

Antimicrobial Electrospun Materials

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Abstract

The fast-growing public health awareness and concern of the devastating problems with bacterial infections and the mounting resistance of bacteria to conventional antibiotic treatments have made this theme the top concern. At the same time the problem will not be solved through solely inventions of antimicrobial materials preventing the prevalence of bacteria resistance. Nevertheless, the fabrication and design of these materials are highly important to find its translational applications in our daily life. In this context, electrospun materials with their inimitable advantages and facile production make them a suitable candidate for various applications. The electrospinning technology represents a versatile and facile approach for the construction of ultrathin electrospun fibers from various materials. Then, it allows the fabrication of electrospun fibers with various and controlled dimensions such as nanosized fibers which have gained significant attention due to their valuable properties such as high surface area, large porosity, and lightweight. Through the combined electrospinning and antimicrobial material employment, a very powerful, robust, and vital strategy for engineered material can be generated. These materials can be employed in many areas such as healthcare (e.g., tissue repair, drug delivery, and wound healing), environmental application (e.g., filters and membranes), energy applications (solar and fuels cells), and in protecting clothing for medical and chemical workers.

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9.1 Introduction

The grand challenges with infections and the prevalence of antibiotic resistance microbes has promoted the topic as imperative subject worldwide due to its significant burden on the public health and global economy [1, 2]. Hereto, the primary focus both in the academia and industry have been devoted to the development of innovate and sustainable solution to this big problem by employing antibiotic-free materials and agents [3, 4]. From this perspective, the employment of electrospun material with induced antimicrobial properties [5] have recently gained increased attention due to their favorable properties, low cost and facile production [6]. There are several strategies for promoting antimicrobial property of electrospun materials [7], for instance, through surface modification [8], integration with nanomaterials [9, 10], addition of antibiotics [11], or addition of antimicrobial agent and materials (Figure 9.1) [12]. Despite, the wide study of this topic, there are still horizons to explore and the desire of invention for more potent solutions. Therefore, in this chapter we will select and discuss some important antimicrobial electrospun materials and their chemistry and applications [13]. Initially, the fundamental and overview of electrospinning technology will be discussed and subsequently some antimicrobial materials will be highlighted. Last, conclusions and some future directions will be discussed.

9.1.1 Electrospinning Technology

Electrospinning technology has emerged as a versatile and vital method for the production of ultrathin electrospun fibers from various materials

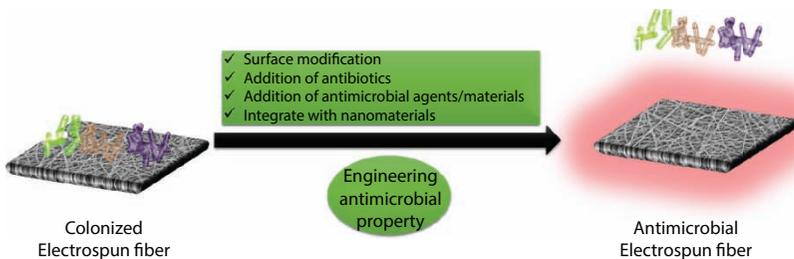


Figure 9.1 Strategies for engineering antimicrobial properties onto electrospun fibers.

(e.g., polymers, composites, and ceramics) in a facile, efficient, low cost, and with relatively high production rate [14]. The principles of electrospinning technology is depicted in Figure 9.2, where strong electric field is applied to a liquid of droplet fluid [15]. The basic traditional setup consists of a pump injecting the solution, high voltage power supply, and a collector (Figure 9.2a). Here the potential is applied between the nozzle (in this case the needle) and the collector, and this electrical field promotes the formation of Taylor cone (a jet of charged particles). Moreover, the electrostatic repulsion endorses motion and the formation of fine fibers that are placed onto the collector (Figure 9.2b) [16]. These allows the preparation of electrospun fibers with various and controlled dimensions such as nanosized fibers [17]. However, the technology is highly influenced from parameters such as applied voltage, solvent employed, concentration, properties of the solution, viscosity, surface tension, conductivity, solvent volatility, temperature and humidity, flow rate, and distance between the collector and nozzle tip [18]. Within this framework, electrospun nanofibers have gained much attention due to their beneficial properties such as high surface-to-volume ratio, large porosity, lightweight, tunable morphology, and small dimensions [19, 20]. Therefore they have shown a wide range of potential applications, such as, in healthcare; tissue repair [21], drug delivery [22], and wound healing purposes [23, 24] in environmental aspects; [25], as filters and membrane, e.g., for water and air purification [26], in energy aspects; solar cells and fuel cells [27], defense material; chemical and biological protection sensor and cloths [28], as catalysts and in batteries (Figure 9.3) [29]. A plethora of various materials have been employed for the engineering of electrospun fibers such as proteins (e.g., collagen and gelatin), hyaluronic acid [18], chitin and polyvinyl alcohol (PVA)-based, etc. [29], and more specifically materials with inherent antimicrobial properties [29], such as chitosan [30], silk fibroin [31], cellulose [32], and poly(ethylene oxide) (PEO) [33].

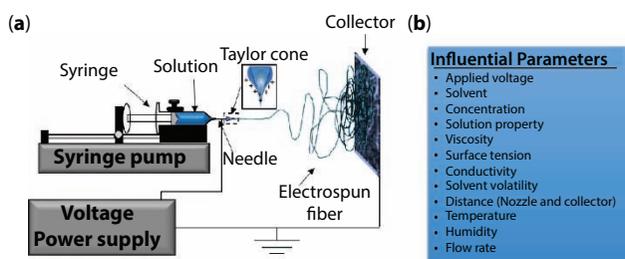


Figure 9.2 (a) The basic setup for electrospinning technology and (b) the influential parameters on the generated electrospun materials.

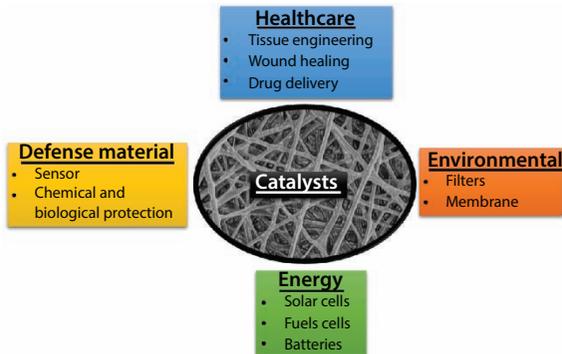


Figure 9.3 The illustration for the application of electrospun nanofibers.

9.1.2 Antimicrobial Materials

To date, the consciousness of the vast growing problem with antibiotic resistant pathogenic microbes has been profound to the society [34]. Therefore, there is an increasing need for the development of antimicrobial materials, which would provide alternatives to the employment of antibiotics [35]. De facto, these materials are important in many different application areas such as in biomedical applications (e.g., medical devices and implants) [36], in protective clothing and textiles [37], food [38], animal feed [39], and packaging [40, 41]. Over the years, a wide range of materials with antimicrobial properties have been developed such as natural based; chitosan [42, 43], and polyphenols [44, 45], carbon nanostructures [46], antimicrobial peptides (AMPs) [47], and various nanoparticles; from elements such as cerium [48], selenium [49], silver [50], and copper [51].

9.1.3 Antimicrobial Electrospun Materials

As vide supra highlighted the urge and huge necessity for the development of materials with antimicrobial properties entail the advancement within the field. In this context, electrospun nanofibers could function as an important candidate in this quest [7]. Here, Lobo, Afewerki, and coauthors devised electrospun nanofibers as a potential scaffold for orthopedic applications by combining the three different polymers polycaprolactone (PCL), polyethylene glycol (PEG), and gelatin methacryloyl (GelMA) and then further crosslinking the fiber blend [52]. The material displayed ultrathin fibers prior to crosslinking ($\sim 0.24 \mu\text{m}$) and a small increase in diameter post UV-crosslinking (of the light sensitive GelMA in the polymer blend) ($\sim 0.66 \mu\text{m}$) (Figure 9.4a–d). The biomaterial

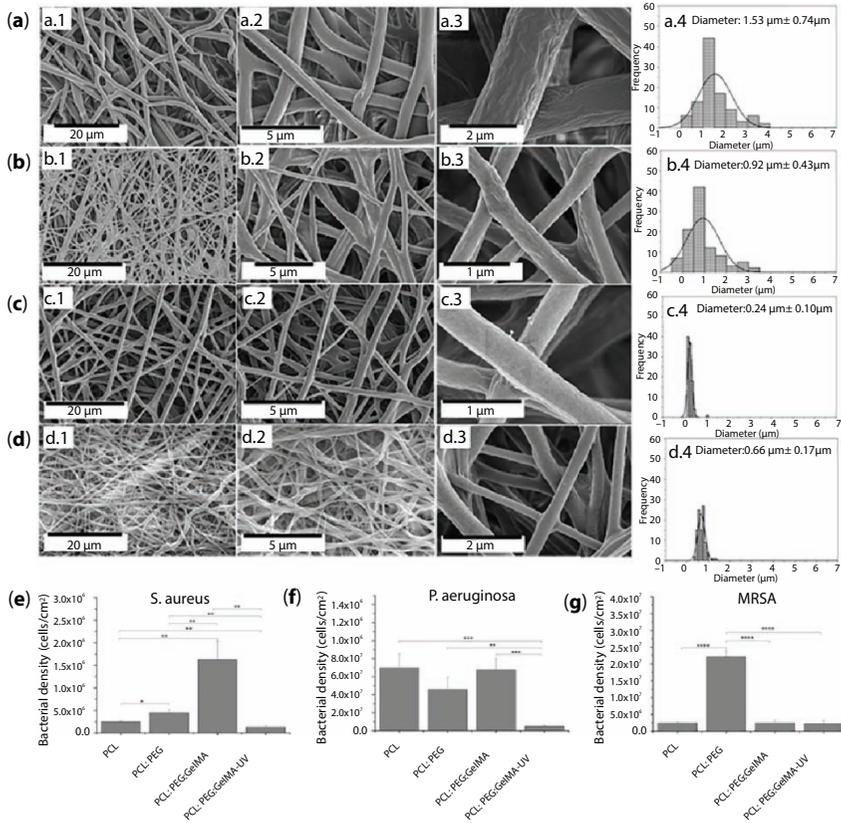


Figure 9.4 Electrospun nanofibers composed of (a) polycaprolactone (PCL), (b) PCL:polyethylene glycol (PEG), (c) PCL:PEG:gelatin methacryloyl (GelMA), (d) crosslinked PCL:PEG:GelMA. The antibacterial study of the nanofibers for 24 h against (e) *Staphylococcus aureus* (*S. aureus*), (f) *Pseudomonas aeruginosa* (*P. aeruginosa*), (g) *Methicillin resistant Staphylococcus aureus* (MRSA). Reproduced with permission from Ref. [53]. Copyright 2018, De Paula (CC BY) license.

demonstrated improved mechanical, hydrophilicity, and biological performance when compared to nanofiber made of only PCL. The nanofiber blend was further evaluated for its antibacterial property against *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Methicillin resistant Staphylococcus aureus* (MRSA) [53]. Interestingly, the crosslinked nanofiber demonstrated the best antibacterial efficacy of all the various nanofiber blends tested with a bacteria reduction of >90% (Figure 9.4e–g). Very recently, the same group further expanded their strategy by designing ultrathin core–shell fiber, where the core consisted of PCL and the shell of PEG and gelatin loaded with

an osteogenic growth peptide [54]. This core-shell strategy did not only provide a controlled and sustained release of the peptide, nevertheless it also demonstrated good biological performance (osteogenic) and most importantly bacteria reduction against *P. aeruginosa*.

Furthermore, Si *et al.* presented a daylight-driven, rechargeable and antibacterial electrospun nanofibrous material comprised of poly(vinyl alcohol-co-ethylene) (PVA-co-PE) [55]. Interestingly, the antibacterial property of the nanofiber is created through the generation of reactive oxygen species (ROS) that is driven by daylight. This was possible due to the incorporation of daylight-active molecules with the ability to generate ROS (Figure 9.5a–d). Fascinatingly, the strategy promotes the antibacterial activity to function under dark condition and still be able to release the ROS (Figure 9.5e and f). The antibacterial performance was demonstrated

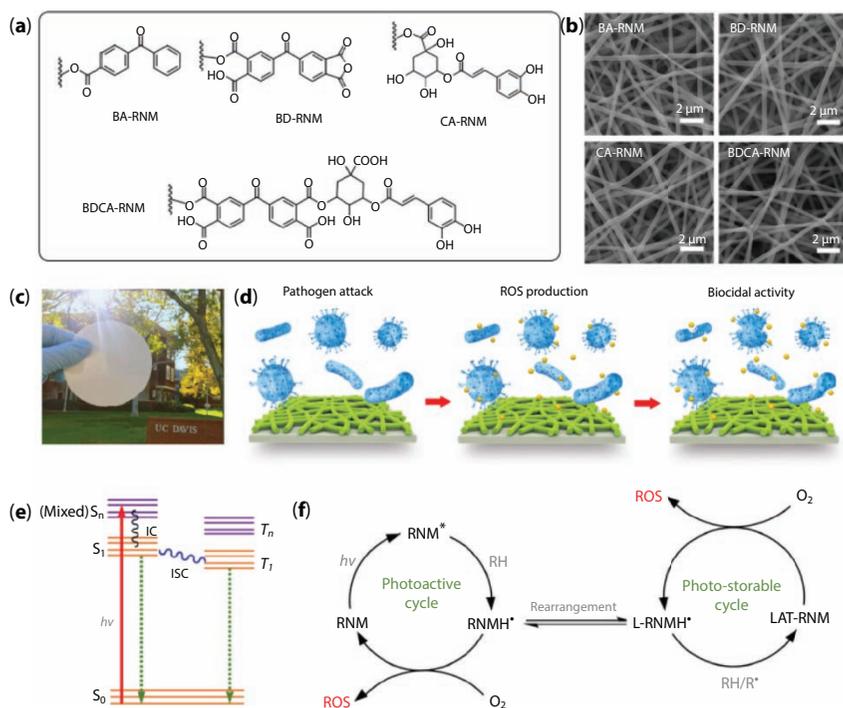


Figure 9.5 (a) The structure of the daylight active molecules. (b) The morphology of the fibers loaded with the active molecules. (c) Image of the nanofiber samples. (d) Scheme demonstrating the mechanism of action for the antibacterial activity. (e) Diagram demonstrating the excitation of the molecules. (f) The plausible mechanism of the photoactivation. Reproduced with permission from Ref. [55]. Copyright 2018 American Association for the Advancement of Science (CC BY-NC) license.

against the Gram-negative *Escherichia coli* (*E. coli*) and the Gram-positive *Listeria innocua* (*L. innocua*) and impressively it showed excellent bactericidal performance (>99.9999%). The practicability of the technology was further verified by applying the material as a surface shielding layer on protective equipment [55]. Moreover, Wang *et al.* envisioned fabricating a three-dimensional (3-D) nanofiber scaffold providing both resistance toward bacterial colonization and simultaneously promoting tissue-cell adhesion and proliferation [56]. These properties were induced by incorporating microgels containing peptides, which were prepared by hierarchical assembly of the anionic microgels and the cationic AMP. Based on the results from the study, the authors suggested that the antimicrobial agent would only be released upon demand, otherwise it would remain within the fibers in case any bacteria appear at a later stage. Furthermore, Li *et al.* employed recombinant silkworm-based AMP that was mixed with poly(*L*-lactic acid) (PLLA) and then further electrospun [57]. The activity was studied against a wide range of bacteria (*Seiatio maicesceis* (*S. maicesceis*), *Bacillus bombysepticus* (*B. bombysepticus*), *E. coli* and *S. aureus*) and the AMP showed a dose dependent performance. The SEM images demonstrated the destruction and deformation of the cell surface and wall. Therefore, it was concluded that the mechanism of action was through electrostatic attraction between the cationic AMP and negative charged cell membrane. This was confirmed through monitoring of the Zeta (ζ)-potential of the cells, which showed a rise of ζ -potential when AMPs were added. Similar mechanism of action for AMPs have previously been proposed [58].

Moreover, the most abundant and sustainable natural polymer on earth cellulose and its nanoform (nanocellulose) have gained great attention due to their unique properties such as biocompatibility, biodegradability, renewable, cost-effective, mechanical resilience, and hydrophilicity [59, 60]. It can be extracted from various sources such as forest, tunicate, algae, and bacteria and have been widely engineered inducing antibacterial properties [61]. Very recently, electrospun made from cellulose acetate blended with the antibacterial agent silver-sulfadiazine as a potential wound dressing was demonstrated [32]. Different strategy was presented by Wahab *et al.*, where they first generated the cellulose acetate and then further coated with silver nitrate (AgNO_3) inducing the antimicrobial activity [62, 63]. Various metal nanoparticles (copper nanoparticles (CuNPs), iron nanoparticles (FeNPs) and zinc nanoparticles (ZnNPs)) as the antimicrobial agent have been integrated with carboxymethyl cellulose (CMC) and then further electrospinning [64]. The electrospun material demonstrated antibacterial activity against *S. aureus* and *P. aeruginosa*

and was proposed as a potential bandage material. In this context, polyvinylpyrrolidone (PVA) based nanofibers have also successfully being incorporated with silver-, copper-, and zinc metals in order to encourage antimicrobial property [65]. Additionally, several other reports have been disclosed employing cellulose based materials solely [66–69] or merged with other materials [70–76]. Another type of interesting saccharide-based compounds for the delivery of various antimicrobial agents is the cyclic oligosaccharides cyclodextrins (CDs) with their unique chemical structure having a hydrophilic outer surface and a lipophilic central cavity [77–79]. A polylactic acid (PLA) and triclosan (TR)-CD inclusion complexation (IC) based electrospun nanofibers have been prepared through electrospinning approach (Figure 9.6) [80]. Interestingly, the employment of the α -CD did not provide any complexation with the TR, probably due to the smaller dimensions, while the β - and γ -CD provided successfully. The nanofibers with the TR-CD-IC demonstrated better antibacterial efficiency against *S. aureus* and *E. coli* compared to the nanofibers loaded with only the TR. In this context it would be interesting to perceive the release profile from the two nanofibers. However, the authors concluded that the increased solubility of the TR emanate from the CD-IC was the underlying mechanism for the improved antibacterial performance, which resulted in a more efficient release [80].

Additionally, PCL-based electrospun fibers integrated with zinc oxide (ZnO) have been designed for periodontal application [81]. Its performance was demonstrated on an *in vivo* rat periodontal defect model; however, the antibacterial performance was not evaluated *in vivo*. Nevertheless the *in vitro* antibacterial study against *Porphyromonas gingivalis* (*P. gingivalis*) presented significant activity compared to fibers in the absence of ZnO.

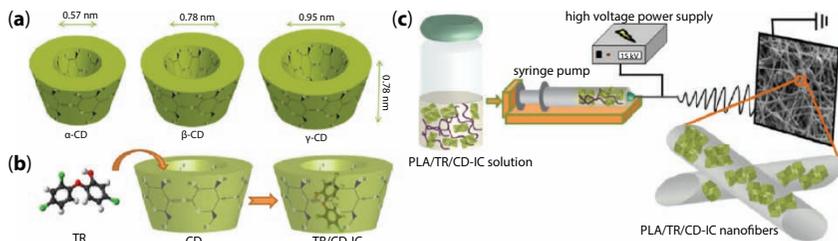


Figure 9.6 (a) The structures and dimensions of the various cyclodextrin (CD) types. (b) The scheme for the production of the triclosan (Tr)-CD-inclusion complex (IC). (c) The illustration of the strategy for the fabrication of the polylactic acid (PLA)-based Tr-CD-IC electrospun nanofibers. Reproduced with permission from Ref. [80]. Copyright 2013 American Chemical Society.

In addition, Faria *et al.* devised electrospun based on a combination of poly(lactide-*co*-glycolide) (PLGA) and chitosan, and then further chemically functionalized with graphene oxide and AgNPs (GO-Ag) to inherent antimicrobial activity [82]. The chemical modification was performed by first activating the GO-Ag with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxysulfosuccinimide (NHS) coupling reaction. Subsequently, the coupling between the NHS activated GO-Ag (GO-Ag-NHS) and the free amines on the PLGA-chitosan fibers finalized the reaction (Figure 9.7). Compared to the unmodified fibers the conjugated showed >98% bacterial inactivation against *E. coli* and *P. aeruginosa*. The strategy behind combining the GO and AgNPs was to obtain synergistic antimicrobial effect [82]. The approach of combining GO-Ag within electrospun prepared from polylactic acid (PLA) have also been disclosed by Liu *et al.* [83]. The addition of the GO-Ag did not only provide bactericidal property, however, it also improved the mechanical property (solely PLA: tensile strength \approx 8.70 MPa and elastic modulus \approx 0.76 MPa, and PLA-GO (1%)-Ag (7%): tensile strength \approx 1211.05 MPa and elastic modulus \approx 5.46 MPa) thermal properties (solely PLA: $T_m = 359.6^\circ\text{C}$ and PLA-GO (1%)-Ag (7%): $T_m = 366.0^\circ\text{C}$) and wettability/contact angle (CA) (solely PLA: CA \approx 131.57° and PLA-GO (1%)-Ag (7%): CA \approx 102.34°). Renege on chitosan based electrospun, the polysaccharide [30, 84] have further been merged with other components such as PEO and AgNO₃ [33], PEO, and Lauric arginate [85] combined with honey, PVA, and the natural extracts *Allium sativum* and *Cleome droserifolia* [86], with PVA and *Bidens pilosa* (a cosmopolitan weed) [87], or chemically modified [88]. Moreover, various other natural based extracts such as Moringa (leaf extracts) [89], and Lanazol

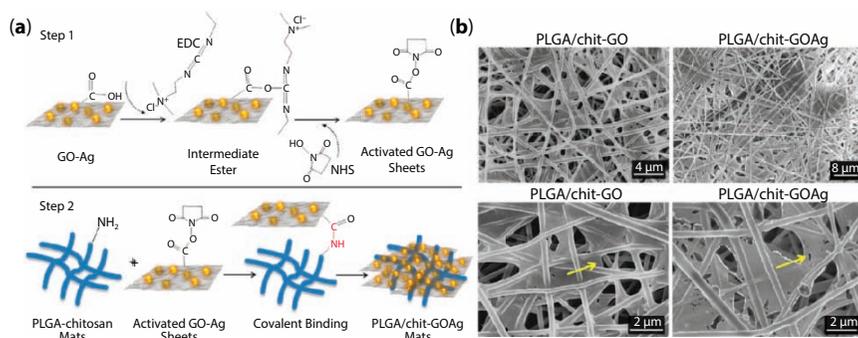


Figure 9.7 (a) The chemical process for the modification generating GO-Ag functionalized PLGA-chitosan electrospun and (b) the scanning electron microscopy (SEM) images [82]. Copyright 2015 American Chemical Society.

(red sea algae extract) [90], limonene [91], mustard (isothiocyanate) [92], or essential oil [93] have been integrated with the electrospun materials in order to provide antimicrobial property. Very recently, Park *et al.* fabricated quaternary ammonium-functionalized amphiphilic co-polymers consisting of a hydrophobic part (poly(methyl methacrylate) (PMMA)) and a hydrophilic fragment (poly(*N,N*-dimethylamino)-ethyl methacrylate (PDMAEMA)), respectively. This block co-polymer was merged with poly(vinylidene fluoride) (PVDF) and GO, and then further fabricated into electrospun blend. Afterward, hydrophilicity was introduced by further coating with PVA providing superhydrophilic and antibacterial nanofiber [94]. The antibacterial study through agar diffusion and dynamic contact tests confirmed the antibacterial activity of the nanofiber blend. Moreover, the antibacterial efficiency was enhanced by increasing the length of the alkyl moieties on the di-block polymer due to the improved interaction with the cytoplasmic membrane of the cells [95]. A superhydrophilic antibacterial nanofiber based on inorganic silica *N*-halamine [96] have also been disclosed by Liu *et al.* [97]. The material was prepared by combining electrospinning technology and sol-gel approach. The silica *N*-halamine-based nanofiber was chlorinated in order to obtain long term, facile, and efficient antibacterial property. The nanofiber was suggested as a profound candidate for water disinfection due to the high chlorine content and highly effective antibacterial property. Nanofibrous antibacterial membrane with 100% (with AgNO₃) or 92% (with poly(catechol)) proficiency in reducing bacteria made from PVA, PVDF and from enzymatic prepared poly(catechol) or AgNO₃ have also been designed for water ultrafiltration applications [98]. In fact, polydopamine based electrospun fibers have very recently been demonstrated displaying many favorable advantages such as excellent antimicrobial properties, good biocompatibility, optimum wettability, enhanced mechanical property, and improved thermal stability [99].

Very recently, Wang *et al.* presented a multifunctional electrospun nanofiber, which besides being bactericidal, performed efficiently as air-filtration device, and with high photocatalytic activity as a scavenger for dye [100]. The nanofiber was made of a mixture of PVA and the polysaccharide konjac glucomannan, and with the antimicrobial ZnO incorporated. The generated electrospun fibers was further processed by a thermal crosslinking step at 140°C, where esterification occurred between the PVA and the polysaccharide by using citric acid as the crosslinking agent. Furthermore, in 2017, Yang and coauthors fabricated an electrospun fiber against multidrug-resistant (MDR) bacteria as a dressing for wound-healing application [101]. The MDR agent comprised of gold nanoparticles

(AuNPs) coated with the compound 6-aminopenicillanic acid (6-APA) and the fibers were made from PCL and gelatin mixture. The characterization of the Au-6-APA-NPs exhibited size at ~ 3 nm with negatively charged particles (ζ -potential = -26.3 ± 0.6). The nanoparticles demonstrated high stability and could be stored without any changes for almost 7 months. The antibacterial activity was tested against clinically isolated MDR strains and the Au-6-APA-NPs demonstrated high efficiency, and even outperformed the antibiotic ampicillin (which was ineffective against the isolated MDR *E. coli*). The practicality of the fibers was further demonstrated in infected skin wound healing application. The *in vivo* model was a dorsal wound model of rat which was exposed to various MDR bacteria. The study showed that the local bacterial levels decreased after 7- and 14-days of treatment. Furthermore, it also demonstrated faster wound healing ability where the wound treated with this fiber decreased to a larger extent compared to the fibers in the absence of the MDR agent [101]. Additionally, PLLA-co-PCL nanofibers loaded with AgNPs have shown to function as a good scaffold for wound healing [102]. Some of the challenging limitations in the design of nanofibers with antimicrobial agents is the tailoring of the delivery system, providing controlled, sustained (thus avoiding systemic toxicity), prolonged and site specific delivery with sufficient encapsulation efficiency [103]. For instance, a cephalexin (antiseptic drug) loaded into alginate based halloysite nanotube electrospun fiber was demonstrated to provide sustained release [104]. Initially, the halloysite nanotube displayed 95% drug release already after 24 h; however, by introducing a crosslinking step the drug released could be prolonged to 76% and 89% after 8 h and 7 days, respectively. Different strategy was presented by Hassiba *et al.*, where they designed a double layered nanocomposite electrospun fibers as wound dressing [105]. The upper layer of the fibers comprised of PVA and chitosan loaded with AgNPs, and the lower of PEO or PVP containing chlorohexidine (antibacterial agent). The underlying mechanism behind the strategy was that the upper layer would function as a protecting deposit against environmental germ invasions and the lower interacting with the injured site and promote the healing and at the same time protects from any conceivable wound infections.

Elasticity is an important feature for the design of stretchable electrospun material for applications such as textiles, wearable electronic, and tissue engineering applications [106]. Very recently, Kang *et al.* devised an engineered trachea made from 3D-printed thermoplastic polyurethane enwrapped with PLA electrospun and loaded with ionic liquid functionalized-GO to provide antimicrobial property. Nevertheless, the

elasticity originate from the 3D-printed thermoplastic and not from the electrospun fibers [107]. However, electrospun materials fabricated from polyurethane and PLGA loaded with the antibiotic tetracycline hydrochloride have been demonstrated as an elastic nanofiber [108].

9.1.4 Conclusions and Future Directions

As have been highlighted in this chapter, electrospun material with inherent antimicrobial property could function as an important candidate in many fields (e.g., environment, biotechnology, food, packaging, medicine, and cosmetics) and for countless of applications. Prominently its facile fabrication and production makes it an interesting technology for industrial and large-scale production [109]. Moreover, since the technology is highly influenced from several experimental factors it is very important investigating those factors, in particularly, when designing an optimal, solid, scalable, and reproducible protocol. Despite all the advancement within the topic and the development of a wide range of various electrospun materials with antimicrobial properties made from various materials, there is still limitations and avenues to explore [110]. Exempli gratia, several challenges need to be further addressed in the antimicrobial strategy, such as the controlled and sustained release of the antimicrobial agents from the nanofibers. Moreover, to date the demonstrations and examples of scalable and translational antimicrobial electrospun technologies are still limited. Despite the several commercially available industry-scale electrospinning apparatuses that are available, the application is still limited and there are still economic challenges that need to be encountered. For instance, electrospun materials in biomedical application is still limited due to the issues with biological safety and efficacy challenges [111]. Moreover, the combined effort for the invention of novel chemistries allowing the facile tailoring of electrospun materials making them robust with antimicrobial activity, and simultaneously the development of highly potent, long-lasting, and broad antimicrobial agents and materials are some features that will boost the field further. Additional future direction is the advancement of the electrospinning technology which hopefully will bring new methods for the production of various electrospun fiber types [112, 113]. For instance, the existing different methods available such as mono-axial [114], co-axial [115], and tri-axial [116] allow the tailoring of various nanofibers. Nevertheless, the invention of new sophisticated approaches could broaden the assortment and fabrication of nanofibers from the electrospinning technology.

Acknowledgments

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