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Review

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Core-shell nanofibers as drug delivery systems

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Core-shell nanofibers have grown in popularity over the last decade owing to their special features and their many applications in biomedicine. They can be produced by electrospinning of immiscible polymer blends or emulsions through a single nozzle or by electrospinning using a coaxial nozzle. Several of the electrospinning parameters allow great versatility for the compositions and diameters of core-shell nanofibers to be produced. Morphology of core-shell nanofibers can be investigated using transmission electron microscopy and, in some cases, scanning electron microscopy. Several studies have shown that core-shell nanofibers have some advantages over monolithic nanofibers, such as better drug, protein, gene or probiotic incorporation into the nanofibers, greater control over drug release, and maintenance of protein structure and activity during electrospinning. We herein review the production and characterization of core-shell nanofibers, the critical parameters that affect their development, and their advantages as delivery systems.

Keywords: coaxial electrospinning, emulsion electrospinning, core-shell nanofibers, nanotechnology, nanodelivery system, drug release

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INTRODUCTION

Nanofibers and microfibers can have diameters of a few nanometers to several micrometers. They are composed of natural and/or synthetic polymers, and are most frequently produced using the electrospinning technique (1). Over the last few decades, electrospinning has grown in popularity and is now used for the production of different monolithic or blended nanofibers for several applications (2). In 2003, coaxial electrospinning was discovered to be a promising method for the preparation of a novel class of nanofibers with core-shell or core-sheath structures using a coaxial nozzle (3). Three years later, emulsion electrospinning using a single nozzle was shown to produce similarly structured nanofibers (4).

Interest in nanofibers rapidly increased in the last decade, with 56 articles published in 2004, while since 2014 more than 1000 articles have been published each year (Fig. 1).

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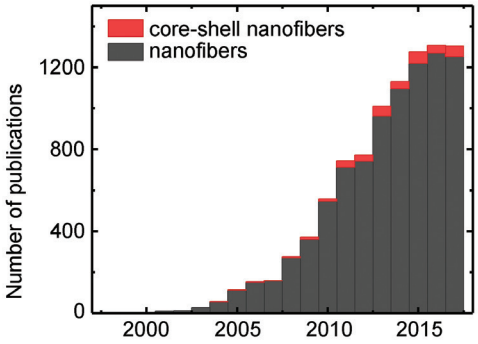

Fig. 1. The number of publications on nanofibers and core-shell nanofibers published between 1996 and 2017 (source: PubMed).

Interest in core-shell nanofibers has also increased, and they have represented 1 to 5 % of all nanofiber publications over these years.

Polymeric nanofibers have several remarkable features, which include nanoscale diameters, unlimited lengths (theoretically), high surface-to-volume ratios, and porous structures, both individually and as mats. These characteristics have provided them with improved mechanical performance and flexibility compared to any other forms of the same material (2, 5, 6). Therefore, in the last 15 years, electrospun fibers have emerged as promising materials in many fields, including filtration (7), packaging (8), electronics (9), and biomedicine. In biomedicine in particular, as nanofibers have the striking feature that they resemble an extracellular matrix, they have grown in popularity as drug delivery systems and as powerful materials for tissue engineering (10, 11).

Although monolithic and blended nanofibers have promising characteristics, there are some disadvantages that provide considerable challenges, such as their incorporation of hydrophilic drugs, proteins, and cells, with their high drug loading and preserved drug or protein activity, and their prolonged release without burst effects (12). Therefore, several studies have developed core-shell nanofibers to solve these problems. This review focuses on the methods for the production of core-shell nanofibers as a single step, parameters that affect these processes, characterization techniques, and presentation of core-shell nanofibers as a drug delivery system.

INTRODUCTION TO ELECTROSPINNING

Electrospinning is the process that produces nanofibers and microfibers from viscoelastic polymer solutions or melts. An electrospinning set-up consists of a pump, a single, coaxial, or triaxial nozzle, a high-voltage source applied to the nozzle, and a grounded or oppositely charged collector (Fig. 2). A polymer dispersion (*i.e.*, solution, miscible polymer blend, immiscible polymer blend, emulsion) is pumped through the nozzle, on the tip of which a drop appears, which is held by surface-tension forces (4, 13–15). When high voltage is applied to the nozzle, charge is induced within the polymer dispersion. When electrical forces overcome those of the droplet surface tension in the charged polymer solution, a

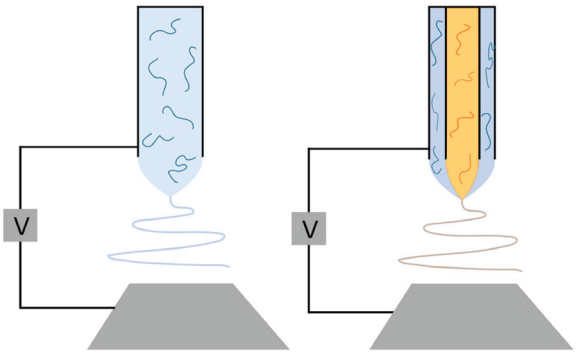

Fig. 2. Schematic representation of electrospinning through a single and a coaxial nozzle.

Taylor cone is formed and the jet is pulled by the electric field toward the grounded collector. The jet undergoes stretching and whipping, and it solidifies due to solvent evaporation before it is deposited as a nanofiber mat on the grounded collector (13). The electrospinning process is influenced by the dispersion used (*e.g.*, polymer type, concentration, viscosity, conductivity, surface tension), the processing factors (*e.g.*, electric field strength, flow rate, collector set-up), and the ambient parameters (*e.g.*, temperature, humidity), which have been previously described in detail (1, 2, 13, 16, 17).

Electrospinning using a single nozzle for the production of core-shell nanofibers

A single nozzle is commonly used for the electrospinning of nanofibers that are formed from one polymer (monolithic) or more polymers (blended). However, in special cases, electrospinning of polymer blends and emulsions can result in core-shell nanofibers (Fig. 3).

Electrospinning of immiscible polymer blends. – Polymer mixtures can be classified as miscible or immiscible polymer blends. Miscible polymer blends can form solutions without any phase separation between the ingredient polymers (Fig. 3b). In contrast, immiscible polymer blends in the same solvent separate into two or more phases (Fig. 3c) due to their thermodynamic incompatibility (15, 18, 19). A few examples of immiscible polymer blends that have been electrospun into core-shell nanofibers include poly(methyl methacrylate) (PMMA)/polyacrylonitrile (PAN) in a 1:1 mass ratio dissolved in dimethylformamide (DMF) (15), and PMMA/polycarbonate, polystyrene/PMMA, polybutadiene/polycarbonate, polyaniline/polystyrene and polyaniline/polycarbonate, all of which were separately dissolved in tetrahydrofuran (THF) in a 1:3 mass ratio (20). Electrospinning of poly(vinyl pyrrolidone) and tetraethyl orthosilicate blends in ethanol produces nanotubes in a single step (21).

Detailed explanation of the formation of core-shell fibers from electrospinning of polymer blends was provided by Bazilevsky *et al.* (16). Briefly, their PMMA/PAN blend in DMF provided 100 to 200 μm PMMA/DMF drops. During electrospinning, on the tip of the nozzle, a drop of the inner polymer is covered with the continuous phase of the other

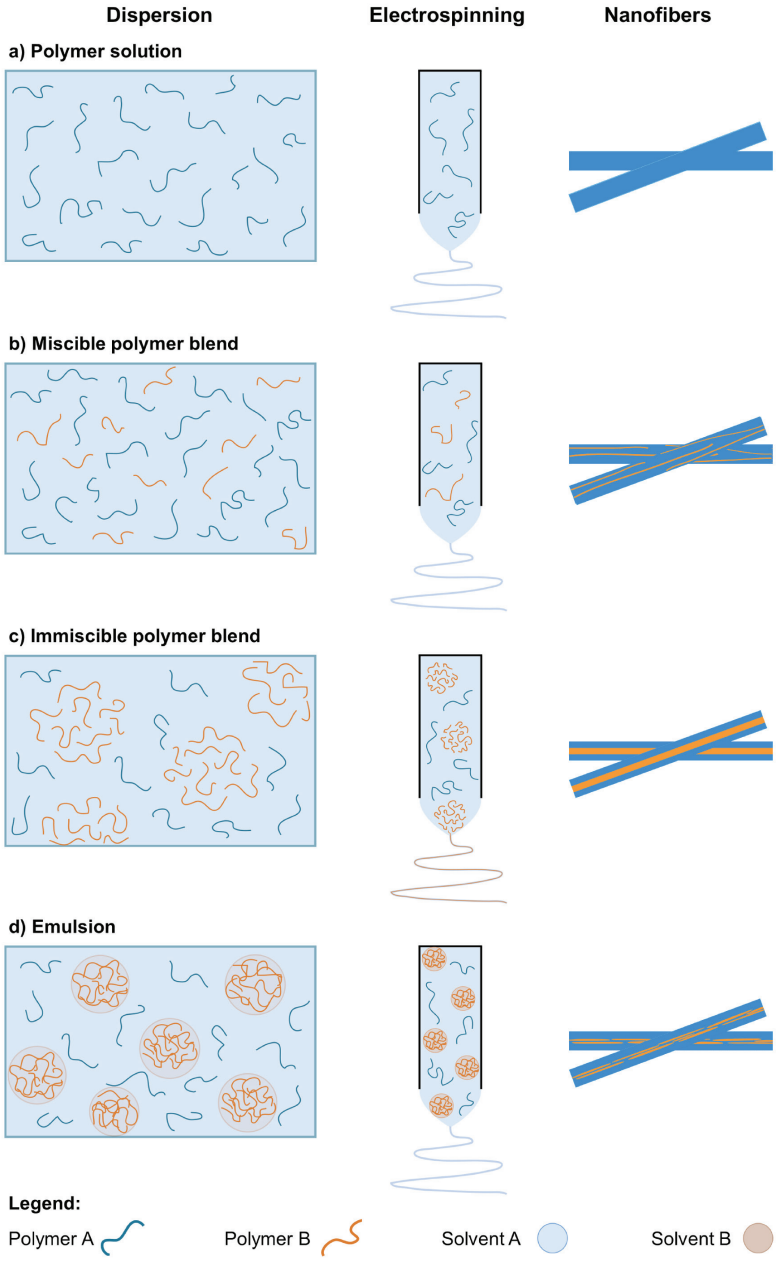

Fig. 3. Schematic representation of polymer dispersions: a) as polymer solution, b) miscible polymer blend, c) immiscible polymer blend, and d) emulsion. These can be electrospun to form: a) monolithic, b) blended, and c, d) core-shell nanofibers. Electrospinning of immiscible polymer blends and emulsions only under optimized conditions results in core-shell nanofibers.

polymer (Fig. 3c). When the electrical forces are sufficiently strong, a continuous phase entraps and sucks the drop into a core-shell jet, which can result in core-shell fibers. Theoretically, after electrospinning, one drop of inner phase with a diameter of 100 μm on the tip of the nozzle can result in a 1-m-long core of core-shell fiber with a diameter of 1 μm. As there are no indications of long fiber sections without a core, which are quite common for core-shell nanofibers produced by coaxial electrospinning, the process appears to be relatively robust (15). In addition, electrospinning of immiscible polymer blends with smaller drops can lead to multi-core or co-continuous fibers (14, 15, 18, 20).

Although polymer blends are often electrospun, formation of core-shell nanofibers from them has rarely been reported. One reason might be that the nanofiber morphology has seldom been studied using transmission electron microscopy when core-shell nanofibers are not expected. Furthermore, there is a narrow window of parameters under which core-shell nanofibers can be formed. The most critical parameter for the formation of core-shell nanofibers is low molecular weight of the polymer, since during electrospinning, polymers with higher mobility will preferentially organize into a core-shell rather than a co-continuous structure. Thermodynamic properties that favor the formation of core-shell structure include incompatibility between the two polymers and a large difference in their solubility parameters. Here, also, the more viscous phase was reported to form the core (20).

Emulsion electrospinning. – Emulsions are mixtures of two or more immiscible liquids, where one liquid is usually dispersed as drops in the other, which is seen as a continuous phase (Fig. 3d). Electrospinning of emulsions through a single nozzle (*i.e.*, emulsion electrospinning) represents one of the ways to prepare core-shell fibers with successful incorporation of drugs, proteins and microspheres (4, 15, 20, 22). For the two types of emulsions, oil-in-water (23) and water-in-oil (4, 22, 24–27), the use of water-in-oil emulsions has been more often reported in the literature. Water-in-oil emulsions frequently consist of an aqueous polymer solution with a dissolved hydrophilic drug or protein as the dispersed phase, with the continuous phase of a solution of hydrophobic polymer in an organic solvent, such as chloroform (4, 27, 28). Surfactants are usually added to increase the physical stability of emulsions (4, 22, 24–27). Emulsion electrospinning of oil-in-water emulsions tends to consist of aqueous polymer solutions, thus avoiding harmful organic solvents, referred to as 'green electrospinning' (29). In such cases, the dispersed phase is an oil (*e.g.*, mineral or plant oil), which is used for incorporation of hydrophobic drugs into hydrophilic nanofibers (23, 29).

In the literature, the formation of core-shell nanofibers prepared using water-in-oil emulsion electrospinning has been explained as follows: during the electrospinning process, the drops stretch into an elliptical shape in the direction of the fiber due to the interfacial shear forces from the outer oil phase. Drops that are too large to resist the electric force break up into smaller drops. Viscosity of the oil phase increases more rapidly than the inner aqueous drops, which directs the drops to be incorporated into the fiber interior, rather than to be at the surface (4, 30). If the drops merge, they form the core, and thus emulsion electrospinning results in core-shell nanofibers (4, 25, 27, 28, 31–33). Instead of core-shell morphology, separate drops embedded into the fibers (4, 34) and discontinuous core (24) have also been reported.

Polymer concentration in an emulsion has a critical role in the formation of nanofibers (35), similar to what has been reported for electrospinning of polymer solutions (17). Elec-

trospinning of emulsions with low polymer concentrations results in beaded nanofiber morphology, due to low viscoelasticity, and thus sufficient polymer concentration and polymer weight are needed for the formation of smooth nanofibers (35). Continuous phase should also show electrospinnability, but if the dispersed phase is not electrospinnable, core-shell nanofibers can still be formed (27). A dispersed phase of the emulsion with a high polymer concentration results in a thicker core and overall fiber diameter after electrospinning compared to lower polymer concentrations (4).

The volume ratio of the dispersed phase to the continuous phase of the emulsion influences the inner structure and diameter of the fibers that can be formed (27, 35). A high water content for the dispersed phase in a water-in-oil emulsion leads to less uniform nanofibers (35) and incomplete movement of the emulsion droplets during electrospinning to the core thus, this results in core material close to the nanofiber surface. In addition, higher water contents in a water-in-oil emulsion results in decreased nanofiber diameter due to higher conductivity of the emulsion (27). In contrast, for an oil-in-water emulsion, the addition of plant oil into an aqueous poly(vinyl alcohol) (PVA) solution increases the emulsion viscosity, and consequently results in thicker fibers (23).

Coaxial electrospinning: electrospinning using a coaxial nozzle for the production of core-shell nanofibers

Coaxial electrospinning is the process that is now most often used for the production of core-shell nanofibers. In terms of immiscible polymer blended and emulsion electrospinning, coaxial electrospinning differs due to the pumping of two separate solutions through a coaxial nozzle, instead of one solution/emulsion though a single nozzle (Fig. 2) (18). The core and shell solutions can be composed of the same (3) or different (18, 36) polymers, and sometimes the core consists of the drug solution only without any polymer added (37). According to some studies, both solutions need to be spinnable, whereas other studies have claimed that coaxial electrospinning is important for core materials that cannot form nanofibers by themselves such as nonpolymeric Newtonian liquids (38). In addition, coaxial electrospinning can result in the formation of nanotubes (39).

For coaxial electrospinning, the core-shell droplet appears at the tip of the coaxial nozzle, which at the applied voltage ejects the core-shell jet. This undergoes similar electrically driven bending instabilities as for the ordinary electrospinning process. Importantly, use of the core-shell nozzle does not guarantee formation of a core-shell jet and later on core-shell nanofibers, since there are also many parameters (*e.g.*, solutions, processing, environmental) involved during coaxial electrospinning. In some cases, the core jet might not enter the shell jet, which will result in a monolithic core and/or shell nanofibers or beads. In particular, two types of capillary instabilities can affect core-shell jets. First, as with standard electrospinning, the whole jet can break up into droplets, due to surface tension and insufficient viscoelastic forces. The other instability is specific to the core-shell jet, where the core solution during coaxial electrospinning can break up into separate droplets inside an intact shell due to the interfacial tension between these two solutions (38).

There are a number of additional parameters that are important in core-shell nanofiber preparation, compared to electrospinning of monolithic nanofibers. These are the miscibility or immiscibility of the core and shell solutions, the flow rate ratio between the two solutions, and the protrusion of the core nozzle outside the shell nozzle (40).

Core-shell nanofibers can be prepared from the same polymer and the same solvent mixture as the core and shell (3), different polymers dissolved in the same solvents as the core and shell (3, 18, 41), and different polymers dissolved in different solvents, which can be miscible, partially miscible, or immiscible (36, 42). Solvent miscibility between the core and shell solutions is an important parameter to consider, as this can affect the core-shell nanofiber formation and their morphology. Zussman *et al.* (43) have reported that a well-stabilized coaxial-electrospinning process can be achieved when both solutions are sufficiently viscous, spinnable, and immiscible. Chakraboty *et al.* (42) indicated that complete immiscibility between the core and shell solutions (*e.g.*, water and chloroform) leads to uneven distribution of the core solution (*i.e.*, water) inside the fiber, which increases the probability of defects. Decreased interfacial tension caused by the addition of a common secondary solvent results in fewer defects, an even distribution of the water inside the fiber, and thinner and uniform core-shell nanofibers (42, 44). Using the same solvent system for shell and core solutions decreases the interfacial tension (18), although in such cases a less sharp boundary between the core and shell systems has been suggested (43). On the other hand, theoretically, a sharp boundary between two identical polymers in core-shell nanofibers can be obtained because the characteristic time of diffusion spreading of a polymer in core-shell nanofibers is 10-fold greater compared to the solidification process (3).

The flow rate ratio between the core and shell solutions in coaxial electrospinning is crucial for the formulation of core-shell nanofibers. At core-shell flow rate ratios of < 1:2, there is insufficient shell solution to coat the core solution, which results in droplets or particles on the collector. Increased core-shell flow rate ratios (1:2 to 1:3) lead to occasional encapsulation of the core solution in the core-shell nanofibers, accompanied by many defects, such as an incomplete shell. The optimum core-shell flow rate ratios are 1:3 to 1:6, which enable the formation of a stable core-shell Taylor cone and result in formation of core-shell nanofibers. Raising the core-shell flow rate ratio from 1:7 to 1:10 does not appear to affect the ability of the core-shell solutions to be electrospun, but reduces the amount of core in the core-shell nanofibers formed (18, 42).

The position of the coaxial needle is also important for successful core-shell nanofiber preparation. It has been shown, both theoretically and experimentally, that the core nozzle needs to be outside its shell counterpart by about half the radius of the shell (40). Coaxial electrospinning can be also typically achieved at an applied voltage similar to that for single electrospinning, although poor miscibility between core and shell solutions can require higher voltage to be applied so as to overcome the interfacial tension between the core and shell (42).

CHARACTERIZATION OF POLYMER SOLUTIONS/EMULSIONS AND CORE-SHELL NANOFIBERS

Polymer solution and emulsion characterization

Optimum solution properties are among the most important aspects for successful nanofiber formation. Solution parameters that are usually measured are shear viscosity, conductivity, and surface tension (Table I) (1, 45, 46). Rheometers with a cone-plate measuring system are used to perform the rotational and oscillatory tests to define the shear viscosity and elastic (G′) and plastic (G″) moduli of solutions (17, 39, 47). Interfacial shear

Table I. Methods that can be used for characterization of core-shell nanofibers

Character	Method	Reference
Solution/emulsion characterization	Bulk viscosity	17, 39, 47, 54
	Interfacial shear rheology	46, 48
	Elongational rheology	49
	Conductivity	43, 59
	Surface tension	60
	Interfacial tension	50
	Optical and fluorescence microscopy	15, 18
Nanofiber morphology and drug distribution	Scanning electron microscopy	18, 24, 53, 54
	Field-emission scanning electron microscopy	56
	Environmental scanning electron microscopy	54
	Transmission electron microscopy	24, 56
	Optical light or fluorescence microscopy	15, 18
	Atomic force microscopy	61
	Energy dispersive X-ray spectroscopy	56
	Electron spectroscopy for chemical analysis	56
Solid state analysis and interactions between nanofiber components	X-ray diffraction	56
	Differential scanning calorimetry	18, 54
	Attenuated total reflectance Fourier transform infrared spectroscopy	18, 54
Porosity	Capillary flow porosimetry	56
	Mercury porosimetry	62
	Scanning electron microscopy images	63
	Micro-computed tomography	64
Hydrophobicity	Water contact angle	24, 53, 54, 65, 66
Permeability	Permeation test	56
Mechanical properties	Atomic force microscopy	65, 67
	Tensile testing	24, 56, 66
Release studies	Pharmacopean apparatus	68
	Modified release tests	69–72

rheology enables even better correlation between the rheological characteristics of the interface and the nanofiber morphology, as this gets closer to actual electrospinning conditions (46, 48). In addition, elongational rheology is a relatively new method for determina-

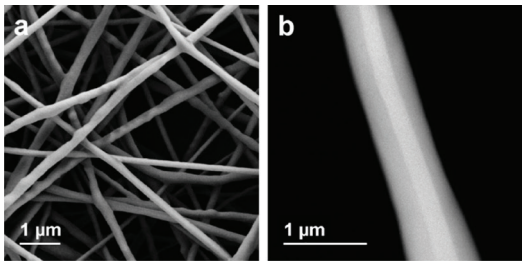

Fig. 4. Representative images of core-shell nanofibers obtained by: a) scanning electron microscopy and b) transmission electron microscopy.

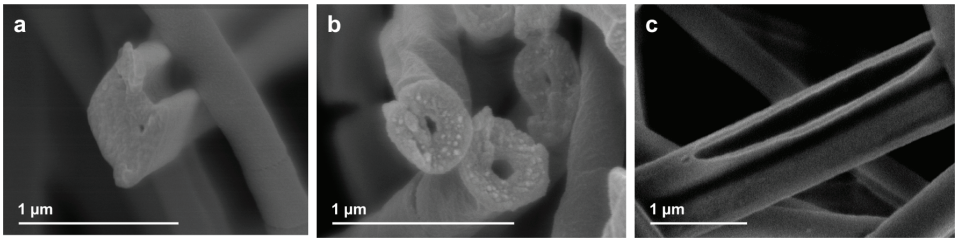

Fig. 5. Scanning electron microscopy images of core–shell nanofibers, where ciprofloxacin was incorporated into a poly(vinyl alcohol) core, with the shell formed with poly(methyl methacrylate) to prolong drug release: a) before and b, c) after ciprofloxacin release. The study is presented in detail in (18).

tion of viscoelasticity; it is based on uniaxial elongational flow that results in self-thinning threads. Elongational rheology can be used to predict solution spinnability and to differentiate between the rheological properties of different polymer solutions (49). Also, special attention needs to be paid to the interfacial tension, which can be measured by a contact-angle system using the pendent droplet method, since it influences the stability of the core-shell jet of two immiscible solutions (50). Phase separation of two immiscible polymers and the size of drops in the emulsion can be determined using optical and fluorescence microscopy (15, 18).

Nanofiber morphology

Nanofiber morphology affects the nanofiber performance, including their drug-release kinetics (51) and the responses of cells to the scaffold (52). Physical characterization of nanofibers in terms of their structure and morphology is usually carried out using electron microscopy. Geometric properties of nanofibers, including their diameter, diameter distribution, orientation, and morphology (*e.g.*, cross-section shape, surface roughness) are most frequently examined using scanning electron microscopy and its variations (Fig. 4a) (18, 24, 53, 54). Cross-sections are used to check the core-shell structure, particularly before and after a particular treatment that can remove the core (*e.g.*, heating, immersion in water) (Fig. 5) (18, 55, 56). Transmission electron microscopy is an alternative for measuring the diameters of extremely small nanofibers (< 300 nm) due to the better resolu-

tion obtained. Indeed, transmission electron microscopy is commonly used to determine the internal structure of core-shell nanofibers, especially to demonstrate the core-shell morphology (Fig. 4b) (32, 57, 58). However, studies often provide only one image of their core-shell nanofibers, which might not be a true representation of all of the nanofibers in a nanofiber mat. With microfibers, the core-shell structure can be also seen using light or fluorescence optical microscopy (15). Fluorescence microscopy, energy dispersive X-ray spectroscopy, and electron spectroscopy for chemical analysis can be also used for determination of the distribution of a drug, dye, or metal nanoparticles incorporated into core-shell nanofibers (56). Atomic force microscopy can be used to visualize the three-dimensional structure of the nanofiber mat morphology, single nanofiber and for nanofiber swelling rate in an aqueous medium (1, 6), although the process of obtaining an accurate measurement is relatively difficult due to tip convolution (2).

Understanding of the solid states of the drug and polymer incorporated into nanofibers can contribute to easier explanation of the drug-release kinetics and their mechanism. For the polymer, drug, and other excipients, crystallinity within the nanofibers can be examined using X-ray diffraction and differential scanning calorimetry, while attenuated total reflectance Fourier transform infrared spectroscopy can be used to determine interactions between the drug and excipients (2, 32, 58).

Porosity of nanofiber mats

Nanofiber porosity is another geometric parameter important to tissue engineering. Pore size measurements can be made using scanning electron microscopy images (63), capillary flow porometry (26), and micro-computed tomography (64, 73), and also using mercury porosimetry (62). Electrospun nanofiber mats are usually highly porous structures with >90 % porosity, total pore volume ~10 mL g^{-1}, total pore area of 23 $m^2 g^{-1}$, and usually nonordered pores with diameters from 2 μm to 465 μm (2). Limitations of mercury porosimetry are the possible collapse and compression of the scaffold during measurements, so this is not advised for electrospun scaffolds comprising fibers with diameters < 3 μm (62, 73). Other methods for measuring nanofiber mat porosity include calculations using the density of the material and the volume of the nanofiber mat (10). Alternatively, porosity can be calculated on the basis of scanning electron microscopy image analysis, where the data can be only used for comparisons between nanofiber mats developed and characterized within a single study. This limitation applies because measurements depend on the contrast and brightness of the images obtained, and the number of layers being analyzed (63). Quynh P. Pham *et al.* compared the porosity of fiber mats using three techniques: mercury porosimetry, liquid intrusion, and gravimetry (62). They showed no statistical differences between these measures. An excellent review of the advantages and limitations of different measurement techniques for porosity was written by Ho and Hutmacher (73).

Hydrophobicity of nanofiber mats

Hydrophobicity of nanofiber mats has a key role in the determination of their overall performance; in particular, hydrophobicity can affect drug release (74–76), degradation of the polymer matrix in aqueous media (77), and cell adhesion, proliferation, and penetration into nanofiber mats (65, 78, 79). Nanofiber mats formed from hydrophobic polymers are usually more hydrophobic compared to polymer films from the same polymers due to

the air captured in the pores between the nanofibers (76, 80). Hydrophobicity is most often determined using the water contact angle (53). In addition, this can detect the surface composition of core-shell nanofibers, especially if a hydrophilic core is incorporated into a hydrophobic shell. With complete core incorporation, the water contact angle does not change, or changes minimally, while the diffusion of the core hydrophilic components to the nanofiber surface reduces the water contact angle (35, 53, 66).

Permeability of nanofiber mats

Nanofiber mats are also studied as physical membranes that can be introduced during tendon or periodontal surgery to create a protective shield between the tendon/periodontium and its surrounding tissues (56, 81). An efficient membrane should prevent fibroblast penetration but still allow nutrient transport through the micron-sized pores. To measure the permeability of nanofiber mats, a permeation test can be performed using a side-by-side permeation chamber and bovine serum albumin as the permeation molecule (56).

Mechanical properties

Mechanical properties of a nanofiber matrix are crucial for biomedical applications such as scaffolds, because a mat must withstand the forces exerted by growing tissue and the physiological activity related to biomechanics, *e.g.*, pulsed blood flow (2). In addition, the mechanical properties of nanofiber mats that resemble the extracellular matrix *in vivo* provide better material performance for tissue regeneration (65). Mechanical characterization involves a variety of approaches, which include nano-indentation, bending tests, resonance frequency measurements, and microscale tension tests (2). Atomic force microscopy can be used for the determination of Young's modulus of a single nanofiber (1, 6, 65).

Drug release studies

Drug release is one of the most important tests for evaluation of novel nanodelivery systems, especially if it predicts the *in vivo* performance at the application site (82). As nanofiber systems do not have their own monography in the European Pharmacopoeia, the standard dissolution method for solid dose forms using a dissolution apparatus such as 'Apparatus 1' can be used to perform release studies (83). Many drug-loaded nanofiber systems have been investigated under 'sink' conditions using smaller volumes (< 500 mL as the minimum suggested in the European Pharmacopeia) and various stirring or shaking devices (69–72). These modified tests can be chosen to resemble more closely the *in vivo* conditions or for practical reasons such as needing lower nanofiber mass for the release test, or problems with the availability of Apparatus I if release studies are longer than a few days or weeks.

CORE-SHELL NANOFIBERS AS DRUG DELIVERY SYSTEMS

Drug release parameters have been investigated for a number of drugs incorporated into monolithic, blended, or core-shell nanofibers. Polymers used for biomedical applications must be biocompatible, with bioadhesive and biodegradable properties usually desir-

able (1). Material selection is of critical importance for the production of such nanofibers, because it affects their morphology, biocompatibility, mechanical strength, degradation rate, and release profile, and also their interactions with cells, which can result in a range of tissue responses (83, 84). Here, some functional properties of core-shell nanofibers as drug delivery systems are presented.

Immediate release of poorly water-soluble drugs

In drug discovery, 70 % of new drug candidates have low aqueous solubility, which results in poor oral bioavailability. The need to improve drug bioavailability by enhancing the solubility and dissolution rate is one of the important challenges to solve in pharmaceutical technology (85). Nanofibers represent a promising nanodelivery system for this application, since their characteristics include high surface-to-volume ratio, high porosity of nanofiber mats, nanometer diameters, and possible drug amorphization due to the rapid solidification process (1), which has been already shown for blended poly(ethylene oxide) (PEO)/poloxamer nanofibers loaded with carvedilol (86). In addition, coaxial electrospinning assists in the preparation of solid dispersions of core-shell nanofibers, with simultaneous incorporation of a poorly water-soluble drug and different functional ingredients, which can improve the dissolution and permeation properties of the incorporated drug. Yu *et al.* (87) showed that using polyvinylpyrrolidone core-shell nanofibers, acyclovir as a model drug can be incorporated into the core, while sodium dodecyl sulfate (a transmembrane enhancer) and sucralose (a sweetener) can be incorporated into the shell. These core-shell nanofibers can rapidly release the acyclovir (< 1 min), with a > 6-fold increased permeation rate compared to acyclovir powder, as shown by *in vitro* dissolution and permeation studies. A slightly different concept was shown in a study of Li *et al.* (88), who prepared core-shell nanofibers with tamoxifen citrate or quercetin, with sodium dodecyl sulfate and poly(vinyl pyrrolidone) K90 as the shell and poly(*ε*-caprolactone) (PCL) as the core. Shell thickness was < 100 nm and thus presented an extremely thin diffusion boundary layer, which increased the dissolution rate of incorporated drugs. Nanofibers that promote rapid release, especially for poorly water-soluble drugs, have the potential to be used as solid dispersions and oral rapid disintegration drug delivery systems (89).

Delayed drug release

The most common examples of delayed drug release are the delivery systems that can ensure drug release in the upper part of the small intestine after the formulation has passed the stomach. Such delivery systems are also known as gastro-resistant and they usually contain polymers that are insoluble at low pH and that can trigger drug release at higher pH, due to their increased solubility under these conditions (90).

Co-axial and emulsion electrospinning have been used to prepare gastro-resistant nanofibers, with the aims to protect acid-sensitive drugs/proteins from the low pH and enzymes in the stomach (91), to provide local treatments of colon diseases (*e.g.*, for delivery of 5-fluorouracil, mebeverine hydrochloride, indomethacin) (92, 93), and to image colon abnormalities using a contrast agent (93, 94). Jia *et al.* used core-shell fibers with a drug-loaded core based on mucoadhesive PEO and a shell based on a pH-sensitive copolymer derived from esters of acrylic and methacrylic acid polymer (*i.e.*, Eudragit S100). This

effectively delayed the release under acidic conditions, as representative of the stomach, whereas when transferred to pH 7.4, there was sustained release up to 22 h. They predicted that after dissolution of the shell, the fibers would adhere to the walls of the intestinal tract and provide sustained local drug release due to the mucoadhesive nature of the PEO from the core (93).

Various examples of monolithic or blended nanofibers that can or cannot delay drug release can be found in the literature (68, 95–97), and although some gastro-resistant core-shell nanofibers have been developed (91, 93), they are not a general solution for all drugs (92). Thus, delayed release does not depend only on the composition, structure, presence of drug on the nanofiber surface, or defects in the shell of the nanofibers, but also on the drug properties, including its solubility at low pH, molecular weight, and interactions with the polymer (18, 68, 92, 95–97).

To sum up, gastro-resistant nanofibers with higher amounts of small hydrophilic drugs incorporated are more challenging to develop compared to the lower loading of nanofibers with larger hydrophobic drugs.

Prolonged drug release

Controlled drug delivery systems that release a drug over a prolonged period can improve therapeutic outcomes through reduction of drug toxicity, increased efficiency, and reduced dosing frequency, all of which will also promote patient compliance (98). An initial burst release often occurs with the use of monolithic and blended nanofibers (51, 99), especially if the drug crystalizes in the nanofibers or is distributed on or near the nanofiber surface (51, 76, 100). The development of core-shell structured nanofibers can reduce this burst release, with prolonged release with minimal burst release shown (18).

Polymers that are often used for prolonged drug release are generally hydrophobic, such as PCL, poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), zein, cellulose acetate and PMMA (18, 44, 99, 101). These polymers can only be dissolved in organic solvents, where some hydrophilic drugs are insoluble (102). In such cases, emulsion or coaxial electrospinning allow incorporation of hydrophilic drugs in the core using water-in-oil emulsions or aqueous core solutions and shell solutions prepared in organic solvents. For example, the hydrophilic drug metoclopramide hydrochloride was successfully incorporated into different core-shell fibers with PVA and the drug as the core and PCL, PLA and PLGA as shell polymers. More prolonged drug release was shown in the case of core-shell nanofibers compared to monolithic nanofibers made from the same polymer (99).

The release rate of a hydrophilic drug can be controlled by varying the physical and chemical properties of the core and shell solutions (18, 99). Each hydrophobic polymer used provides slightly different release kinetics (99). Drug release can be tailored by varying the flow rate ratio between the core and shell solutions. Co-axial electrospinning with the core-shell flow rate ratio of 1:5 resulted in core-shell nanofibers with fewer imperfections, which can include an incomplete shell (for *e.g.*, see Fig. 5c), or cracks and open ends, and longer release (30 days) compared to a flow rate ratio of 1:3, which showed release over 1 day (18). Similar effects of the flow rate ratios between cores and shells have also been confirmed by other studies (54). Drug release can be additionally controlled by blending hydrophilic and hydrophobic polymers in the core (18).

In some cases, two-phase drug release can be desired, especially when an initial burst release can relieve symptoms as soon as possible, with prolonged drug release then providing the required effects for several more hours or days. These release kinetics are especially advisable for nonsteroidal anti-inflammatory drugs, antihistamines, and anti-psychotics (103). Jiang *et al.* (44) reported 12 h biphasic release of ketoprofen encapsulated in both a fast releasing shell of polyvinylpyrrolidone and a prolonged releasing core of zein. For peripheral nerve tissue engineering, lycium barbarum polysaccharide was incorporated into core-shell nanofibers, where the core solution was an aqueous solution of this polysaccharide and the shell solution was from PLGA. Two stages of release kinetics were shown, with a fast burst release (in the first 7 days) and sustained and constant release for the following 53 days (66).

Core-shell nanofibers also enable incorporation of two or more drugs, as seen for example for core-shell nanofibers composed of a core of ibuprofen, hyaluronic acid, and PEO, and a shell of polyethylene glycol, PCL and Ag nanoparticles. All active ingredients (*i.e.*, ibuprofen, hyaluronic acid, Ag nanoparticles) were slowly released over 20 days. The development of this multi-functional anti-adhesion nanofiber mat can reduce fibroblast attachment and penetration while simultaneously preventing post-surgical infection and inflammation (56).

CORE-SHELL NANOFIBERS FOR INCORPORATION AND RELEASE OF BIOPHARMACEUTICALS

Biopharmaceuticals are defined as protein- or nucleic-acid-based pharmaceutical substances that are used for therapeutic or *in vivo* diagnostic purposes and are produced by means other than direct extraction from a native (non-engineered) biological source (104). The biopharmaceutical market is rapidly increasing, and it is now the fastest growing segment of pharmaceuticals, due to their specific therapeutic effects (105). However, formulations of proteins still remain a major challenge, since they are prone to chemical and physical degradation, such as oxidation, deamidation, hydrolysis, conformational changes, undesirable adsorption to surfaces, precipitation, and aggregation (106). The problem of protein instability in aqueous solutions can be solved by freezing or drying because proteins in the solid state are more stable than the same substances in solution (107–109). Electrospinning is a promising method, which in one step enables drying of proteins and formulation of a dosage form. Several advantages of core-shell nanofibers over monolithic or blended nanofibers have been shown in terms of protein delivery (110). Coaxial electrospinning of core-shell nanofibers can reduce to a minimum the direct contact of bioactive agents in an aqueous core solution with the potentially dangerous solvents of a shell, leaving only the core-shell interface (38). Jiang *et al.* implied that the coaxial-electrospinning process had no detectable negative impact on either the structure or stability of lysozyme (111), whereas Ji *et al.* reported reduced activity of alkaline phosphatase after coaxial-electrospinning (110, 112). With emulsion electrospinning, small morphological changes in lysozyme were observed, which indicated some aggregation with composite fibers with higher protein loading, which was probably due to inefficient encapsulation of the protein within the fibers. In addition, the emulsification procedure resulted in a 16 % reduction in protein activity, while electrospinning did not show any further increased loss of activity (32). Similarly, Frizzell *et al.* reported reduced protein activity after emulsion electrospin-

ning, whereas electrospinning at a higher flow rate and inclusion of PVA increased the recovered bioactivity from 90 to 100 % for horseradish peroxidase, and from 60 to 75 % for alkaline phosphatase (91). It appears that protein stability during the emulsion or coaxial electrospinning processes depends on many different factors, which include the protein properties, the composition of the emulsion or core and shell solutions, and the processing parameters. In addition, it is difficult to compare various studies due to different analytical methods used, with some methods being more sensitive for the detection of protein degradation than others.

Controlled protein release is needed for several biomedical applications, such as the delivery of growth factors. Core-shell nanofibers enabled sustained release of growth factors over 30 days and decreased the burst effect of the released growth factors compared to blended nanofibers (58). Encapsulated platelet-derived growth factor-bb and lysozyme were released from core-shell nanofibers over 20 days, and they maintained high bioactivities over this period (32, 36). Platelet-rich plasma is a natural source of growth factors, and it has been successfully incorporated into blended chitosan/PEO nanofibers, with release over 1 day (113). More recently, platelet-rich plasma was also incorporated into core-shell nanofibers together with PVA in the core, with the shell composed of silk fibroin and PCL (11).

In some cases, a hydrophobic polymer can form an intact shell, and the active component cannot be released. To overcome this obstacle, porogens (*i.e.*, materials that rapidly dissolve in water to open pores, such as (poly)ethylene glycol) have been added to the shell (36) or biodegradable shells have been used (32). Drug release can then be regulated by the amount and type of porogen used (36).

Protein can be released through different shell imperfections (*e.g.*, cracks, pores, incomplete shell) (38), which more frequently occur when there is a higher volume ratio of aqueous to organic phase in the case of water-in-oil emulsion electospinning (27), and a higher ratio of core to shell solution flow rate in the case of coaxial electrospinning (18). If an incomplete core-shell structure is observed and some unencapsulated protein located close to or loosely associated with the fiber surface, the protein is likely to be released immediately (27).

Core-shell nanofibers have also been shown to be a promising option for gene delivery, especially for tissue engineering (31, 114). Plasmid DNA (pDNA) together with poly(ethylene glycol) was incorporated into the core of core-shell nanofibers using coaxial electrospinning. The shell consisted of the non-viral gene delivery vector poly(ethylenimine)-hyaluronic acid and PCL. Complexes of pDNA with poly(ethylenimine)-hyaluronic acid were released over 60 days from core-shell nanofibers and successfully transfected cells and induced the expression of enhanced green fluorescent protein (114). Yang *et al.* successfully incorporated either pDNA or pDNA and poly(ethylene imine) polyplexes into the core of core-shell nanofibers using emulsion electrospinning. Poly(DL-lactide)-poly(ethylene glycol) formed the shell, with poly(ethylene glycol) as the porogen to enhance the release of pDNA polyplexes, which were released over 25 days (31).

CORE-SHELL NANOFIBERS FOR INCORPORATION OF PROBIOTICS

Probiotics are live microorganisms that are beneficial for the health of the host and represent a new way of combating various infectious diseases (115). Electrospinning has been shown to be a promising single-step process to dry such bacteria and incorporate

them into a delivery system (116, 117). Lopez-Rubio *et al.* successfully incorporated *B. animalis* Bb12 into PVA microfibers using coaxial electrospinning, without any changes in viability before or after the electrospinning process (118). The advantage of coaxial electrospinning here is that the effects of the electric charges in the core (where the bacteria are) were lowered due to the rapid escape of the charge to the outer surface of the shell at the very beginning of the core-shell jet formation (38).

CONCLUSIONS

The greatest advantage of emulsion and coaxial electrospinning is their versatility in terms of the types and sizes of the core-shell nanofibers that can be developed. They have several advantages over monolithic nanofibers, *e.g.*, they can provide incorporation of water-soluble drugs or organic-solvent-sensitive proteins into nanofibers from hydrophobic polymers, improved drug stability, more complete drug encapsulation and greater control of the release kinetics due to a number of variable parameters. Changes in the shell and core material properties relate to variations in molecular weight, polymer type and porogen substances used, which can fine-tune the drug-release profiles and drug stability. However, fabrication of core-shell nanofibers includes several known and unknown parameters, which can be challenging in nanofiber preparation and result in low reproducibility. On several occasions, different laboratories were not able to produce similar core--shell nanofibers. Also, analytical techniques for nanofiber characterization are often time consuming and are not straightforward. In addition, in some cases, drug release from monolithic and core-shell nanofibers is very similar, and thus there is no special advantage of using the more complex processes.

Additional studies are needed in the future to better understand the effects of multiple parameters on the electrospinning process, nanofiber morphology, drug distribution in core-shell nanofibers, drug release, and *in vivo* effects. Use of monolithic and blended nanofibers in nanomedicine will be the first to arrive on the market, due to their easier production, higher reproducibility and already developed scale-up processes. Later, core-shell nanofibers will emerge for biomedical cases, where monolithic or blended nanofibers cannot offer such functionality with regard to drug/protein/probiotic incorporation, stability and the desired release kinetics.

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