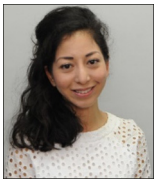

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systems highly efficient and enables the use of lower drug doses with subsequently fewer adverse side effects [1,2]. Compared with their particulate counterparts, micro/nanofibrous scaffolds display several advantages: (i) their physical structure mimics naturally occurring extracellular matrix (ECM), thus supporting cell adhesion, proliferation, migration and differentiation better than particulate scaffolds; (ii) they exhibit a higher surface-area: volume ratio and higher interconnected porosity with tunable pore sizes, enabling them to release biofactors such as proteins or genes and facilitate nutrient and oxygen diffusion as well as waste removal [5–9]. These unique characteristics render these micro/nanofibers superior for drug delivery and tissue regeneration applications.

To manufacture such micro/nanofibers, electrospinning, self-assembly or phase separation have been employed, among which electrospinning is the most popular because of its simplicity, cost-effectiveness, scalability and versatility [10]. Most importantly, the ability to electrospin a variety of materials (ranging from nondegradable to fully degradable) enables the fabrication of scaffolds with controllable drug-release profiles. Polymers of natural, synthetic or composite origin are commonly used in electrospinning owing to their different degradation kinetics and remarkable ability to encapsulate hydrophobic as well as hydrophilic drugs and biomacromolecules [10]. By (i) selecting an appropriate polymer–drug–solvent system and (ii) optimizing electrospinning techniques and processing parameters factors influencing drug release including fiber chemistry and diameter, surface charge, drug diffusion coefficient and degradation rate can be finely tuned to obtain long-term drug-eluting scaffolds. Moreover, the limitless creativity of drug-loading designs endows such systems with even tighter control over sustained drug release. For instance, drug-loaded nanoparticles (NPs) dispersed in the polymer solutions can be electrospun to form composite nanofibers. The extended drug diffusion route for drug particles encapsulated within the NPs results in a prolonged release period of up to 90 days [11,12]. Details on drug-loading and -release profiles will be discussed below.

Being an incredibly versatile technique, electrospinning of polymeric micro/nanofibrous scaffolds has the potential for widespread applications in traumatic or disease states requiring effective and sustained drug action such as in skin regeneration or treatment of cancer [10]. In this paper, we critically appraise the status quo of electrospun drug-eluting polymeric materials and their potential for clinical applications. First, we summarize the classification of polymers into natural, synthetic and composite polymers and their respective benefits and disadvantages. We then critically appraise the current literature pertaining to drugs loaded onto micro/nanofibers and discuss in detail commonly used drug-loading protocols for long-term drug release. These include surface modification of micro/nanofibrous scaffolds, blending of drug particles with scaffolds, as well as coaxial and emulsion spinning of solutions containing drugs and polymers. We subsequently discuss the mechanisms of drug release from the micro/nanofibrous scaffolds to explain how the rate and extent of drug release is customizable according to needs. Finally, we highlight the potential applications of drug-loaded systems in the biomedical arena including the regeneration of bone, skin and neural tissues as well as the treatment of cancer. We focus our attention on drug-eluting fibers that can release drugs at minimum effective concentrations for periods longer than 20 days to satisfy clinical needs. The aim of this paper is to provide the readers with a broad understanding of our current knowledge of electrospun polymeric micro/nanofibrous scaffolds used for long-term drug release and their biomedical applications, with this information they shall be better placed to shape their research in the future.

Polymers used for fiber fabrication

Electrospinning offers great flexibility in the choice of polymers used for drug delivery applications. Polymers make for an ideal carrier material for long-term controlled drug release considering their compatibility with various drugs, and capacity to be modified to suit a variety of delivery routes. When designing an effective polymer micro/nanofibrous system for drug release, various properties of polymers should be considered, including biocompatibility, biodegradability, hydrophilicity and mechanical properties [13]. Polymers used for electrospinning are broadly classified into three categories: (i) natural polymers, (ii) synthetic polymers and (iii) blends of the two. The biological, physicochemical, mechanical and degradation properties of the most commonly used natural and synthetic polymers are shown in Table 1.

Natural polymers

Natural polymers are among the most popular base materials used for tissue engineering scaffolds owing to their similarity to many macromolecular substances found in the human body. With the advantages of low toxicity, favorable biocompatibility and remarkable physicochemical properties, many natural polymers, such as collagen, gelatin, silk fibroin, chitosan and hyaluronic acid (HA), have been extensively studied as drug delivery systems [14].

Collagen, the basic building block of ECM, is the most prevalent and intensely studied natural biomaterial [15,16]. Gelatin, a hydrolyzed form of collagen, maintains the properties of biocompatibility and biodegradability, in addition to being more readily modifiable compared with collagen [1]. They both display excellent biocompatibility with various cell types and are considered as ideal scaffold materials for drug delivery in tissue engineering. Another natural polymer, silk fibroin, is considered a favorable scaffold material for the incorporation and delivery of a diverse range of drugs in tissue regeneration applications, owing to its biocompatibility, relatively slow biodegradability, facile processability and better mechanical properties [17,18]. In addition, chitosan and HA, which can be found in large quantities in mammals, are the most extensively studied polysaccharides for micro/nanofibrous scaffold production. In particular, their excellent biocompatibility, biodegradability and many other characteristics contribute to their broad range of biomedical applications [19]. However, despite these important advantages of natural polymers, some characteristics remain suboptimal – their limited mechanical strength and relatively rapid degradation profile, as well as a potential for immunogenicity, batch to batch variation, their limited supply, high cost of production and susceptibility to cross-contamination, limit their clinical applications [14,20]. In addition, because they are generally hydrophilic and rapidly degradable, the use of natural polymers for long-term drug delivery is restricted.

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TABLE 1

Most commonly used natural and synthetic polymers for the development of electrospun polymeric micro/nanofibers for drug delivery.

Polymer[a]	Integrin-binding sites	Physicochemical properties		Mechanical properties		Biodegradation	
		Hydrophilic	Hydrophobic	Strong	Weak	Hydrolytic	Enzymatic
Natural							
Collagen	√	√			√		√
Gelatin	√	√			√		√
Silk fibroin	×	√		√			√
Chitosan	×	√			√		√
HA	×	√			√		√
Synthetic							
PVA	×	√		√			√
PLA	×		√	√		√	
PGA	×		√	√		√	
PLGA	×		√	√		√	
PCL	×		√	√		√	

Abbreviations: HA, hyaluronic acid; PVA, polyvinyl alcohol; PLA, polylactic acid; PGA, polyglycolic acid; PLGA, polylactic-*co*-glycolic acid; PCL, poly-ε-caprolactone.
[a] All listed polymers possess favorable biocompatibility.

Synthetic polymers

Synthetic polymers, compared with natural polymers, have tunable biodegradability and are abundantly available because they can be manufactured easily and at low cost. They are classified into nondegradable and biodegradable polymers. Polyurethanes (PU), for example, are nondegradable and have excellent chemical stability, abrasion resistance and high mechanical strength, rendering them a widely used material as drug delivery devices and artificial organ systems [21,22]. In comparison, biodegradable polymers, such as polyvinyl alcohol (PVA), polylactic acid (PLA), polyglycolic acid (PGA), polylactic-*co*-glycolic acid (PLGA) and poly-ε-caprolactone (PCL) which are broken down by enzymolysis or hydrolysis, have drawn great attention for drug delivery because they offer tunable degradation rates that can match the new tissue formation [23]. Moreover, the ability to self-absorb over time renders a second operation to remove the implant obsolete, widening their range of clinical applications.

Specifically, PVA, a linear water-soluble polymer that possesses satisfactory spinnability, biocompatibility and excellent physical properties, has diverse biomedical applications such as in rapid drug delivery systems or as temporary tissue engineering scaffolds [24,25]. PLA and PGA are biodegradable materials with desirable biocompatibility and configurable physical properties that allow facile incorporation of various medications. PLA and PGA have been approved by the FDA as scaffolds for application in drug delivery [26]. PLGA, a synthetic copolymer made of PLA and PGA, possesses tunable biodegradability by adjusting the PLA:PGA ratios, rendering *in vivo* degradation and drug release from PLGA carriers highly controllable [24]. PCL is another biodegradable synthetic polymer. It is superior not only because of its slow biodegradation rate but also as a result of its excellent spinnability and good mechanical properties, making it an ideal long-term delivery system for drugs that require extended body retention times [24]. Although synthetic polymers possess advantages such as durability, relative inexpensiveness and tunable degradation time, they lack cell-specific recognition and attachment affinities. This has urged the development of composite polymers, which can maximize the advantages of natural and synthetic materials and minimize their disadvantages.

Composite polymers

Composite polymers are made of two or more polymers including natural and synthetic components, which combine advantages of both types of polymers such as their resemblance to the extracellular microenvironment and excellent mechanical properties and/or tunable biodegradability, respectively. For instance, PLGA–gelatin nanofibers were prepared by blending electrospinning for the delivery of fenbufen (FBF) [27]. Such scaffolds were found to have suitable mechanical and degradation properties as well as bioactivity. Moreover, the release rate of FBF from this nanofibrous scaffold could be tailored by altering the ratio of PLGA and gelatin, with increased PLGA content enhancing scaffold hydrophobicity, resulting in slower FBF release. In another study, PCL–gelatin composite nanofibrous scaffolds were fabricated such that the PCL component conferred tunable hydrophobicity and degradation properties, and the gelatin component provided a favorable extracellular environment for adhesion and proliferation of bone-marrow-derived human mesenchymal stem cells (hMSCs). Such controllable physicochemical properties have made this nanofibrous scaffold a promising drug delivery system for tissue engineering applications [28].

When designing electrospun polymeric micro/nanofibrous scaffolds, the type of polymer is of crucial importance because it affects the wettability and degradation rates of the scaffolds, which are key factors controlling the drug-release profile. In addition to the properties of polymers, other factors including the types of drugs and drug-loading techniques are equally important for designing long-term drug delivery vehicles. In the following sections we will review these factors in detail.

Drug loading and release from electrospun polymeric micro/nanofibrous scaffolds

In addition to the polymer properties, the types of drugs and the drug-loading techniques play a vital part in fabrication of

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polymeric micro/nanofibrous scaffolds with long-term drug-release profile.

Types of drugs incorporated

Electrospun polymeric micro/nanofibrous scaffolds are capable of encapsulating and releasing a wide variety of therapeutic agents including chemicals (e.g., hydrophobic and hydrophilic drugs) and biologicals (e.g., proteins, nucleic acids). In general, drugs exhibiting similar physicochemical properties to their carrier polymers dissolve better than those showing opposing properties. Hydrophilic drugs like doxorubicin and chloroquine are effectively encapsulated within hydrophilic polymers including gelatin [29] and PVA [30,31], whereas hydrophobic drugs such as paracetamol and ibuprofen (IBU) are better incorporated into and released from hydrophobic polymers like PCL [32], PLGA [33] and PLA [34]. The long-term release of hydrophilic drugs is, however, more challenging compared with that of hydrophobic drugs. This is because hydrophilic drugs exhibit poor dispersion within hydrophobic polymers, which usually make up at least part of the carrier vehicle, and are highly soluble in the release media (usually water based), leading to a higher risk of burst release. These issues can be counteracted by measures such as using low loading doses, which decrease localization of drugs to the surface of fibers thus avoiding burst release. Also, improvements in drug-loading designs with the aim of establishing a barrier to isolate drugs from the incompatible polymers have been explored, such as core–shell fibers in which drugs locate to the core. Such designs have successfully demonstrated the capacity to achieve sustained release of hydrophilic drugs. In comparison, hydrophobic drugs are relatively easy to load and release over a longer period owing to their poor solubility in the release medium and good dispersion within hydrophobic polymers.

In addition to chemicals, biologicals have also been incorporated into electrospun polymeric micro/nanofibrous scaffolds for different purposes. Growth factors (GFs) are a group of bioactive proteins capable of regulating proliferation, migration and differentiation of cells by transferring signals between cells and their ECM [35]. Thus, the incorporation of GFs into ECM-mimicking micro/nanofibers is advantageous in tissue engineering applications. However, retaining the bioactivity of GFs is still challenging because they can quickly become inactive during the electrospinning process. Previous studies have suggested that GFs, such as nerve GFs (NGFs), fibroblast GFs (FGFs) and vascular endothelial GFs (VEGFs) are suitable for incorporation into different polymers for steady release using various techniques [36–38]. Nucleic acids, another group of biomacromolecules with the ability to interfere with biological processes by integrating into the cellular genome, can modulate the secretion of signaling molecules on a long-term basis to enhance or prevent specific biological functions. For instance, sustained release of DNA from the electrospun scaffolds can expedite cell transfection or increase transfection efficiency to promote secretion of bioactive molecules, resulting in improved therapeutic efficacy [39]. In addition, siRNA, a type of bioactive macromolecule that suppresses the expression of certain proteins, has been used in cancer treatment for inhibition of tumor-inducing genes, thus reducing the secretion of specific factors and hence tumor size [40].

Because regeneration of damaged or diseased tissues (e.g., bone fracture, skin burns) can take several weeks or even months, it is important to synthesize scaffolds that are capable of releasing drugs over an extended period of time to achieve optimum therapeutic efficacy. To attain a sustained release profile of the above-mentioned therapeutic agents, various drug-loading strategies have been developed.

Drug-loading techniques

Different drug-loading techniques including surface modification, blending, emulsion and coaxial electrospinning have been employed to encapsulate therapeutic molecules into various electrospun structures (e.g., single fiber, core–shell structured fibers). Different drug-loading techniques will produce fibers with different structures and different drug-release kinetics (Fig. 1) [10].

Surface modification

The surfaces of electrospun micro/nanofibers can be chemically and physically modified with a variety of bioactive molecules including anticancer drugs, GFs, nucleic acids and carbohydrates (Fig. 1a). Modification of the polymer fiber surface with such molecules can avoid their uncontrolled dispersion within the bulk phase of the polymer fibers, so that they do not need to pass through the harsh electrospinning conditions. This is particularly the case for fragile molecules such as proteins and nucleic acids, which would otherwise rapidly degrade in such conditions. An additional benefit of surface modification includes the ability to deliver certain macromolecules such as heparin that, owing to their charged nature, are extremely difficult to be homogeneously dissolved within the polymer matrix during conventional electrospinning [41,42]. Such biofunctionalization of surfaces renders the micro/nanofibers morphologically and biologically similar to natural ECM, thus enhancing cell adhesion, proliferation and differentiation. Moreover, these functionalized scaffolds provide a robust delivery platform as a result of an extremely high surface-area:volume ratio, leading to a very high drug-loading capacity. Several studies have demonstrated that immobilization of biomolecules onto fiber surfaces via chemical bonds could attenuate the initial burst release and maintain a steady release rate for over 15 days [43,44]. Many disease states, however, require a much longer-term drug action, thus rendering surface-modified delivery platforms suboptimal. To achieve longer-term release of therapeutic agents, other techniques including blending, coaxial and emulsion electrospinning have been developed.

Blending electrospinning

In blending electrospinning, drugs or molecules are encapsulated by direct dissolution within the polymer solution before electrospinning (Fig. 1b). Drug encapsulation efficiency, drug distribution inside the micro/nanofibers and drug release kinetics are dictated by the polymer–drug interaction and physicochemical properties of the polymer. Poor solubility of the drugs can result in nonuniform drug distribution throughout the polymer solution as well as drug migration toward the fiber surface, which can ultimately lead to an undesirable initial burst release [45]. Therefore, when selecting blending electrospinning, it is essential for the drug and polymer to have similar physical properties with respect to wettability for a better drug solubility and distribution in the polymer solution. When incorporation of drugs into polymers of different properties is necessary, either surfactants can be

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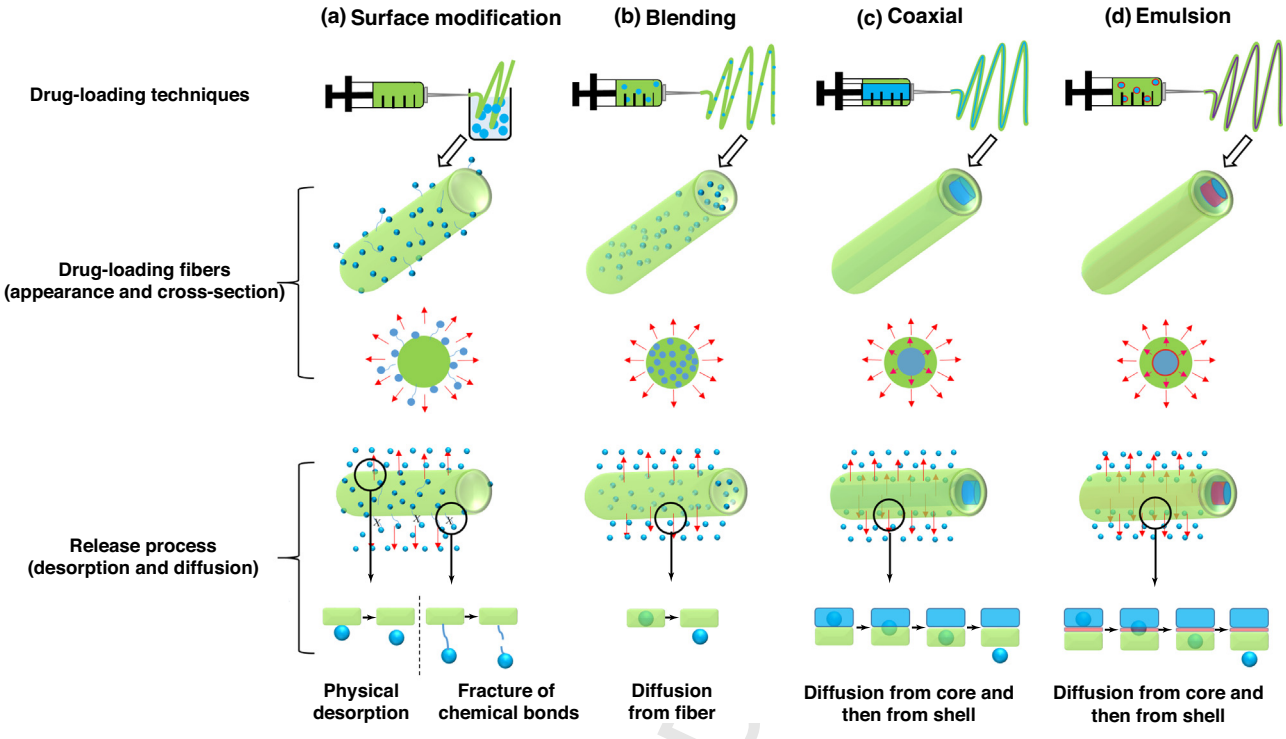

FIGURE 1

Drug loading and release (desorption and diffusion) from polymeric micro/nanofibers fabricated by **(a)** surface modification, **(b)** blending, **(c)** coaxial and **(d)** emulsion electrospinning. The green color stands for polymer, blue for drugs and maroon for surfactant. The red arrows represent the direction of the drug release.

introduced or the drugs can be incorporated into NPs or microspheres before electrospinning to create a physical barrier between the drug and the polymer [11,46]. Blending electrospinning creates only single-layered micro/nanofibers, and drugs blended into such fibers are shown to be released for up to 48 h only [47]. To fabricate micro/nanofibers with a core–shell structure in which fragile biological reagents are protected and drugs can be released for a prolonged period [48,49], coaxial and emulsion electrospinning are possible options (see sections below) [50].

Coaxial process

When incorporating biomolecules into micro/nanofibers using coaxial electrospinning the biomolecule is located in the inner portion (the core) and the polymer solution in the outer portion (the shell) of the solution jet (Fig. 1c) [51]. In this way, the polymeric shell can effectively protect the biomolecule core against exposure to environmental insults, which preserves the bioactivity of the incorporated biomolecules. Other types of pharmaceutics, like antibiotic or antioxidant drugs, can also be loaded inside polymer fibers using this technique [52]. Moreover, compared with blending electrospinning, coaxial electrospinning can prolong the drug release period because it can create fibers with a core–shell structure that has been shown to extend the drug diffusion route by modulating the thickness and composition of the shell [39].

Emulsion electrospinning

In emulsion electrospinning, droplets composed of drug molecules are dispersed in the polymer solution before electrospinning [46]. A core–shell fibrous structure is configured as macromolecules aggregate in the aqueous phase forming the core and the polymers form the shell (Fig. 1d) [53–55]. The core–shell structure formed by emulsion and coaxial electrospinning has a drug release time of up to 60 days because the outer shell layer can act as a barrier for diffusion of the encapsulated drug [56,57]. Furthermore, emulsion electrospinning does not require a common solvent for the drug and the polymer in comparison to blending electrospinning. Therefore, a solution containing therapeutic reagents and polymers with drastically different hydrophilic–hydrophobic properties can be easily electrospun without significant contact of the drug with the organic solvent [58].

Other drug-loading techniques

Another technique to achieve long-term drug release is via incorporation of nano or micro drug delivery devices, such as NPs, nanotubes, micelles, microspheres and liposomes into electrospun fibers [59]. For instance, electrospun polymeric micro/nanofibers hybridized with NPs have been developed to improve drug-loading efficiency, release manner and drug stability. In addition, modification of NPs with multifunctional ligands does not only achieve active target recognition but enhances therapeutic efficacy as well and reducing side effects [60]. Similar to the above-mentioned drug-loading techniques, NPs can be integrated into micro/nanofibers by assembly onto their surface or within the micro/nanofibers because they can maintain drug stability in organic solvents to which they are exposed during the fabrication process. Moreover, owing to the existence of the two barriers provided by NPs and micro/nanofibers, the drug diffusion route and release time from

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scaffolds are relatively extended compared with naked electrospun micro/nanofibers. At present, the use of NPs such as silver NPs [61–63], mesoporous silica NPs (MSNs) [1,12,64,65] and biopolymer-based NPs (e.g., albumin NPs [66] or HA NPs [56]) has brought about tremendous progress in long-term controllable drug release in electrospun polymeric micro/nanofibers.

Another advanced drug-loading design is to fabricate layer-by-layer micro/nanofibers by stacking fibrous sheets. Like coaxial micro/nanofibers, such a stacked structure can control drug release by forming sheet barriers. In a study by Okuda *et al.*, a tetra-layered nanofibrous mesh was fabricated via a sequential electrospinning process of different solutions to achieve time-programmed dual-drug delivery (Fig. 2a–c) [67]. Poly(L-lactide-*co*-ε-caprolactone)

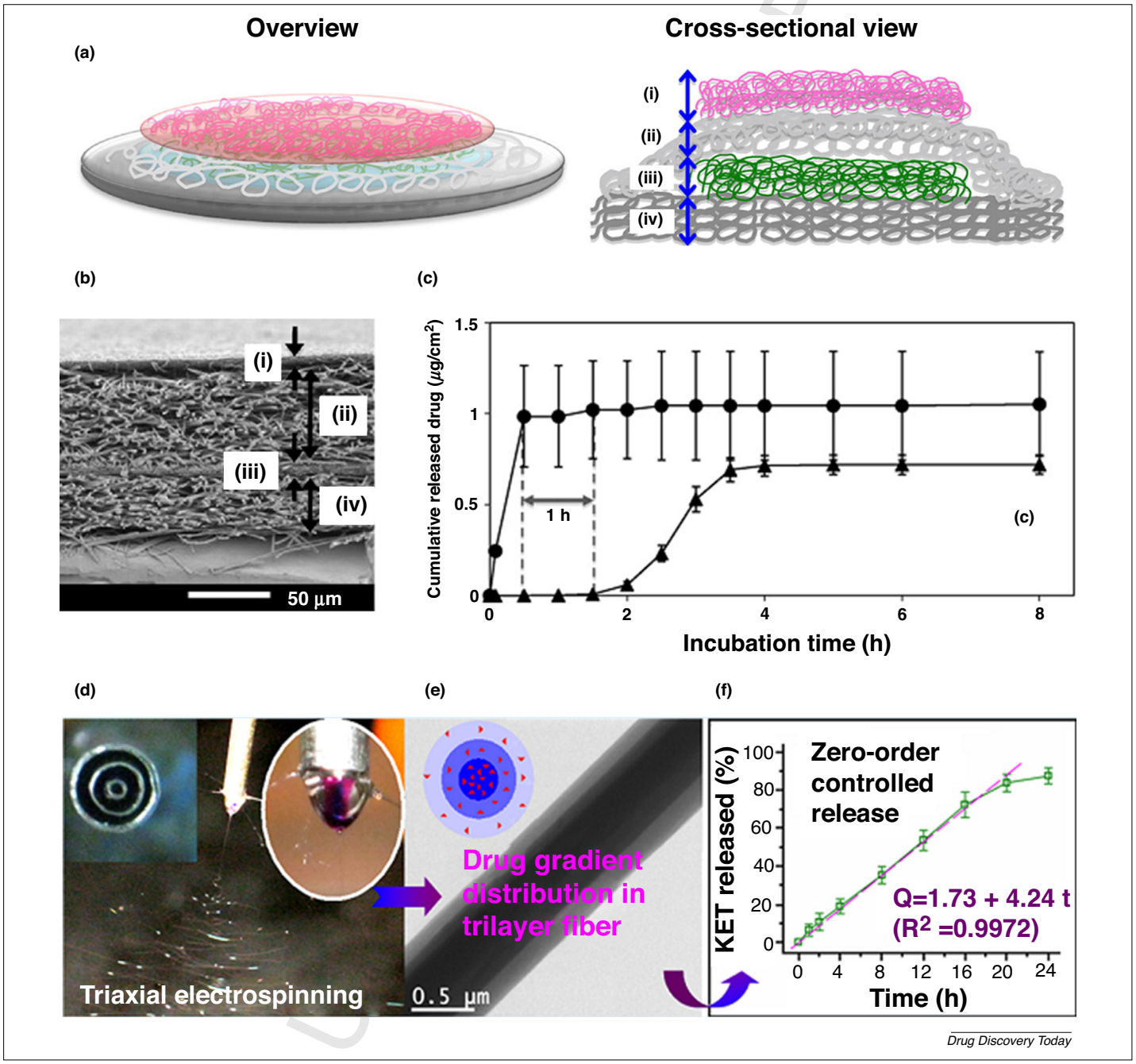

FIGURE 2

Q10 Multilayered drug-loading techniques. **(a)** Graphical demonstration of overview and cross-sectional view of tetra-layered nanofiber meshes. (b) Scanning electron microscopy (SEM) images of cross-sections of tetra-layered nanofiber meshes. Layers are (i) chromazurol B (ChroB) mesh, (ii) barrier mesh, (iii) 5,10,15,20-tetraphenyl-21H, 23H-porphinetetrasulfonic acid disulfuric acid (TPPS) mesh, and (iv) basement mesh. **(c)** Release profile of drug-loaded meshes of the tetra-layered nanofiber meshes. Closed circles and triangles represent release profiles of TPPS and ChroB, respectively. **(d)** Digital photographs showing the triaxial electrospinning process. The upper left and right insets show the structure of spinneret and a typical compound Taylor cone, respectively. **(e)** TEM image of a trilayer nanofiber. The upper-left insets show drug gradient distribution in trilayer fiber. **(f)** *In vitro* dissolution test results for the trilayer nanofibers. Images (a–c) reproduced, with permission, from Ref. [67] and images (d–f) reproduced, with permission, from Ref. [68].

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(PLCL) solutions containing two different model drugs were prepared and sequentially electrospun to fabricate the first drug-loaded mesh (top side) and then the second drug-loaded mesh. Electrospun PLCL meshes without drugs were layered between those containing different drugs to form a physical barrier to drug release. It was found that the drug release rate could be controlled by varying the fiber diameter and the mesh thickness. This tetra-layered system not only prolonged the drug-release time of the second drug-loaded mesh but also provided a delayed release of the respective drugs. These features render this tetra-layered system a useful scaffold for combined treatment of multiple drugs requiring respective administration at different times.

Triaxial electrospinning is another novel strategy to create functional trilayer micro/nanofibers for drug release. For example, ethyl cellulose (EC) was used as the filament-forming matrix in the outer, middle and inner working solutions, which were combined with ketoprofen (KET) such that the KET concentration gradually increased from the outer to the inner layer (Fig. 2d–f) [68]. This method successfully eliminated the initial burst release of KET. Also, it was found that the KET released from these layered fibers exhibited a linear release pattern that could be widely exploited in clinical settings, for example to treat painful conditions such as rheumatoid arthritis. These basic drug-loading techniques mentioned above can be integrated with each other to obtain novel polymeric micro/nanofibrous scaffolds with controllable release profiles. To better design and control the drug release from electrospun polymeric micro/nanofibrous scaffolds the release mechanisms should be understood. Details of these are presented below.

Mechanisms of drug release

The drug release mechanism from micro/nanofibers can be described mainly through desorption from the surface, diffusion through fibers, as well as fiber degradation [6]. All three release mechanisms can co-exist simultaneously and significantly influence drug release kinetics throughout the entire release period. The release profiles of drugs from nanofibers formed by different electrospinning methods are shown in Fig. 1. When nanofibers containing various drugs are surrounded by aqueous solution, the drugs attached to the fiber surfaces are released initially by desorption. The desorption mechanism is not limited to drugs adhering to the outer surface of the nanofibers but also includes drugs attached to the internal surface of nanopores inside nanofibers [24]. Among the three release mechanisms described, desorption of the drug from the fiber surface results in a relatively quick burst-release pattern owing to the close proximity between drugs on the fiber surface and the surrounding liquid. This mechanism of drug release is not generally considered useful as a controlled means of sustained release of bioactive agents. Hence, surface modifications, especially physical modification, have been considered in the design of carrier scaffolds to obtain a more controlled and sustained release profile. As mentioned above, a variety of techniques including coaxial and emulsion electrospinning have been developed to reduce the amount of drug localized to the surface and thus burst release. A different type of release mechanism involving the diffusion of drug through channels or pores is dependent on the concentration gradient between these channels and pores and the outside medium, the degree of diffusivity of the drug within the polymer matrix and on the diffusion route. The mechanism of drug release for blending electrospinning is mainly by diffusion down concentration gradients, which results in an unfavorable burst-release pattern. This has been attenuated by creation of a barrier layer (e.g., by incorporation of drug-loaded NPs into electrospun fibers, coaxial and emulsion electrospinning) between the drug and the aqueous phase to prolong the release time by extending the diffusion routes. The degradation pattern of micro/nanofibers greatly affects drug-release profiles regardless of the method of synthesis of such fibers. For most nonbiodegradable polymeric matrices, drug-release rates only correlate with the diffusion distance through the polymer or through the formed aqueous pores caused by water sorption [21]. However, when using a biodegradable carrier system, drugs can be released via diffusion and via spaces created by degradation of the micro/nanofibers. Through fiber degradation, drugs entrapped inside the polymer materials are released into the surrounding medium. This is more pronounced in rapidly degrading polymers such as chitosan or PVA, adding further complexity to controlled drug release. Thus, the drug-release routes and material degradation manners warrant careful consideration when selecting biodegradable materials for a drug-carrier scaffold. More details about drug-release mechanisms can be found in reviews by Leung and Ko [24] and Szentivanyi *et al.* [69]. In summary, a satisfactory drug-release profile over the whole period of release is eventually determined by a certain balance among the release mechanisms, and in turn correlates with corresponding drug-loading techniques and the polymer selection, which can be exploited in the development of long-term release drug carriers for biomedical applications as will be explained in the next section.

Biomedical applications of electrospun polymeric micro/nanofibrous scaffolds for long-term drug release

Increasing efforts have been made to fabricate electrospun polymeric micro/nanofibrous scaffolds for long-term drug release. Such scaffolds have been applied in the field of regeneration of damaged tissues like bone, skin, neural tissues and disease treatments such as cancer and adhesion formation (a frequent complication following surgery). In the following paragraphs, we introduce the latest applications of electrospun polymeric micro/nanofibrous scaffolds as long-term drug-release carriers in the field of tissue engineering and disease treatment.

Tissue engineering

Bone tissue engineering

Being a rigid organ, bone makes up our skeleton which serves multiple functions including supporting and protecting our vital organs, enabling mobility, producing red and white blood cells, as well as storing minerals [70]. Collagen and hydroxyapatite (HAp) are the main organic and inorganic components of bone ECM, respectively. Electrospun fibers incorporating HAp, tricalcium phosphate and dicalcium silicate are promising scaffolds for bone tissue engineering owing to their similarity to the structure and composition of naturally mineralized collagen fibers found in bone ECM [71–73]. To further facilitate the regeneration of fractured bones, a variety of GFs like bone morphogenetic protein-2 (BMP-2) and VEGF have been encapsulated into the electrospun fibers. For example, Chiu *et al.* covalently conjugated heparan-sulfate-decorated recombinant domain I of perlecan–HSPG2 onto

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an electrospun PCL scaffold to facilitate binding and control the release of recombinant human BMP-2 (rhBMP-2) [74]. This PCL scaffold provided sustained release of rhBMP-2 over 23 days *in vitro*. Additionally, rhBMP-2 released from the modified scaffolds enhanced alkaline phosphatase (ALP) activity in W20-17 mouse bone marrow stromal cells, suggesting its osteogenic potential and rendering it suitable for bone tissue regeneration [74].

Although previous studies have shown that direct delivery of GFs showed great therapeutic potential *in vitro* or *in vivo*, few studies have successfully completed clinical trials owing to the requirement of high amounts of therapeutic reagents and insufficient local retention [75]. Maintaining a localized high concentration of different GFs is crucial because during natural bone healing varying GFs and cytokines are excreted from different functional cells in a temporospatially predetermined way. Thus, a scaffold capable of efficient and long-lasting delivery of multiple therapeutic agents to mimic and accelerate natural healing is needed [75]. For instance, incorporation of BMP-2 and dexamethasone (DEX) into PLCL–collagen nanofibers by coaxial electrospinning has demonstrated excellent control over their drug-release profile, thereby promoting osteogenic expression of hMSCs [76]. This scaffold with the shell layer loaded with DEX and the core layer loaded with BMP-2 exhibited an initial fast release of DEX and a subsequent steady long-term release of BMP-2 for over 22 days. This dual GF-loaded scaffold combined the biological functions of both drugs, synergistically inducing the differentiation of hMSCs into osteogenic cells and improving osteogenic efficacy.

Compared with GF delivery, gene delivery represents a more fundamental approach to achieving controlled long-term release for bone regeneration via transferring local nucleic acids into somatic cells for sustained therapeutic expression of osteoinducive factors [77]. In their study, Xie *et al.* [78] designed a core–shell electrospun fibrous scaffold made of PLGA (shell) and polyethylenimine (PEI) (core) incorporating BMP-2 plasmids (pBMP-2) to protect pBMP-2 from direct exposure to organic solvents and to control its release. Compared with single axial scaffolds, this scaffold showed higher transfection efficiency and stable BMP-2 expression for over 28 days in human periodontal ligament stem cells (hPDLSCs). To date, there have been many achievements in bone tissue engineering, and electrospun polymeric micro/nanofibrous scaffolds count among the most versatile and promising scaffolds capable of controlled and sustained delivery of different bioactive factors for a predetermined period of time to not only mimic natural bone healing but further accelerate bone repair.

Skin tissue engineering

Skin is the largest organ of our body. Loss of integrity of our skin can lead to disability and even death. Most superficial skin wounds can heal by themselves. This is not the case, however, in wounds involving the deep dermis or wounds affecting large areas of the body (greater than 20% of the total body surface area [79]) caused by, for example, traumatic burns and chronic skin ulcers in patients with diabetes mellitus. Such wounds are difficult to heal completely in a relatively short period because of the lack of cellular and molecular signals necessary for normal wound repair. Additionally, the presence of inflammation with or without infection can result in abnormal wound healing and even develop into chronic wounds. To promote healing, the wound bed requires a 3D supporting structure such as ECM, as well as the external administration of therapeutic agents at a controlled rate and concentration over a period of weeks or months [80,81]. Therefore, artificial matrices such as electrospun polymeric fibrous membranes containing various therapeutic agents (e.g., anti-inflammatory and antimicrobial drugs, or GFs) have been trialed in skin repair strategies. Q6

In the early healing phases, especially for burn injuries, rapid and effective neovascularization plays a significant part. Thus, angiogenic factors [e.g., VEGF, basic fibroblast growth factor (bFGF), endothelial growth factor (EGF) and platelet-derived growth factor (PDGF)] have been incorporated into the electrospun scaffold, resulting in an efficient way of increasing the formation of blood vessels [82,83]. However, common loading techniques such as blending electrospinning are not considered to be effective in most cases owing to their tendency for severe burst release, which reduces effectivity. Nanofibrous scaffolds prepared by emulsion electrospinning with integral core-sheath structures, by contrast, can increase encapsulation efficiency, retard initial burst release and maintain bioactivities within the local tissue microenvironment for a longer timeframe. For instance, Yang *et al.* [82] adopted emulsion electrospinning to embed bFGF into poly (ethylene glycol)-poly(D,L-lactide) (PELA) fibers to promote wound healing. Such ultrafine fibers with a core-sheath structure have been shown to attenuate the initial burst release (as low as 14.0 ± 2.2%), rather exhibiting a sustained release of bFGF for ~4 weeks, which is in accordance with the duration for skin wound recovery. The gradual release of bFGF from PELA scaffolds was found to stimulate fibroblast growth and enhance collagen deposition and ECM remodeling, which resulted in significantly higher wound recovery rates with complete re-epithelialization and regeneration of skin appendages in the skin wounds of diabetic rats when compared with empty scaffolds or sham. Although these *in vivo* findings are suggestive of a potentially significant role for bFGF-loaded electrospun fibrous scaffold in the rapid restoration of structure and function of skin wounds, clinical trials in the past have shown that scaffolds with only one type of angiogenic factor are insufficient to induce a mature, stable, vascular structure because this usually requires the interaction of a number of stimulating factors that act at different stages of angiogenesis [84]. To tackle this issue, Lai *et al.* [83] designed a composite electrospun scaffold containing collagen–hyaluronic-acid–gelatin NPs (Col-HA-GNs), which can deliver multiple angiogenic growth factors in stages for functional vascularization of full-thickness skin. In this particle-in-fiber delivery system, multiple GFs including bFGF, EGF, VEGF and PDGF-BB were encapsulated either in the nanofibers or in the NPs and showed a stage-wise release pattern lasting for as long as 1 month. bFGF and EGF directly embedded in the nanofibers exhibited an initial rapid release, whereas VEGF and PDGF-BB encapsulated in the gelatin NPs showed a gradual slow release pattern. The sequential release of GFs, which is analogous to the natural physiological environment, offered precise control of the proliferation and migration of human umbilical vein endothelial cells (HUVECs) during the vascularization process, as well as epidermal and dermal tissue regeneration in diabetic rats.

In addition to neovascularization, attenuation of inflammation is an essential process in wound healing because persistent inflammation can lead to failure of normal skin wound repair mechanisms, cause pain and restrict the mobility of the patient. Various

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anti-inflammatory agents are therefore integrated into the electrospun scaffolds to decrease local inflammation of the skin wound. In one study, ibuprofen, a frequently used nonsteroidal anti-inflammatory drug (NSAID), was loaded into PLA nanofibrous scaffolds by blending electrospinning [34]. Owing to its slow *in vivo* degradation, this PLA scaffold released its cargo over a period of 14 days, which helped in reducing wound contraction and promoting healing of full-thickness incisional skin wounds.

Another concern of wound healing is potential wound infections, which can occur throughout the process of wound healing and are often due to a compromised immune system associated with the trauma itself or a more systemic disease such as diabetes. It is therefore important to consider incorporating not only GFs and anti-inflammatory drugs into wound healing scaffolds but also antibiotics or other antimicrobial substances to further reduce the likelihood of complications delaying healing. For example, Chen *et al.* [85] developed sandwich-structured, nanofibrous, drug-eluting membranes via sequential electrospinning for use as a wound dressing. This nanofibrous membrane with PLGA–collagen as the surface layer and PLGA–drugs at the inner layer released high concentrations of vancomycin and gentamicin (well above the 90% minimum inhibitory concentration) and lidocaine *in vivo* for at least 3 weeks. Owing to this prolonged antibacterial activity, these nanofibrous membranes were functionally active in treating wound infections and effective at accelerating wound-healing accelerators in the early stages.

It is known that skin injuries extending deep into the reticular layers of the dermis usually take up to 20 days to heal. Throughout this period there is a greater than 70% risk of hypertrophic scar formation [86]. Hypertrophic scars mainly form as a result of abnormal wound healing and often cause physical and psychological distress for the patient stemming from problems such as pruritus, pain and contractures. Consequently, it is desirable to develop a wound dressing that can facilitate wound healing and simultaneously suppress scar formation. For example, Cui *et al.* [87] have successfully developed Ginsenoside Rg3 (G-Rg3)-coated electrospun poly(L-lactide) (PLLA) fibrous scaffolds to reduce scar formation in a rabbit ear model. The G-Rg3–PLLA electrospun fibrous scaffolds exhibited an initial burst release of G-Rg3 during the first 2 days and then continued to gradually release the drug for up to 40 days (Fig. 3). The long-term release of G-Rg3 significantly inhibited proliferation of human hypertrophic scar fibroblasts (HSFs) *in vitro*, and the *in vivo* results showed significant prevention of hypertrophic scar formation by decreasing the thickness of the neodermis and the number of proliferative cells as well as collagen fibers. Overall, scar-free skin regeneration can be achieved using electrospun fibrous membranes that can release drugs on a long-term basis, thus reestablishing lost functions of skin. Similarly, a study by Cheng *et al.* [88] showed that PLGA electrospun fibers carrying Rg3 and coated with HA on the surface exhibited a relatively low initial burst release during the first 4 days, followed by a constant slow release of Rg3 within the next 36 days. Such scaffolds have exhibited an ability to promote wound healing in its early stages and inhibit scar formation in the latter stage.

Because wound healing is a dynamic, complex, multicellular process affected by many pathological and physiological factors, the sustained delivery of a combination of various bioactive agents *in vivo* has been hypothesized to be a promising multifunctional treatment modality to achieve active wound healing [89]. Xie *et al.* developed a composite nanofibrous delivery system containing chitosan-, poly(ethylene oxide)-, VEGF- and PDGF-BB-loaded NPs for wound healing applications [90]. This dual growth-factor-releasing nanoparticle-in-nanofiber system successfully enhanced angiogenesis by burst-releasing VEGF (63% released within 1 h and almost 100% within 1 day), supported fibroblast growth in a sustained manner via long-term release of PDGF-BB (a small initial burst release of 28% at 2 h and 40% release at day 7) and also demonstrated antibacterial properties of chitosan. This unique delivery device demonstrated that, by synergistically integrating several important key factors involved in the healing process, tissue regeneration and remodeling were accelerated, rendering this device highly suitable for the treatment of chronic complex wounds. Overall, despite significant achievements in skin regeneration using electrospun scaffolds, more research needs to be done to elucidate the complexities of wound healing to develop platforms for the simultaneous delivery of multiple therapeutic agents.

Neural tissue engineering

The nervous system (NS) is a complex collection of specialized excitable cells known as neurons which receive, process, store and transmit information gathered from within and outside of a cell body [91]. Nerve injuries including peripheral nerve injury (PNI), spinal cord injury (SCI) and traumatic brain injury (TBI) are very common and are a leading cause of morbidity and mortality due to resultant neurological deficits and gliosis [92–94]. The subsequent burden on the individuals, their families, as well as society is understandably grave [95]. Nanofibrous scaffolds fabricated by electrospinning have several advantages including an ECM-like structure as well as control over fiber diameter and scaffold porosity, which makes them desirable candidates for the investigation of treatments for neurological injuries and diseases. Many studies have already demonstrated the effects of electrospun fiber topography and density, surface coating and supporting substrates on nerve regeneration [96–98]. Here, we aim to emphasize electrospun scaffolds that have undergone drug loading to support improved nerve regeneration.

Neurotrophic factors, such as NGFs, widely exist in the developing peripheral and central nervous systems and have vital roles in dominating the survival, migration, proliferation and differentiation of various neural cell types [99]. Unfortunately, NGFs, like most other GFs, are chemically and physically unstable, thus suffering from short half-lives. Regeneration of neural tissues, however, takes a relatively long time depending on the extent of defect and would thus require prolonged exposure to NGFs. Delivery systems capable of preventing rapid loss and prolonging delivery of their cargo need to be developed to accelerate nerve regeneration. Recently, NGFs have been successfully encapsulated within various polymers by electrospinning to treat different nerve injuries. For example, in PNI, which involves the loss of long segments between proximal and distal nerve endings, electrospun polymeric micro/nanofibrous scaffolds containing therapeutic reagents have demonstrated favorable results [100]. In one study, Hu *et al.* [101] developed electrospun PCL scaffolds loaded with NGF and BSA (used as stabilizer to avoid initial burst release) by emulsion electrospinning. NGF and BSA were encapsulated within the core, whereas PCL formed the shell (Fig. 4). It was found that

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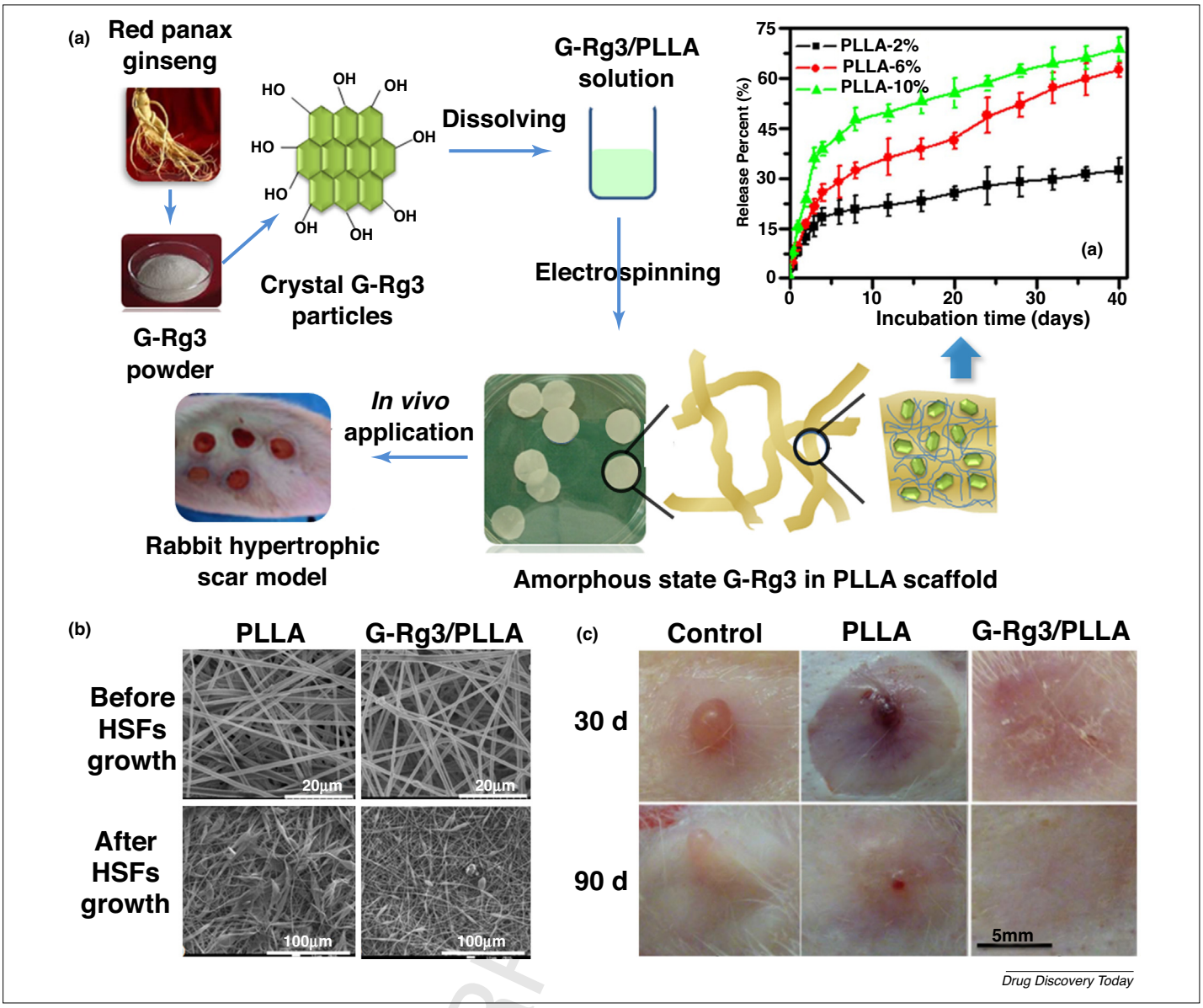

FIGURE 3

Q11 Ginsenoside Rg3 (G-Rg3)-coated electrospun poly(L-lactide) (PLLA) fibrous scaffolds for inhibition of hypertrophic scar formation. (a) Schematics showing fabrication and application of G-Rg3-coated electrospun PLLA fibrous scaffolds for inhibition of hypertrophic scar formation. The inset shows *in vitro* cumulative percentage release of G-Rg3 (2%, 6% and 10%) from electrospun fibers after immersion in PBS. **(b)** Scanning electron microscopy (SEM) images showing the adhesion of human hypertrophic scar fibroblasts (HSFs) on the naked PLLA and G-Rg3-coated PLLA scaffolds. **(c)** Therapeutic efficacy of G-Rg3-coated electrospun PLLA fibrous scaffolds for inhibition of hypertrophic scar formation using a rabbit ear model. Images adapted, with permission, from Ref. [87].

the presence of the stabilizer prolonged the release of NGF for over 28 days, which was beneficial for axonal regeneration and neurite outgrowth. Moreover, these scaffolds can be successfully applied to situations where cell apoptosis as opposed to cell growth is required such as in the management of glioblastoma [102]. In another study, NGF encapsulated into the cores of coaxially electrospun aligned silk-fibroin–poly(L-lactide-*co*-ε-caprolactone) [P(LLA-CL)] nanofibers exhibited a sustained release over 60 days, which was beneficial for promoting peripheral nerve regeneration [103].

In addition to GFs, the electrospun fibers can provide prolonged delivery of other therapeutic agents for the treatment of nerve injuries [104,105]. For instance, methylcobalamin (MeCbl), one of the active forms of vitamin B12, is known to promote nerve regeneration and neuronal cell survival [106]. However, it is difficult to achieve prolonged administration and effective drug concentrations because of the short half-life of MeCbl. To overcome this, Suzuki *et al*. developed an electrospun PCL nanofibrous sheet incorporating MeCbl to locally deliver a high concentration of the compound to the PNI site [107]. This sheet showed a sustained release of MeCbl for 8 weeks *in vitro* as a result of the slow degradation of PCL, which resulted in accelerated functional recovery and nerve regeneration *in vivo* without adverse effects on the nervous system.

Another treatment avenue for nerve damage is regeneration via nonviral delivery of nucleic acids using electrospun fibers. Following nerve injury and axonal division, a specific miRNA, miR-222, is induced to support nerve regeneration by regulating protein

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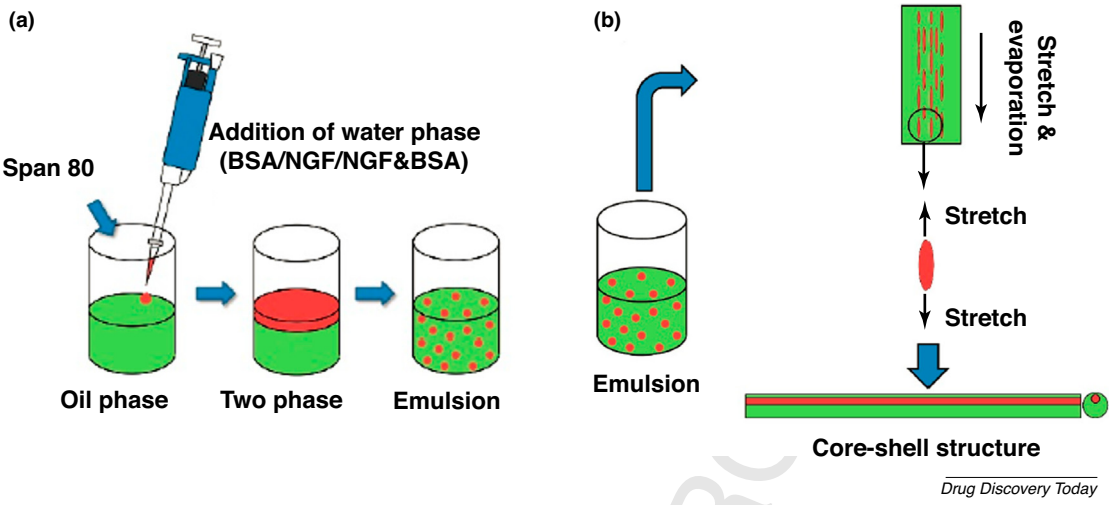

FIGURE 4

Schematic illustration of emulsion electrospinning of nerve growth factor (NGF)-loaded electrospun poly-ε-caprolactone (PCL) fibrous scaffolds. **(a)** The process of emulsion preparation. **(b)** The formation of core–shell fibers using emulsion electrospinning. Red color represents water phase containing water-soluble drugs and green color indicates PCL phase. Images adapted, with permission, from Ref. [101].

synthesis at the distal axon and supporting proliferation and migration of Schwann cells [108]. Thus, the delivery of miR-222 to injured nerve cells could enhance nerve regeneration. Nguyen *et al*. designed an aligned poly(ε-caprolactone-co-ethyl ethylene phosphate) (PCLEEP)–collagen hybrid scaffold and incorporated miR-222 into nanofibers and/or collagen hydrogels using electrospinning [109]. The miR-222 was rapidly released within the first month and reached a steady release for a further 2 months due primarily to slow diffusion of miR-222 through the scaffold and the relatively slow degradation of PCLEEP. This scaffold provided not only biochemical signals to enhance axon regeneration and remyelination but also topographical signals to effectively direct neurite extensions and support remyelination within the lesion sites after spinal cord injuries. It is envisioned that the synergistic effect of the favorable characteristics of fibers and meshes and sustained delivery of various therapeutic agents could be conducive to the novel design of a multifunctional artificial scaffold for guiding nerve tissue growth and promoting nerve regeneration in future.

Other tissues

In addition to bone, skin and neural tissues, electrospun polymeric micro/nanofibrous scaffolds can find broad applications in regeneration of blood vessels [110], cartilage [111] and cardiac tissue [112,113]. For example, Zhou, *et al*. [114] developed an efficient intracellular delivery system for miRNA-126 (miR-126), a regulator of vascular integrity, by encapsulating it within a bilayered electrospun fibrous structure resembling a blood vessel (encapsulation efficiency of 68%). This entrapment method enabled the direct intracellular delivery of miR-126 to vascular endothelial cells seeded onto the luminal surface of the scaffolds for a period of 56 days, resulting in significantly improved proliferation of vascular endothelial cells and enhanced endothelialization *in vivo*. In another study, Wang *et al*. [111] developed a bioactive PLCL–collagen nanofibrous scaffold via coaxial electrospinning for cartilage regeneration. The scaffold was loaded with transforming growth factor β3 (TGF-β3) (encapsulation efficiency of 45%) which was released from the scaffolds in a sustained and stable manner for over 57 days while retaining bioactivity throughout. This slow release pattern was mainly attributed to the slow degradation of PLCL, the shell layer of the scaffold. This study indicated that PLCL–collagen scaffolds containing TGF-β3 could promote the chondrogenic differentiation of human umbilical cord stem cells (WMSCs) for cartilage regeneration in trachea repair. For enhancing cardiac regeneration and angiogenesis following acute myocardial infarctions, Chung *et al*. [115] developed electrospun nanofibrous PLLA mats for targeted epicardial delivery of VEGF and cardiac stem cells to localized infarcted myocardium. The VEGF was immobilized onto the mats via emulsion electrospinning and showed steady and long-term release kinetics over 4 weeks. The sustained VEGF release from the mat to cardiac stem cells significantly promoted the migration and proliferation of endothelial cells and cardiac stem cells *in vitro*, resulting in marked improvement of angiogenesis and cardiac functional recovery. All in all, electrospun polymeric micro/nanofibrous scaffolds are ideal candidates for tissue regeneration owing to their structural similarity to natural ECM, adequate drug loading capacity and controllable release kinetics. The following sections will further discuss their suitability as drug delivery systems with sustained and controlled release of GFs, proteins or other drugs for treatment of diseases such as cancer.

Therapeutic delivery

Cancer therapy

Cancer recurrence is a recognized complication of inadequate medical and/or surgical treatment and can happen at any time (weeks, months or even many years) after treatment [11]. The mechanism of recurrence is either by *de novo* transformation of dysplastic cells or the multiplication of malignant cells, which have somehow remained in the body after treatment. It is therefore important to develop implantable scaffolds capable of releasing drugs long after primary cancer treatment or resection. Recently, electrospun PLLA scaffolds containing MSNs capable

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of releasing drugs for up to 90 days have been developed (Fig. 5a) [116]. The long-term drug release is mainly the result of the extended drug diffusion route provided by the MSNs – the entrapped drugs must first be released from the MSNs, and then diffuse through the polymeric fibers to be released into the medium. In this way, therapeutic agents can be continuously delivered at a controlled rate over a period of weeks and months. Not only is this beneficial for the sustained fight against cancerous cells but it also improves patient compliance and comfort, reduces fluctuations in drug blood levels, decreases adverse effects at the doses required for clinical efficacy and improves overall upon existing pharmacotherapies.

A major drawback of conventional drug release systems involves the nonspecific release of drugs upon encountering a cellular environment, which is not necessary for the cancerous tissue. Thus, it is desirable to develop a drug-eluting strategy responsive to the tumor microenvironment to improve treatment. Because tumor cells secrete acids, it would be more sensible if the electrospun fibers were sensitive to acids and thus released their cargo in response to an acidic environment only. To develop a tumor-triggered drug delivery system, Zhao *et al*. constructed electrospun PLLA fibers incorporating drug-loaded MSNs capped using $CaCO_3$ (Fig. 5b) [11]. The inorganic $CaCO_3$ caps were shown to control the opening of pore entrances of MSNs encapsulated inside the PLLA fibers. This is because $CaCO_3$ being stable at the physiological pH of 7.45 is readily dissolved into biocompatible Ca^{2+} (cations) and CO_2 in response to an acidic environment (pH < 6.8) which is frequently encountered in and around cancer tissues. This system was not responsive at normal pH ranges, thus reducing the risk of damage to healthy cells. Furthermore, this system released the drug over a period of 40 days owing to the existence of MSNs that could extend the drug diffusion route, demonstrating effective antitumor efficacy.

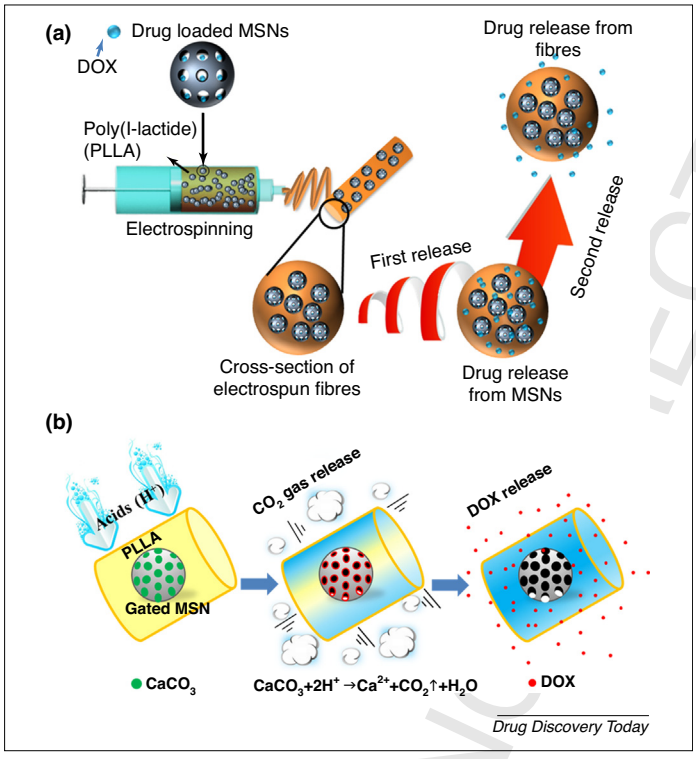

FIGURE 5

Q12 Electrospun polymeric fibers containing mesoporous silica nanoparticles (MSNs) for extended drug delivery. (a) Schematic illustration of a composite drug release system for extended drug release. The chemotherapeutic agent doxorubicin (DOX) was loaded onto MSNs, which were dispersed within poly (L-lactide) (PLLA) solution and then electrospun to obtain PLLA fibers. DOX was released first from the MSN core to the PLLA shell and from the PLLA shell into the surrounding medium. By extending the drug diffusion route, the drug release was prolonged. **(b)** Schematic illustration of a tumor-triggered controlled drug release system. Infiltration of protons (H^+) into the electrospun PLLA fibers results in the unplugging of the $CaCO_3$ cap to unveil the DOX molecules trapped inside the MSN pores. Water penetration into the PLLA fibers facilitates the DOX release. Image reproduced, with permission, from Ref. [11].

Adhesion prevention

Adhesion formation is a physiologically important part of wound healing. However, adhesion formation after surgery, in particular bowel surgery, can result in serious complications including pain and functional obstruction, which might require difficult reoperations. Taking tendon injury and/or surgery as an example, peritendinous tissue adhesion is a common complication and is associated with various factors including severe infection and foreign-body reaction caused by the degradation products of biomaterials inserted as tendon adjuncts [117]. These factors are prone to producing an inflammatory response because inflammatory cells and fibroblasts migrate to the site of injury, forming new capillaries during the process of tendon healing, which results in the development of an undesirable healing microenvironment [118]. Recently, various studies looking into the long-term prevention of peritendinous adhesion formation have reported good outcomes when NSAIDs and HA were added to electrospun polymer fibers as they induced fibroblast apoptosis and inhibited collagen secretion [117,119,120]. In one study, Zhao *et al*. [56] developed PLLA fibers encapsulating mitomycin-C (MMC)-loaded HA hydrosols by micro-sol electrospinning for accelerated healing and prevention of adhesion formation in a tendon wound model. In this system, drug-loaded electrospun fibers achieved continuous and controlled release of MMC over several months to inhibit fibroblast proliferation via upregulation of the apoptotic protein Bax and downregulation of proteins Bcl2, collagen I, collagen III and α-SMA. The HA hydrosols provided nutrients required for tendon healing and acted as a lubricant for tendon gliding. The relatively slow burst release of MMC during the early stage combined with its long-term sustained release over 40 days resulted in faster tendon regeneration compared with other carrier systems while preventing significant adhesion formation. In another study, Jiang *et al*. [121] incorporated celecoxib (a drug to suppress fibroblast proliferation and collagen expression) into PELA fibrous membranes via blending electrospinning (Fig. 6). The results of *in vitro* studies showed celecoxib-loaded PELA membranes effectively sustained the drug release over 20 days because of slow drug diffusion and inhibited the proliferation and adhesion of rabbit fibroblasts and tenocytes. *In vivo* studies demonstrated that celecoxib released from this membrane downregulated ERK1/2 and SMAD2/3 phosphorylation, leading to decreasing collagen I and collagen III expression and reduced inflammation and fibroblast proliferation, and thus successfully preventing tissue adhesion. This research has demonstrated that the celecoxib-loaded PELA membranes, which could release celecoxib long-term, could effectively prevent tendon adhesion formation.

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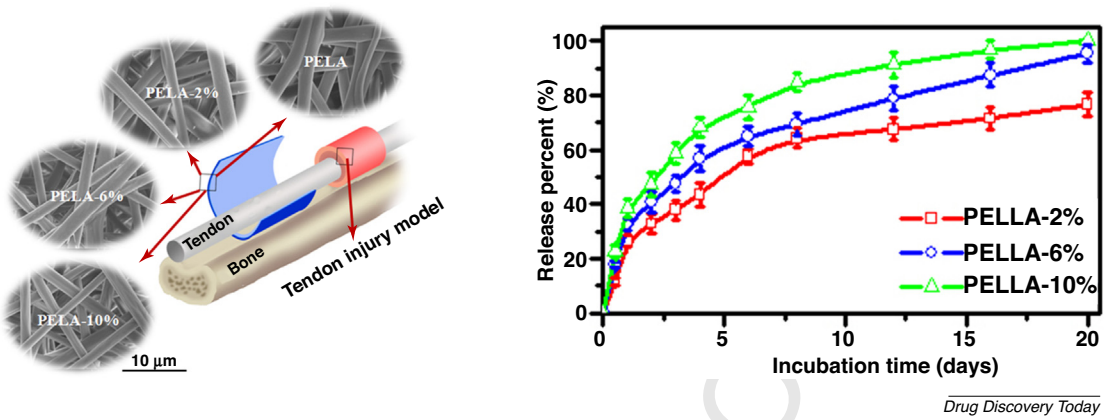

FIGURE 6

Q13 Electrospun poly(ethylene glycol)-poly(D,L-lactide) (PELA) nanofibers for prevention of tendon adhesion formation. (a) Schematic illustration of celecoxib-loaded PELA fibrous membrane and scanning electron microscopy (SEM) images showing the morphology of drug-loaded PELA fibers. **(b)** Cumulative celecoxib release from electrospun PELA fibers after incubation in PBS at 37°C. Image reproduced, with permission, from Ref. [121].

Other diseases

Apart from treating cancer and inhibiting adhesion formation with sustained and long-term drug release, electrospun polymeric micro/nanofibers have also served as drug delivery vehicles in other disease states, such as periodontal disease [122], rheumatism [123] and esophageal stenosis [124]. For example, Zamani *et al.* [122] constructed electrospun PCL nanofibers containing metronidazole benzoate (MET) and evaluated their therapeutic efficacy in the treatment of periodontal disease. Sustained release of MET from the nanofibers was achieved for about 20 days *in vitro* with a low initial burst release. This could be a desirable treatment period for periodontal diseases owing to more-prolonged drug availability and sustained drug action. Siafaka *et al.* [123] encapsulated teriflunomide (the active pharmaceutical ingredient of leflunomide used for the treatment of rheumatoid arthritis) into a range of novel electrospun fibrous mats using PLA and poly(butylene adipate) (PBAd) polymer blends to demonstrate their potential as pharmaceutical patches for transdermal and continuous delivery of antirheumatic agents. Compared with PLA fibers, PLA–PBAd blends showed significantly longer release profiles because of slower diffusion of the drug from the polymer matrix. In another study, Zhu *et al.* [124] employed blending electrospinning rotating-collection to develop a biodegradable paclitaxel–PCL microfibrous membrane-covered stent for the treatment of benign esophageal strictures. Paclitaxel was released from the stent mainly via diffusion and showed stable release profiles for up to 32 days at pH 4.0. Furthermore, significant inhibition of proliferation of smooth muscle cells was observed *in vitro*. An *in vivo* esophageal stricture model showed that the drug-loaded stent resulted in decreased tissue inflammation and collagen fiber production and easy removal of the stent from the esophagus, with no detrimental effects on the normal esophageal tissues. Thus, this novel stent is considered as a potential clinical esophageal stricture therapy to effectively attenuate stent-induced inflammation and scar formation. In summary, electrospun polymeric micro/nanofibrous scaffolds play a crucial part in drug delivery owing to the large specific surface area and ECM-like physical structure which promote cell adhesion, migration, proliferation and differentiation. Owing to their widespread popularity as drug delivery vehicles, electrospun fibers are increasingly applied in tissue regeneration and therapeutic delivery.

Concluding remarks and future perspectives

Electrospun polymeric micro/nanofibrous scaffolds have gained significant popularity for use as long-term drug delivery vehicles because of several advantages including an inherently high surface-area:volume ratio, tunable fiber diameter, high porosity and ECM-like structure. In addition to these factors, their cost-effective production renders these scaffolds highly applicable to a multitude of disease states. A rich variety of materials, including natural, synthetic and their composite polymers have been successfully electrospun into micro/nanofibers with different properties to deliver an array of hydrophilic and hydrophobic drugs as well as biomacromolecules like GFs and genes. Different loading techniques such as surface modification, blending, coaxial and emulsion electrospinning, and encapsulation of drug-loaded NPs into micro/nanofibers have been shown to greatly influence the drug-release profiles, rendering such systems very controllable and attenuating common issues such as an initial burst release of drugs. Thus far, these electrospun scaffolds have proven to be a promising long-term drug delivery platform for the support of bone, skin and nerve regeneration as well as for cancer and adhesion treatment. Despite significant progress in the field of electrospun scaffolds, four important issues remain to be solved for clinical translatability: (i) polymers require further optimization for better controllable degradation rates; (ii) new electrospinning strategies will have to be developed to improve loading and extend release periods of hydrophilic drugs; (iii) core–shell structured scaffolds need to be further evaluated in the context of multi-drug delivery and preservation of bioactivities of unstable drugs; and (iv) scaffolds require further investigation in *in vivo* and then clinical settings to confirm their applicability. In conclusion, disease treatment and

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tissue regeneration are complex processes, which in most cases require the accurate orchestration of various drugs and GFs at various concentrations for different periods of time. By selecting the appropriate polymer type and designing effective drug-loading strategies and release routes, so-called 'smart' devices could be developed for successful clinical applications.

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