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REVIEW

Electrospun Antibacterial Nanofibers: Production, Activity, and *In Vivo* Applications

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ABSTRACT: Electrospinning is an economical and relatively simple method to produce continuous and uniform nanofibers from almost any synthetic and many natural polymers. Because of the high specific surface area, tunable pore size, and flexibility, the nanofibrous membranes are finding an increasingly wide range of applications. Some particular attention has been devoted to antibacterial nanofibers for applications such as wound dressings. A variety of biocides, e.g., antibiotics, quaternary ammonium salts, triclosan, biguanides, (silver, titanium dioxide, and zinc oxide) nanoparticles and chitosan have been incorporated by various techniques into nanofibers that exhibit strong antibacterial activity in standard assays. However, the small diameters of the nanofibers also mean that the incorporated biocides are often burst released once the materials are submerged in an aqueous solution. Nevertheless, several strategies, such as core-sheath structure of the nanofiber, covalent bonding of the biocide on the fiber surface and adsorption of the biocide in nanostructures, can be utilized to sustain the release over several days. This review summarizes recent development in the fabrication of antibacterial nanofibers, the release profiles of the biocides and their applications in *in vivo* systems. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40797.

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INTRODUCTION

Polymer nanofibers, an important class of nanomaterials, have been attracting increasing attentions in the last 15 years or so. Nanofibers generally refer to fibers with diameters less than several hundred nanometers, although those with a diameter less than 1 μm are also broadly regarded as nanofibers. As the diameter of the fibers reduces from 12–20 μm (i.e., in cotton, wool, and conventional synthetic fibers) to less than 1 μm, the specific area of the material increases exponentially.[1] This intrinsic feature makes nanofibers attractive for many applications where high specific surface area is highly desirable or necessary.

Fabrication methods to produce nanofibers have been widely explored. Several techniques such as melt blowing and force-spinning,[2] template synthesis,[3] and electrospinning,[1,4,5] have been reported to produce suitable polymer nanofibers for different applications, with electrospinning being the most popular. Electrospinning has been known since 1930s but only gained widespread recognition from mid 1990s when the term electrospinning was coined.[6] Numerous synthetic and natural polymers have been successfully electrospun.[1,4,5,7] Such popularity is due to its simplicity, cost-effectiveness in the process, and its applicability to seemingly any synthetic polymers and many natural polymers (e.g., proteins and carbohydrates) to produce continuous and uniform nanofibers. In addition, electrospinning appears to be the only method that can be scaled up for industrial productions. And indeed, several companies, including Inovenso and Elmarco, have recently been manufacturing and marketing industrial scale electrospinning machines.[8]

In a basic laboratory setup, an electrospinning apparatus includes a syringe with a metal needle (or spinneret) mounted on a syringe pump, a high voltage power supply that is connected to the needle, and a metal collector plate. The polymer, together with any additives such as antibiotics, is dissolved in a solvent at a suitable concentration and loaded into the syringe. During the electrospinning process, the polymer solution is slowly pushed to the needle tip by the syringe pump. The electrical field provided by the high power supplier induces charges within the polymer solution at the tip and causes a jet of the polymer solution to fly towards the collection plate and form nanofibrous membranes.[5] A slightly more complex variation of this simple setup is the coaxial electrospinning where, by using two concentrical needles (or spinnerets), two different solutions are co-electrospun without being mixed to form a core/sheath nanofiber. The Spanish company Yflow has developed semi-large scale core-sheath electrospinning machines. Such structure

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can combine different properties of the two polymers into the same fiber, embed drugs in the core for slow release, or create a surface that is suitable for further functionalization.[9] Figure 1 shows a schematic setup of an apparatus and a typical SEM image of electrospun nanomembrane.

Nanofibers produced by electrospinning have found applications in many areas, including biomedical areas (e.g., scaffolds for tissue engineering, drug delivery, wound dressing, and medical implants), filtration, protective textiles, and battery cells.[1,7,10] Out of these, wound dressing is one of the most widely regarded applications.[11–13] An important role of the nanofibers is to prevent bacterial growth or infection. To this aim, a large body of work has been devoted in the last several years to the fabrication of antibacterial nanofibers by incorporating various antibiotics or biocides. In this review, we examine the recent development in the production of antibacterial nanofibers through electrospinning, the release of the antibacterial agents from the nanofibers, the efficacy of their antibacterial activity and their applications.

FABRICATION OF ANTIBACTERIAL NANOFIBERS BY ELECTROSPINNING

Fabrication of antibacterial nanofibres generally adopts the strategy of incorporating a biocide in the fibers. This can be achieved by evenly blending the active agent in the polymer solution prior to electrospinning, confining the active agent in the core of the fiber through coaxial electrospinning, encapsulating the active agent in nanostructures before dispersing them in the electrospinning solution, post-treatment of the fiber after electrospinning to convert a precursor to its active form, or attachment of the active agent onto the fiber surface (Figure 2). Various well-known active agents have used, including antibiotics, triclosan, chlorhexidine, QACs, biguanides, silver nanoparticles, and metal oxide nanoparticles.

Antibiotics

Kenaway et al. were one of the first to report the incorporation of an antibiotic in nanofibers through electrospinning for antibacterial nanofibers, although the antibacterial activity of the

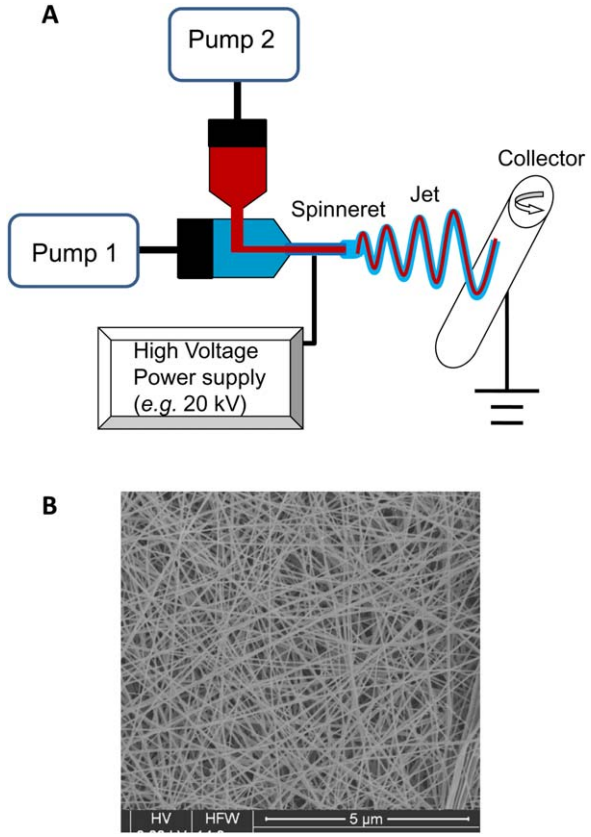

Figure 1. A: A schematic diagram showing a basic setup of electrospinning. In coaxial electrospinning, two pumps deliver two solutions without mixing them to the two concentrically aligned spinnerets to produce sheath/core structured nanofibers. B: A typical electron microscopy image of electrospun nanomembranes. The scale bar is 5 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

resultant fibers was not examined in their study.[14] Since then, numerous hydrophilic and hydrophobic antibiotics have been incorporated into various polymeric nanofibers by this simple method (see Table I). In general, the polymer is dissolved in an organic solvent such as DMF, chloroform, methanol, or hexafluoroisopropanol. The antibiotic agent is firstly dissolved in the same solvent or a different kind, and slowly added to the polymer solution while stirring to produce a homogeneous solution prior to electrospinning.

This method can accommodate a large range of amount of the antibiotic to be loaded in the nanofibers by adjusting the initial concentration of the drug in the electrospinning solution. The concentrations of antibiotics used have varied considerably. At the lower end, tetracycline was blended at 250–500 μg/mL with PCL and PLA solutions of 6–15% (wt/vol).[15] At these values, the antibiotic represented less than 1% of the polymer weight in the final nanofibers. In the higher range, 1% sodium cefoxitin was blended in PLGA fibers, 3.75–7.5% mupirocin in PLA fibers and 1–20% tetracycline hydrochloride in PLGA fibers.[16–18] Much larger quantities have also been reported, including up to 30% of Moxi in coPLA[19] and >30% tetracycline in PLA and PEVA.[14]

The inclusion of antibiotics in the polymer solution can have some effect on the electrospinnability of the polymer and the morphology of the nanofibers, due to the changes in viscosity, surface tension, and conductivity of the solution. For instance, sodium cefoxitin increased the conductivity and improved the electrospinnability of PLGA/PLA/PEG-b-PLA solution, enabled the production of more uniform nanofibers, and decreased the fiber diameter in a concentration dependent manner.[16] Similar results have been observed for moxifloxacin hydrochloride in coPLA solution.[19] The presence of 1–20% tetracycline hydrochloride in PLGA affected the fiber diameter but no clear trend could be concluded.[18]

To confer antibacterial activity while at the same time provide favorable physical properties in the nanomembranes, a two-stream electrospinning setup has been used to simultaneously produce two different kinds of nanofibers onto the same membrane. One stream contained the biodegradable PEUU while the other contained PLGA loaded with tetracycline hydrochloride.[18] The resulting composite sheets exhibited high elasticity, tensile strengths, and suture retention capacity, but markedly reduced shrinkage.

While mixing antibiotics in the polymer solution prior to electrospinning is a simple and versatile method to load large quantities of drugs into practically any polymeric nanofibers, the drawback is also obvious. That is, the antibiotics in the nanofibers tend to leach out rapidly in an aqueous solution, a phenomenon that has been termed burst release (see Section 3). Presumably, this phenomenon would reduce the effectiveness of the nanomembranes against bacteria once the releasable amount has reached below a critical level. Several strategies have been employed to provide more sustained release. One way is to use the coaxial electrospinning technology in which the outer solution contains the polymer and the inner solution contains the antibiotic. The polymer forms a sheath (or shell) to encapsulate the antibiotic component (the core) in the nanofibers. This technique has been used to encapsulate gentamycin in PCL[20] and PLA,[21] ampicillin in PMMA/nylon,[22] and tetracycline hydrochloride in PLLACL fibers.[23]

Another approach to achieve sustained release is to adsorb or encapsulate the drug in a nanostructure before dispersing it in the polymer solution. Amoxicillin was encapsulated in laponite nanodiscs or adsorbed on hydroxyapatite nanoparticles by dispersing the nanostructures in amoxicillin solution.[24,25] The drug-loaded nanostructures were then dispersed into PLGA solution for electrospinning. In these studies, the amounts of drug loaded to the nanodiscs or nanoparticles were 10–20% of the mass of the nanomaterials, and represented 0.5–1% of the polymer mass in the electrospinning solution. Such loading was substantially lower than those accomplished in the simple mixing method, but still provided strong antibacterial activity.

Biocides

Many biocides, including QACs, triclosan, chlorhexidine, and PHMB, have been developed for various industrial and household disinfection.[26] They are potent and broad spectrum biocides against both gram positive and gram negative bacteria, yet with low toxicity to humans. Most of these biocides have been applied to conventional fibers and textiles for antibacterial

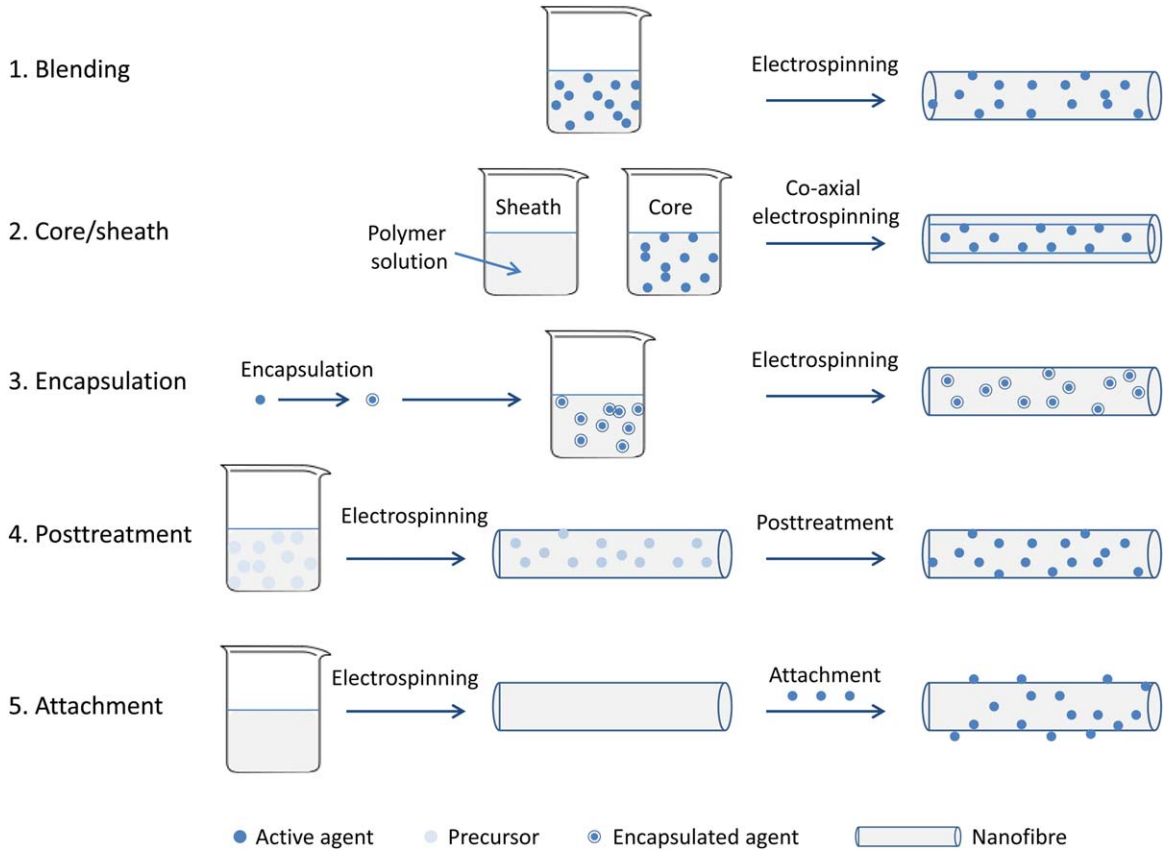

Figure 2. Various methods of incorporating biocides into electrospun nanofibers. 1, Blending/dispersion of the active agent in the polymer solution prior to electrospinning; 2, Confinement of the active agent in the core of the fiber through co-axial electrospinning; 3, Encapsulation/adsorption of the active agent in nanostructures before dispersion in the electrospinning solution; 4, Conversion of a precursor to active agent in the nanofibers after electrospinning; 5, Attachment of the active agent onto the nanofibers after electrospinning. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

finishing.[27] Given such wide applications, it is not surprising that several of them have been incorporated into nanofibers.

As with antibiotics, these small molecule biocides are typically resuspended in the polymer solutions prior to electrospinning. Benzalkonium chloride (10% relative to polymer weight) has been added to PLA or PLA/PEG solutions.[28] Similarly, 0.1–5% of a mixture of two QACs (*N, N, n, n,*-didecyl-*N,N*-dimethylammonium chloride and bis-(3-aminopropyl)-dodecylamine) were blended in 15% PAN.[29] These cationic substances greatly increased the conductivity of the electrospinning solutions and resulted in up to 20% reduction in fiber diameters, but did not significantly affect the crystallinity of the fibers. SEM imaging has showed that the drug was evenly distributed in the fibers, without drug crystals and aggregates. Triclosan of as much as 3% (w/v) was resuspended in PCL, PLA or their blends (10% w/v) (i.e., the drug accounted for up to 30% of the polymer mass).[30] The presence of such large amounts of triclosan in the solution did not affect the diameter of the nanofiber, although it tended to cause surface roughness. Triclosan has been complexed with the inclusion body β-CD before being electrospun into PLA nanofibers, and such complexation appeared to increase antibacterial activity of the membrane.[31] PHMB (10% relative to the polymer mass) has been electrospun in PEU or CA.[32] PHMB reduced fiber elasticity but did not significantly reduce the tensile strength. Potassium 5-nitro-8-quinolinolate (K5N8Q, 1% relative to polymer mass), a broad spectrum antibacterial and antimycotic agent, has been introduced to chitosan/PEO blend.[33] A cyclic *N*-halamine precursor (50% relative to polymer mass) has been added to PAN nanofibers.[34] While the precursor had no antibacterial activity, a treatment of the nanofibers with a dilute hypochlorite solution chlorinated the *N*-halamine compound and conferred the fibers with high antibacterial activity.

As with antibiotics, such simple mixing almost invariably results in a burst release of the active agents from the nanofibers in aqueous solutions. However, some of these biocides have functional groups in their structures that can be utilized for attachment to fiber surface to slow the release process. Chlorhexidine has been attached to CA nanofibers which had been electrospun with the aid of small amount of high molecule PEO.[35] Chlorhexidine was then crosslinked onto the surface using a crosslinker that reacted with the amino group in chlorhexidine and hydroxyl group in CA to achieve a yield of 7–9% (w/w). Similarly, PAN nanofibers have been chemically modified by reducing the nitrile groups to amino groups using lithium aluminium hydride in predried diethylether.[36] After activation

Table I. Some Representative Studies Where Antibacterial Nanofibers Have Been Produced Through Electrospinning and the Incorporation of Various Biocides

Electrospun Polymer	Antibacterial agents	Method of incorporation	References
	Antibiotics		
PLA, PEVA, PLA/PCL, PEUU/PLGA	Tetracycline	Mixing	14,15,18
PLGA	Cefoxitin	Mixing	16
PLA	Mupirocin	Mixing	17
coPLA, coPLA/PEG, PU	Ciprofloxacin	Mixing	19,88
PLAGA	Cefazolin	Mixing	89
PLGA	Amoxicillin	Mixing	90
PLA, PLA/Collagen, PCL	Gentamycin	Core/sheath	20,21
PLLACL	Tetracycline	Core/sheath	23
PMMA/nylon	Ampicillin	Core/sheath	22
PLGA	Amoxicillin	Adsorption/Encapsulation on nanostructures	24,25
	Nonantibiotics		
PCL/PLA	Triclosan	Mixing	30
PLA	Triclosan	Complexing with βg-CD	31
CA	Chlorhexidine	Mixing	35
PAN, PLA, PLA/PEG	QACs	Mixing	29,91
CA/PEU	PHMB	Mixing	32
PAN	PHMB	Covalent immobilization	36
PAN	*N*-Halamine	Mixing	34
PEO/Chitosan	K5N8Q	Mixing	33
PDLLA, PEO	Antibacterial peptides	Mixing	92
	AgNP		
PVDF, PVA/PU, Nylon 6, PVP, PLGA, PBS	AgNP	NP Dispersion	40–45,93
Nylon 6, PAN, PLLCL, PCL, PVA	AgNP	Synthesis in polymer solution	48–53,55,94–96
PLA, PCL, PAN, PVA, PEO	AgNP	*In situ* synthesis	49,58,71,82,83,116
PLA/Chitosan	AgNP	*In situ* synthesis	54,71
PEO/Chitosan	AgNP	*In situ* synthesis	82
PVA/chitosan	AgNP	*In situ* synthesis	83
PVA/chitosan	AgNP	NP Dispersion	84
PEO/Chitosan	AgNP	NP Dispersion	97
	Metal oxide NP		
PU, PVA, Silk fibroin	ZnO, TiO_2	Dispersion	63,64,94
PU	TiO_2	*In situ* synthesis	66
Nylon 6	ZnO	Electrospray on surface	67
PMMA	ZnO/TiO_2	Synthesis in solution	45
	Chitosan		
PLA, PVA	Chitosan derivatives	Blending	86,87,98
PET, PCL, PEO	Chitosan	Blending	73–76
PLA	Chitosan	Core/shell	99

of the amine groups with the bifunctional crosslinker glycerol diglycidyl ether, PHMB was then attached to yield the antibacterial product.[36]

Silver Nanoparticles (AgNP)

Silver (ions and metals) has long been known as an effective antibacterial agent and has been used in medical applications and food preservation.[37] The use of AgNP as an antibacterial agent have attracted particular interest, as the nanoparticles themselves may have antibacterial activity and their large surface area facilitates the release of the metal ions.[38,39] AgNP can be introduced to nanofibers at different stages, i.e., (1), by the blending of pre-synthesized AgNP to the polymer solution prior to electrospinning, (2), through *de novo* AgNP synthesis

in the polymer solution from a precursor, and (3), through *in situ* AgNP synthesis in the nanofiber after electrospinning.

Blending. The simplest and most commonly used method for producing AgNP incorporated nanofibers is by fully dispersing premade AgNP in the polymer solution prior to electrospinning. AgNP colloidal solutions are preferred. If nanoparticles powders are used, care should be taken that they do not form large aggregates in the solution. PVDF,[40] water soluble PVA/waterborne PU blends,[41] Nylon 6,[42,43] PLGA,[44] and PVP[45] have all been blended with AgNP in such a manner. Apparently, the AgNP is compatible with many solvents, as water, dimethylacetamide, hexafluoropropanol, and formic acid have all been used in the preparation of the solutions. The amount of AgNP can vary considerably from 0.5 to 5% of the polymer mass. The addition of AgNP increased the conductivity of the solutions, and consequently resulted in fibers with smaller diameters.[40,42] The AgNP were usually distributed evenly inside the nanofibers or on the surface, and could make the surface appear rough under electron microscopy, particularly when large amounts were used.[40]

Synthesis of AgNP in the Polymer Solution. While adding premade AgNP to a polymer solution before electrospinning is a straightforward way of producing antibacterial nanofibers, the method does require the preparation of the nanoparticles beforehand, and it could be an issue to fully disperse the AgNP in the polymer solution. Many studies have therefore reported a one-step preparation of the AgNP/polymer solution, using $AgNO_3$ as the precursor and the solvent as the reducing agent for AgNP synthesis. This in-solution synthesis produces a uniform dispersion of AgNP, partially due to the stabilization effect of the polymer.

The most explored system was PAN in conjunction with the solvent DMF. In an early study, hydrazine hydroxide was used to convert $AgNO_3$ to AgNP in the PAN/DMF solution.[46] It was later realized that DMF itself could serve as a reducing agent, albeit at a slow rate, to allow the synthesis at ambient conditions without using any additional chemicals. A solution of PAN and $AgNO_3$ in DMF was simply aged at ambient conditions for up to 10 days for the AgNP synthesis to complete.[47,48] However, heating the PAN/$AgNO_3$/DMF solution by refluxing at 80–90°C for up to 2 h,[49,50] exposing it to a xenon arc light for 15 min[51] or UV light for 10 min,[48] atmospheric plasma treatment for 5 min[52] or the inclusion of β-cyclodextrin in the solution,[50,53] all appeared to accelerate the synthesis process. In most of these cases, the concentrations of $AgNO_3$ were in the vicinity of 0.5–1% of the mass of PAN. The AgNP formed had narrow distributions in diameters around 10 nm, and were evenly distributed in the nanofibers or on the surface after the electrospinning process.

Other systems have also been used to synthesize AgNP in the polymer solution at ambient conditions. An aqueous PVA/CM-chitosan solution containing $AgNO_3$, in which carboxymethyl-chitosan acted as the reducing agent, was stirred for 12 h to produce AgNP.[54] AgNP was formed in Nylon 6 solutions using formic acid or formic acid/methoxy poly(ethylene glycol) as the solvent and reducing agent after stirring for 24 h.[55,56]

Post-Treatments of Fibers. Finally, AgNP can be synthesized *in situ* in post-treatments of the nanofibers from $AgNO_3$ that has been included in the electrospinning solution. The most common post-treatment is heating the nanofibers. PVA/regenerated silk fibroin blend fibers have been heated at 155°C for 5 min or treated with UV for 3 h,[57] PLA fibers at 80°C for 48 h in a hydrogen atmosphere,[58] or PAN fibers at 160°C for 2 h[49] to induce the transition of silver ions to metallic metal. However, such post-treatments are not as effective as the synthesis of AgNP in the polymer solution.[49]

Silver can also be loaded to preformed nanofibers, either using AgNP or $AgNO_3$ as the precursor. Nylon 6 nanofibers were immersed in a solution of AgNP that had been coated with citric acid.[59] Under acidic conditions, hydrogen-bonding interaction between the amide groups in the nylon fibers and the carboxylic groups on the nanoparticles was able to hold the AgNP on the fiber surface. In a separate study, PAA/β-cyclodextrin nanofibers were briefly immersed in a 0.1*M* $AgNO_3$ solution to take up silver ions, and a subsequent immersion in a 0.1*M* dimethylamine borane solution converted the ions to metallic silver nanoparticles.[60]

Metal Oxide Nanoparticles

In addition to their well-known photocatalytic activity and UV light absorption, zinc oxide (ZnO), and titanium dioxide (TiO_2) nanoparticles also exhibit excellent antibacterial activity after or during UV illumination.[61,62] However, compared with AgNP, a major limitation of metal oxides is that they need UV treatment to achieve antibacterial activity, a condition that may be difficult to satisfy during the applications of the nanofibers (e.g., as biomaterials or filtration media). As in the case of AgNP, these metal oxide nanoparticles can also be introduced in nanofibers by several approaches. First, presynthesized ZnO or TiO_2 nanoparticles can be added to the polymer solution prior to electrospinning.[63,64] Second, metal oxide nanoparticles can be synthesized in the polymer solution from precursors. Nanoparticles of ZnO and TiO_2 were synthesized in PMMA solution by sequentially stirring the solution with the precursors of titanium isopropoxide and zinc acetate in DMF/acetic acid at 60°C for several hours.[65] In a slight variation, the precursor tetrabutyl titanate for TiO_2 nanoparticle synthesis was dissolved in PU solution. Rather than using a metal collector, the fibers were projected into a water bath at pH 4. Under the acidic condition, the precursor was hydrolysed and condensed to form TiO_2 nanoparticles of 30–60 nm in the fibers.[66] Up to 5% of TiO_2 could be incorporated into the nanofibers. Third, a dual electrospinning-electrospraying hybrid process has been reported to produce nanoparticle decorated nanofibers.[67] In this process, Nylon 6 nanofibers were electrospun onto a surface, and simultaneously ZnO nanoparticles were electrosprayed from a ZnO suspension onto the nanofibers. The amount of ZnO loading can be adjusted by tuning the flow rates in the electrospinning and electrospraying jets. The ZnO nanoparticles in the nanomembranes are solely located on the fiber surface and are exposed to the environment for immediate actions against pathogens.

Chitosan

Chitosan, the deacetylated derivative of chitin from the shells of crustaceans such as shrimps, crabs, and lobsters, has been found to inhibit the growth of microbes in a large body of work.[68]

This antibacterial ability, coupled with its nontoxicity, biodegradability, and biocompatibility, is facilitating chitosan's emerging applications in food science, agriculture, wound dressing, pharmaceuticals, and textiles.[69]

The inherent antibacterial ability of chitosan makes it possible to produce antibacterial nanofibers without the use of any biocides. However, due to its polycationic nature in solutions, chitosan cannot be fabricated easily into nanofibers by electrospinning.[70] Furthermore, pure chitosan nanofibers tend to be physically weak and susceptible to swelling in a solution unless stabilized e.g., by crosslinking with glutaradehyde.[70] To overcome such issues, chitosan has been blended with various other polymers to produce stronger antibacterial composite nanofibers. These polymers include PLA,[71] PCL,[72,73] PET,[74] and PEO,[75–77] PVA,[78–80] and nylon 6.[81] Chitosan in the composite fibers can range from 10 to 90% by mass. Under those conditions, uniform fibers were formed with fiber diameter in the range of 200–400 nm. A small amount of surfactant (e.g., Triton X-100) could be included in the solution to further improve the spinnability.[76]

Chitosan, or its derivatives, has frequently been used in combination with AgNP to further enhance antibacterial activity in the composite nanofibers with another polymer.[71,82,83] Synergistic effect between chitosan and AgNP in antibacterial activity has been observed.[84]

Given the low solubility of chitosan in aqueous solutions and organic solvents, it is usually dissolved in a strong organic acid, with trifluoroacetic acid, formic acid and acetic acid being the most commonly used.[70,71,73,74] Such volatile and corrosive acids may pose practical issues during electrospinning, especially on a large scale. Many studies have therefore converted chitosan into quaternized derivatives in order to increase its solubility as well as the antibacterial efficacy. Derivatives such as *N,N,N*-trimethylchitosan iodide,[85] *N*-butyl-*N,N*-dimethylchitosan iodide[86] and *N*-[(2-hydroxy-3-trimethylammonium)propyl] chitosan chloride[87] have been synthesized and have been electrospun with PLA or PVA into composite fibers in common solvents such as DMF, DMSO, or water.

RELEASE OF THE ACTIVE AGENTS FROM NANOMEMBRANES

For many applications, the release profile of the drug from the nanofibers is an important consideration. For instance, the release should preferably last at least a few days during the use of wound dressings.

In the manufacturing of antibacterial nanomembranes, the active agents are often conveniently doped in the polymer solution prior to electrospinning (Table I). In such fibers, the release of the agent in an aqueous environment was found to follow a biphasic profile: an initial burst release followed by a much slower process thereafter.[14–19,30,32] The high burst release can be ascribed to two reasons. First, the very small diameter and the high surface area in the nanomembranes provide short diffusion pathway and are conducive to mass transfer of the drug. Second, during electrospinning, the majority of cationic drugs (e.g., tetracycline hydrochloride) is likely to be localized on the surface of the fibers due to their ionic strength.[16] In such a spatial arrangement, the drug can easily be dissolved and released into a solution. This spatial mechanism may also explain the fast release of PHMB from PEU nanofibers.[32] PHMB is a small polymer with a MW of ~2500 Da, but is highly cationic.[100] Its incorporation in the fibers had originally been expected to result in some entanglement of the molecules in the fiber matrix and lead to its slow release. But instead, PHMB was almost instantaneously released from PEU nanofibers (up to 60% release in the first 5 min in water at 37°C).[32]

The type and composition of the polymer(s) and structure of the nanofiber can influence the release rate. PEVA fibers allowed more sustained release of tetracycline hydrochloride than PLA fibers or PEVA/PLA blends,[14] PLA fibers released triclosan more efficiently than PCL fibers,[30] and the lactidyl/glycolidyl unit ratio in the PLGA copolymer nanofibers had an effect on the release of tetracycline.[101] Adding a water soluble polymer to the electrospinning solution has great effects on the drug release. PLGA/PLA/PEG-*b*-PLA (80 : 5 : 15) blend fibers showed a more sustainable release profile of cefoxitin than the pure PLGA polymer.[16] This effect was attributed to the amphiphilic PEG-*b*-PLA block copolymer which may have complexed with the drug and entrapped it in the nanofibers. Such an effect appears to be dependent on the correct match of the hydrophobicity/hydrophilicity of the drug and the blending polymer. The inclusion of hydrophilic PEG to coPLA polymer (coPLA : PEG 70 : 30) transformed a hyperbolic release profile of three hydrophobic fluoroquinolone antibiotics into an almost instantaneous release profile.[19]

A few strategies, such as adsorption and encapsulation in nanostructures, have been adopted to minimize the burst phase [Figure 3(A,B)]. The antibiotic amoxicillin has been adsorbed on hydroxyapatite nanoparticles or laponite nanodiscs and then dispersed in PLGA solution for electrospinning.[24,25] Mesoporous silica and halloysite nanotubes have also been used to adsorb/encapsulate active agents in electrospun nanofibers for controlled release.[102,103] In an aqueous environment, the adsorbed drugs would have to first dissociate from the nanostructures to the PLGA matrix before being released to the outer liquid phase. This process significantly slowed the release rates and achieved a sustained period up to two weeks or longer.[24,25,103]

Physical confinement of the active agent in the core of the fibers through the method of co-axial electrospinning has also been utilized to control drug release [Figure 3(C,D)]. The containment of tetracycline hydrochloride in the PLLACL core reduced the burst release to only 10–20% and extended the release to over 160 h.[23] This was in contrast to simple blending in which 60–80% of the drug was released in the burst phase. Similarly, the confinement of ampicillin in the core/sheath structure of the PMMA/nylon nanofibers led to a very short burst release phase (6 h) followed by a gradual release phase over the next 30 days.[22] The encapsulation of gentamycin in PCL nanofibers almost totally eliminated the burst release phase and resulted in smooth release over 180 h.[20]

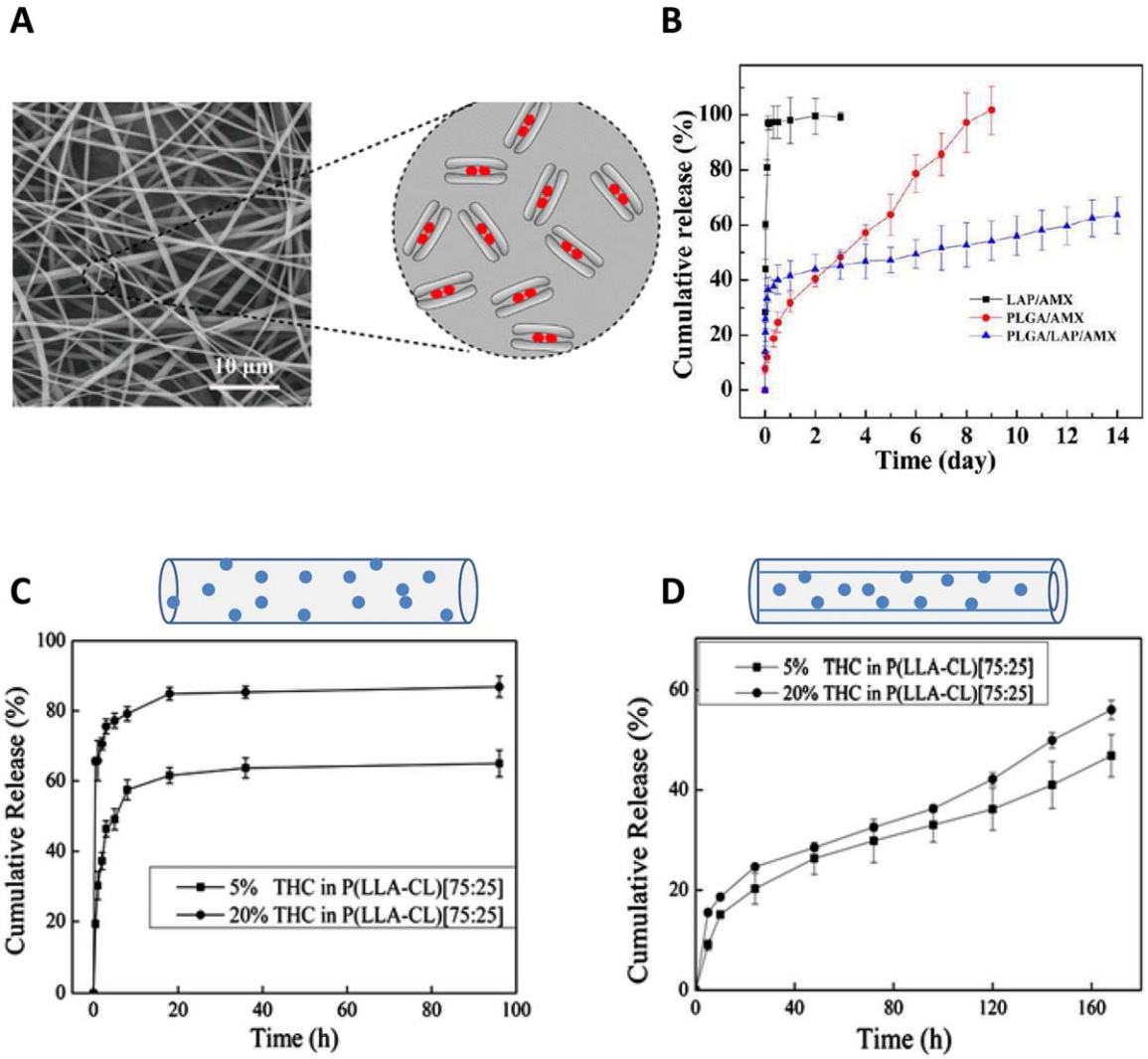

Figure 3. Controlled release of active agents from electrospun nanofibers through encapsulation in nanostructures (A, B) and core/sheath structure of the nanofiber (C, D). A, amoxicillin (AMX) was encapsulated in laponite (LAP) nanoparticles before being incorporated into PLGA nanofibers and B, the release profiles of AMX from the nanofibers.[24] C and D, the release of tetracycline hydrochloride (TCH) from blended and core/sheath structured PLLACA nanofibers, respectively.[23] Permissions to reproduce these figures granted by Springer and ACS Publications. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The release of silver from AgNP-loaded nanofibers appears to be slower and more sustainable than that of antibiotics and other small molecule biocides that have been simply blended into the polymers, perhaps due to the confinement of silver in the nanoparticles. Nevertheless, a burst phase, *albeit* longer (i.e., ~24 h), was still observed.[51,52] It was unknown whether the burst release was due to any residual $AgNO_3$ in the nanofiber from AgNP synthesis in the polymer solution. After this initial burst, the release became gradual and could sustain 6–10 days.[51,52,55]

EVALUATION OF ANTIBACTERIAL EFFICACY

The studies on the antibacterial efficacy of the electrospun fibrous materials have adopted established methods developed in the textile industry. These methods generally fall into three categories: the agar diffusion test, dynamic contact test and intimate contact test. The bacterial species *Staphylococcus aureus* (Gram positive) and *Klebsiella pneumoniae* (Gram negative) are recommended in most test methods. These two species are potentially pathogenic and therefore require proper physical containment facility in their handling. Many studies have instead used *Escherichia coli* (Gram negative) as the test microorganism which can be cultured and handled in a standard laboratory with minimal health risk.

Agar Diffusion Test

The semi-qualitative agar diffusion tests are exemplified by the AATCC 147-2004, the JIS L 1902-2002, and SN 195920-1992 methods. In practice, a dilute bacterial inoculum is spread or streaked on nutrient agar plates. The nanomembranes, typically in squares or circular discs of 10 mm, are firmly laid over the agar before the plates are incubated at 37°C for 18–24 h. The leachable antibacterial agent in the nanomembrane will diffuse

into the surrounding agar and inhibit the growth of bacteria (if its local concentration has reached the MIC) to form a zone of inhibition. The size of the zone is indicative of the level of antibacterial activity in the nanomembrane, and is affected by the potency of the antibacterial agent, the amount that has been leached into the agar and the rate of release. However, zone of inhibition should not be expected if the antibacterial agent in the sample cannot diffuse into the agar, such as the inherently antibacterial chitosan nanomembranes, or textiles on which the antibacterial agents have been durably attached.

This zone of inhibition method has been used to examine the antibacterial activity of electrospun nanomembranes loaded with various antibiotics such as mupirocin,[17] tetracycline,[15] and ciprofloxacin,[19] as well as AgNP.[48,49,60] A clear zone of inhibition in the order of 5–10 mm was often observed, indicating the effectiveness of the antibacterial agents leached into the agar during the incubation period.

Dynamic Contact Test

The test follows the guidelines of ASTM E2149-Determining the Antibacterial Activity of Immobilized Antibacterial Agents Under Dynamic Contact Condition. In principle, the antibacterial specimen is immersed in a dilute bacterial solution and shaken for a certain period of time. During this period, dynamic contact between the bacteria and the specimen will deactivate the bacteria. A small volume of the suspension is withdrawn at designated times for the determination of bacterial concentrations. This method was originally designed to measure antibacterial activity of non-leaching (immobilized and not water-soluble) antibacterial products or surfaces. But it has since been widely used for leaching products as well, due to the ease in the procedure. It should be noted, however, that if the biocide is leachable, the free biocide in the solution will probably play a more important role than the dynamic contact in deactivating the bacteria, particularly when the biocide is released early in a burst nature. This was indicated in a study where the nanomembrane had similar effects as the free drug at comparable concentrations.[16]

ASTM E2149 test method has been used to examine the antibacterial activity of nanomembranes loaded with various leachable agents, e.g., cefoxitin,[16] tetracycline,[23] amoxicillin,[24,25] PHMB,[36] triclosan,[30] chlorhexidine,[35] AgNP[42,52,54,56] as well as nonleachable chitosan blends.[71,98,99] These studies followed the guidelines of the method but the actual test conditions varied widely in terms of the amount of the nanomembrane used, the volume of the cell suspension and the cell suspension media (e.g., a rich media broth or a saline solution), all of which could have an influence on the assay results. Nevertheless, strong antibacterial activity, often in the order of >99% bacterial reduction, was reported in the nanomembranes.

The antibacterial assay for TiO_2 nanoparticle-based materials requires UV illumination. The selection of the UV illumination conditions and the inclusion of appropriate controls are critical to ascertaining that the biocidal effect is indeed arising from the TiO_2 in the material. ISO has developed a specific method for this purpose (ISO 27447, 2009). UV illumination can be performed during the incubation of bacteria with TiO_2-nanomembranes.[104] Alternatively, the TiO_2-nanomembrane can be UV-irradiated to activate the TiO_2 immediately prior to the antibacterial test.[64]

Intimate Contact Method

This type of test is exemplified by the AATCC 100-2004 (AATCC Technical Manual) which is designed for antibacterial examination of textiles under intimate contact conditions. Typically, a small volume (e.g., 0.1 mL) of dilute bacterial inoculum is fully absorbed into a small amount of test material to ensure the intimate contact between the material and the bacteria. After incubating the inoculated samples in humidified jars at 37°C for up to 24 h, the bacteria are eluted and counted by serial dilution and plating on nutrient agar plates. The method is best suited for nonleaching biocides on fibers where the biocides act from the outside of the bacterial cells. For example, textiles durably finished with QACs and PHMB have commonly adopted this method in the antibacterial assay.[88,105,106] When used on samples loaded with leachable biocides, the leaching biocides could reach high concentrations surrounding the cells and kill them a relatively short incubation time. This has been shown with PHMB-loaded PEU and PEU/CA blend nanofibers.[32]

Regardless of the antibacterial test methods used, it should be noted that the test materials (e.g., nanomembranes) are always in direct contact with the bacteria, or are confined in small volumes in which the bacteria are inoculated. As such, the active agents leached out from the nanofibers can quickly reach the MIC or lethal concentrations surrounding the bacteria. This may explain why strong antibacterial results are almost always obtained with the nanomembranes in the studies described above. However, such test conditions may not be found in the environments in which the nanomembranes are to be used, either as wound dressings, medical scaffolds or liquid filters. In these situations, the nanomembranes will likely experience large volumes of dynamic fluid (e.g., wound exudates, circulating blood, or filtration liquid), which will effectively elute or dilute the local concentration of the leached biocide.

While the antibacterial efficacy of the drug-loaded nanomembranes has been well reported, the kinetics of bacterial deactivation is also an important consideration. For some applications (e.g., in wound dressing), it is highly desirable that the nanomaterials kill bacteria quickly as well as sustain the action over a long period of time in order to control bacterial growth. The kinetics is affected not only by the properties of the nanomaterials (e.g., the amount and type of the biocide, the release of the biocide and the structure of the fiber), but also by the antibacterial test method used. Using the direct contact method, CA nanofibers loaded with silver ions or AgNP (0.5% or 1% on polymer wt) were found to kill *E. coli* quickly, with a killing rate of >99% after a contact time of 5 min, and 100% after 30 min contact.[107] This kinetics is very similar to that reported in conventional microfibers coated with PHMB.[100] Using the dynamic contact method described above, PBS nanofibers containing 0.29% AgNP killed most of the bacterial cells only after 3-h incubation.[93] An interesting kinetics was observed in another study in which 10 mg of PVA/chitosan/AgNP

nanofibers were incubated with 50 mL *E. coli* solution under dynamic contact conditions. The nanofibers were able to kill ~99% of the bacteria in the first 8–12 h. However, such ability diminished with time and by 24 h, cells started growing again, presumably due to depletion of silver in the bacterial solution.[54]

APPLICATIONS OF ANTIBACTERIAL NANOMEMBRANES

Although nanofibrous membranes have been suggested for a wide range of applications,[1,4,7,70,108,109] the applications of antibacterial nanofibers is largely focused to biomedical materials (e.g., as wound dressing, implants and sutures) and filtration where antibacterial activity is a necessity or advantage.[11–13] However, it should be noted that the claimed applications for antibacterial nanofibers are largely based on *in vitro* studies which simply show the antibacterial activities of the nanomembranes. Studies on antibacterial nanomembranes in *in vivo* systems are far and few, and have not consistently demonstrated their effectiveness and advantages. This review examines some of these *in vivo* studies.

Filtration

Electrospinning allows the production of nanomembranes with small and tunable pore sizes suitable for filtration. A number of companies, including DuPont, Amsoil Inc. and Donaldson, have been producing electrospun nanofiber-based filter products for automobile fuel filter, liquid filtration, HVAC, and defence applications.

Biocide-containing nanomembranes have been suggested for antibacterial filters for sanitization or sterilization purposes, including polyurethane cationomer that contained quaternary ammonium groups,[110] CA, PAN, and PVC polymers containing AgNP[111] and blends of PCL-chitosan.[73] However, these studies presented little evidence that the membranes actually deactivated bacteria during filtration.

Daels et al.[112] analyzed the antibacterial performance of polyamide nanomembranes containing five different biocides and compared them with control nanofibers during filtration of hospital wastewater. Because of physical removal, the control nanomembranes could reduce bacterial numbers in the filtrate by 1.5–2 $\log_{10}$, whereas all the antibacterial nanomembranes caused 4–6 $\log_{10}$ reduction. Such results appeared to support the claim that antibacterial nanomembranes were more effective in removing or deactivating bacteria during filtration. However, it should be noted that the addition of a biocide in the electrospinning solution can often change the diameter of the nanofibers, and therefore the pore size and porosity of the nanomembranes. As such parameters were not compared in the nanomembranes, it is unknown whether the enhanced efficiency in the filtration was due to physical differences in the antibacterial nanomembranes (e.g., smaller pores).

Biomaterials

Only a limited number of studies have examined the performance of electrospun antibacterial nanomembranes *in vivo*, and these studies haven't conclusively demonstrated the effectiveness or advantage of the materials over their conventional counterparts.

Lui et al.[113] investigated the effect of antibacterial nanomembranes on wound healing in Sprague-Dawley rats. A variety of nanomembranes, some of which contained AgNP, were produced from the polymers PVA, PAN and PCL and PVDF. These membranes differed in thickness, density, porosity, and hydrophobicity, and were applied as wound dressings onto the wounds in the rats that had just been created by incision through the skin. Subsequent examinations found no direct relationship between the antibacterial activity of the wound dressing and the wound healing performance. Instead, wound healing was mainly influenced by the porosity, air permeability and surface wettability of the nanomembranes.[113] This conclusion was supported by a separate study, which made wounds in Wistar rats by steaming the skins at 99°C for 13 s and used pure PEU, antibacterial PEU (containing 1% PHMB based on polymer wt) and hydrophilic PEU/CA (4 : 1) composite nanomembranes as the wound dressing materials.[32] Again, the PEU/CA composite membrane, owing to its high wettability, good moisture retention and air permeability, produced the best healing. No clear evidence could be observed that the antibacterial membrane (i.e., the PEU-PHMB) improved the healing beyond that observed for the PEU membrane alone.[32]

Hu et al.[114] produced antibacterial nanofibers by blending PLLA with the antibiotic cefotaxime or creating a core-sheath structure during the electrospinning process. The fibers were subsequently braided into yarns and used as sutures on Sprague Dawley rats on which wounds were made by incision. Two commercial sutures, a PLLA and a silk, were included for comparison. The trial could not reach a clear conclusion that the antibacterial sutures were advantageous, as the commercial PLLA suture and the blended PLLA-cefotaxime suture performed equally well but better than the silk suture and core-sheath PLLA-cefotaxime suture in helping wound healing.

One early study examined the effect of antibacterial nanomembranes on the prevention of abdominal adhesion in female Wistar-Albino rats.[115] PCL nanomembranes were first prepared and then loaded with an antibiotic (Biteral). The antibiotic membranes, or plain control membranes, were implanted on one side of the abdominal wall while leaving the other side as a control. Macroscopical and histological analyses found that the antibiotic-embedded membranes significantly reduced postsurgery abdominal adhesion and improved the healing process.

Gilchrist et al.[116] examined the effect of antibacterial nanomembranes on the colonization of bacteria on titanium implants in Sprague-Dawley rats. Antibacterial PLGA nanomembranes (loaded with the biocides fusidic acid and rifampicin) or the controls were implanted alongside a titanium disk into pockets made in the dorsum of the rats. The rats were then injected with 10^8 CFU of Methicillin-resistant *Staphylococcus aureus* (MRSA) onto the surface of the titanium implant. After 7 days, it was found that the antibacterial nanomembranes were able to prevent the adhesion of bacterial to the titanium implant.

Sumitha et al.[117] examined antibiotic release from biodegradable PLGA nanomembranes in animals. The drug-loaded membrane was placed on bone defects created on New Zealand White rabbits, and the intralesion fluid was aspirated by

a syringe over a time course for the determination of amoxicillin concentration by HPLC. It was found the local amoxicillin concentration sustained above the MIC for 28 days. This profile was similar to that of *in vitro* studies where amoxicillin was gradually released from the membranes over more than 20 days, and suggested long-term effects of the drug-loaded material *in vivo*.

CONCLUSIONS AND FUTURE DIRECTIONS

Since the mid 1990s, great advances have been achieved in the further understanding of the electrospinning process, the development of specialized electrospinning techniques (e.g., coaxial electrospinning, dual spinneret electrospinning, and the combination of electrospinning with other techniques such as electrospray), and the emergence of industrial electrospinning machines. As a result, it is now possible to produce uniform nanofibers from most synthetic polymers and many natural polymers in the laboratory and some at industrial scales. Such ability has allowed the production of antibacterial nanofibers through the incorporation of biocides in the electrospinning solutions or the functionalization of the nanofibers after electrospinning. Numerous biocides, such as antibiotics, small molecule biocides and silver nanoparticles have been successfully incorporated into nanofibers. The antibacterial nanofibers can potentially be used as medical implants, scaffolds, wound dressings, or as filter media.

Despite the significant advances, several issues still need to be further addressed in antibacterial nanofibers. First, the production of antibacterial nanofibers, which to date is largely limited to the laboratory using simple setups (e.g., syringe needles) to produce small-sized nanomembranes, needs to be demonstrated on large scales. The inclusion of a biocidal agent generally does not hinder the electrospinning process in the laboratory. However, high throughput industrial machines are needleless, it is unknown whether the biocides, particularly ionic ones (e.g., tetracycline, QACs, and PHMB), will adversely affect the electrospinning process on these machines, and if yes, what modifications need to be made in order to produce uniform drug-loaded nanofibers.

Second, the slow and controlled release of the antibacterial agents from the nanofibers is an important consideration during the applications of the nanomembranes. In most studies to date, the active agents are simply blended in the polymer solutions prior to electrospinning. While this technique is simple and versatile to accommodate a large range of concentrations of the active agents, the resultant fibers tend to release the active agents in a burst fashion in solution. The current strategies to ameliorate the burst release, such as core/sheath structure of the nanofibers through coaxial electrospinning or multi-step chemical immobilization of the active agents on the fiber surface, may not be applicable to needleless industrial machines or are too costly to scale up. Nevertheless, semilarge scale equipment for core/sheath structured nanofibers has emerged (e.g., Yflow), and further development in specialized equipment and processes will likely increase the production rate and improve the drug release profile from the nanofibers.

Third, the biocide-loaded nanofibers generally show strong antibacterial activity when assayed in the laboratory under standard conditions. However, it should be noted that in such assays, the nanomembranes are either in direct contact with the bacteria or are confined in small volumes of bacterial solution. These conditions may be very different from the situation during the applications of the materials, where the nanomembranes are likely to experience large volumes of dynamic fluid which may effectively dilute or even wash away the biocide. In contrast to the numerous *in vitro* studies, few studies have examined the antibacterial performance of the nanomembranes during their applications, e.g., in reducing wound infection or deactivating bacteria during filtration. The limited number of studies carried out so far on the use of antibacterial nanofibrous materials as wound dressings, sutures, implants, and filtration media have not consistently demonstrated the effectiveness of the antibacterial materials. Further studies are needed if antibacterial nanofibers are to fulfill their perceived potentials.

In conclusion, great advances have achieved in electrospinning for the production of nanofibers in general and antibacterial nanofibers in particular. Nevertheless, more research and development is required if the potential of antibacterial nanofibers is to be fully realized.

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