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ELECTROSPUN NANOFIBERS AS ADVANCED ANTIBACTERIAL PLATFORMS: A REVIEW OF RECENT STUDIES

Masoud Aman Mohammadi [1], Mohammadreza Rostami [1], Samira Beikzadeh [2], Mojtaba Raeisi [3], Mahnaz Tabibiazar [4] and Mohammad Yousefi [* 1]

Student Research Committee [1], Nutrition Research Center and Department of Food Science and Technology, Faculty of Nutrition and Food Science [4], Faculty of Nutrition and Food Science, Tabriz University of Medical Sciences, Tabriz, Iran.
Department of Food Technology [2], Faculty of Nutrition Sciences and Food Technology/National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Cereal Health Research Center [3], Golestan University of Medical Sciences, Gorgan, Iran.

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Correspondence to Author:
Mohammad Yousefi

Student Research Committee, Faculty of Nutrition and Food Science, Tabriz University of Medical Sciences, Tabriz, Iran.

E-mail: Mahnaz_tabibiazar@yahoo.com

ABSTRACT: This study aimed to introduce antibacterial nanofibers, produced by electrospinning as a novel technique in constructing nanostructured materials. In the electrospinning technique, a high voltage is applied to a polymer solution to overcome the surface tension. A charged polymer jet is ejected to a grounded electrode. As the solvent is evaporated, the electrospun fiber with diameter from micrometer to nanometer is formed. One of the most important features of electrospun nanofibers stated in this paper is their activity as effective carriers of antibacterial substances, which leads to the use of them in many fields such as food packaging, drug delivery, wound healing, filtration, and food sciences. An electrospun nanofiber containing antimicrobial agents indicates excellent antibacterial functions against a wide spectrum of bacteria and sustain the antibacterial activity over a long period. In general, several aspects of antibacterial nanofibers, including synthesize, applications, types of antibacterial agents combined with them such as essential oils, herbal bioactive components, metals, drugs, and peptides have been stated.

INTRODUCTION: Nanofibers are ultrafine solid fibers having very small diameters, they are generally defined as nanostructured materials with a diameter under 1000 nm and typically a length-to-width ratio greater than 50 [1, 2, 3, 4]. Nanofibers are of remarkable interests for a multiplicity of applications due to their worthwhile properties such as high surface area and porosity with small pore sizes [5].

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These traits make nanofibers suitable for the production of several technologically advanced products within many areas such as drug delivery, scaffolds, wound dressings, and filtration [6, 7, 8, 9].

Electrospinning is a simple, versatile, and cost-effective method which can produce nanofibers with excessive surface area, flexibility, better mechanical properties, and morphologically improved forms than those obtained using traditional methods. In the electrospinning technique, a high voltage is applied to a polymer solution to overcome the surface tension. A charged polymer jet is ejected to a grounded electrode. As the solvent is evaporated, the electrospun fiber with diameter from micrometer to nanometer is formed.

One of the important applications of electrospun nanofibers is the usage of them to the encapsulate / entrapment of antibacterial agent [10, 11]. Bacterial contamination is a serious worry in the food industry and healthcare, packaging, and synthetic textiles products. Among the several types of pathogenic bacteria, *Staphylococcus aureus* and *Escherichia coli* are responsible for a variety of infections and diseases such as diarrhea and gastrointestinal disease [12, 13]. Food contamination may happen during slaughtering, processing, packaging, and marketing, also it is a chief public health concern and an economic problem for the food industry. Hence, the development of methods to control the growth of bacteria that contaminate foodstuff is gaining a lot of interests among the community. Therefore, the development of antibacterial carriers such as nanofiber with antibacterial activity is of the proposed methods to deal with the food contamination [14, 15, 16]. Many natural substances, including organic acids, metals, bacteriocins, spices, enzymes, and polysaccharides have been proposed and tested for antimicrobial nanofiber systems [17]. The antibacterial property of electrospun nanofibers can be evaluated qualitatively or quantitatively against model bacteria using minimum inhibitory concentrations, diffusion (agar disc diffusion, agar well diffusion, and epsilometer test), dilution (agar dilution and broth micro/microdilution), and metabolic-based assay methods [18].

Various antibacterial test methods likewise can be summarized in three steps: (1) preparation of bacterial medium; (2) direct contacting of bacteria and antibacterial electrospun nanofibers; and (3) the evaluation of antibacterial properties by observing the growth of model microorganisms. The most bacteria used in the antimicrobial tests are *Escherichia coli* as gram-negative and *Staphylococcus aureus* as gram-positive bacteria [19]. Antibacterial electrospun nanofibers are divided into two main groups: (1) electrospun nanofibers which inherently have antibacterial activity, (2) electrospun nanofibers containing antibacterial agents.

The main purpose of this study is representing the several antibacterial nanofibers, with explanations about their activity mechanisms, applications, and their synthesizing methods.

2. Chitosan Electrospun Nanofiber with Inherent Antibacterial Activity: Chitosan is a natural cationic biopolymer obtained from chitin by deacetylation (above 60%) *via* thermochemical reactions [20, 21]. It is linear and contains repeating units of N-Acetyl-D-glucosamine and N-glucosamine (1-4) linked through glycosidic bonds [22, 23]. Chitosan is soluble in acetic acid (mainly) and other weak organic acids. It can form a gel, nanocomposite, fiber, *etc*. [24] Among the biopolymers, chitosan and its derivatives are regarded as one of the most used substances in nanofibers. Nanofibers containing chitin or chitosan have specific applications in areas such as drug delivery, tissue engineering, wound healing, active food packaging, and filtration [25, 26, 27].

Several beneficial properties of chitosan such as non-toxicity, biocompatibility, biodegradability, and its inherent antibacterial features have attracted scientists attentions [28]. Antibacterial activity of chitosan has been proved against many bacteria, filamentous fungi, and yeasts [29]. Chitosan has a wide spectrum of activity and high bactericide rate against gram-positive and gram-negative bacteria. Many factors affecting on the antibacterial property of chitosan are molecular weight, concentration, and solubility, among these, the most important factor is the degree of deacylation, because the amount of free amino groups in the molecule is related to its activity, in order to enhance the antimicrobial efficacy of chitosan and its derivatives, higher degree of deacylation and higher molecular weight with more amino groups are desired [30, 31, 32, 33].

However, due to its polycationic nature in solutions, chitosan cannot be fabricated easily in the fiber form by electrospinning. Pure chitosan nanofibers with a satisfactory quality are obtained using high concentrations of the polymer in the solution. Factors such as applied voltage, the concentration of polymer in solution, flow rate, and particularly the viscosity of the solution affect the morphology of nanofibers [34, 35]. To facilitate the nanofiber formation, different blends of chitosan with other polymers such as polycaprolactone, poly (ethylene oxide) (PEO), poly (vinyl alcohol) (PVA), and collagen have been used. These polymers help to overcome bead defects in chitosan nanofibers, which are attributed to an insufficient stretching of the fibers during the bending of the polymer solution jet [36, 37, 38, 39]. It has been proved that over 99% of microorganisms reduce with pure chitosan nano-fibers and above 50% reduction has been seen with the blend of chitosan/PEO nanofibers [40]. Arkoun *et al*., investigated the antibacterial activity of nanofibers based on chitosan (CNFs) achieved by the electrospinning process. The bactericidal efficiency of CNFs was determined against gram-positive bacteria, *Listeria innocua* and *S. aureus* and gram-negative bacteria, *Salmonella typhimurium*, and *E. coli*. Their findings showed a reduction in a range of 98.97% for *S. typhimurium* and 100% for *E. coli* and *L. innocua*. The results strongly suggested that CNFs interact with negatively charged bacterial cell walls causing the rupture of membrane and the leakage of intracellular components like proteins and DNA. The creation of pores on cell walls was observed in transmission electron microscopy (TEM) **Fig. 1** [41].

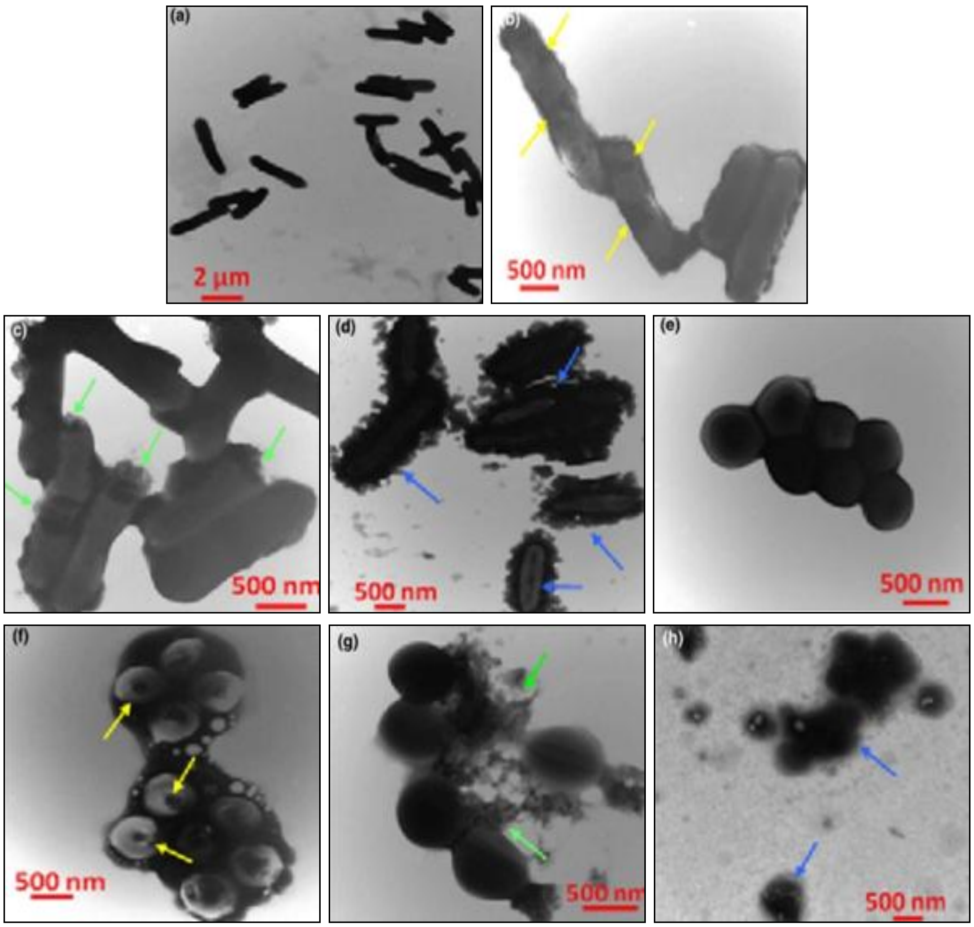

FIG. 1: TEM MICROGRAPH OF A, B, C D: T_0, 10, 20 AND 30 min CONTACT OF *E. COLI* BACTERIA TO CNFS, AND E, F, G, AND H: T_0, 10, 20 AND 30 MIN CONTACT OF S. *AUREUS* BACTERIA TO CNFS, RESPECTIVELY. THE YELLOW, GREEN AND BLUE MARKS, SHOW MEMBRANE PERFORATION, LEAKAGE OF CYTOSOL, AND BACTERIA LYSIS, RESPECTIVELY

3. Electrospun Nanofiber Containing Antibacterial Agent: Recently, nano-scale antibacterial agents (essential oils, bioactive component, antibacterial drug, and metallic nanoparticle) have attracted more and more attention owing to their improved antibacterial properties compared with traditional substances [42]. Electrospinning can produce nanofibers with unique properties, including high surface-to-volume ratio, very low weight, porous and flexibility nature. Electrospun nanofiber is having antibacterial properties can be achieved by encapsulation/entrapment of several types of antibacterial agents in the nanofiber matrix through the electrospinning process, which is satisfactory for the sustained release of antibacterial agents [6, 43, 44]. For the blended polymer used in electro-spinning, environmentally-friendly polymers are desirable compared with traditional petroleum-based polymers [23]. Electrospun nanofibers protect antibacterial agents from temperature, oxidation, degradation, and ambient conditions [45].

3.1. Electrospun Nanofibers Containing Essential Oils: In recent years, concerning the health worries associated with the side effects of synthetic compounds used in medicine and food industries and the advent of antibiotic-resistant pathogens, researchers have focused on biomaterial towards the development of nanofibers used for encapsulating essential oils [15]. Essential oils are concentrated hydrophobic liquids, including volatile compounds extracted from plants. Hydrophobic terpenoid and phenolic compounds exist in essential oils. Essential oils are mixtures of different chemical compounds such as cymene, eugenol, and carvacrol extracted from aromatic plants [46, 47]. The activity mechanism of essential oils against microorganisms depends on their hydrophobic nature, on the other words, they can be partitioned into the lipid bilayer of the bacterial cell membrane, with subsequent disruption of its structure. The membrane is then made permeable to ions and other cellular contents, and this finally leads to the collapse of the proton pump and cell death [47, 48, 49]. Wen *et al*., produced a novel antibacterial electrospun nanofibrous film based on PVA to encapsulate the cinnamon essential oil (CEO). The results showed that the minimum inhibitory concentration (MIC) of PVA/CEO nanofibrous film against *S. aureus* and *E. coli* were approximately 0.9 and 1.0 mg/mL, respectively. Also, the minimum bactericide concentration (MBC) of PVA/CEO nanofibrous film against *S. aureus* and *E. coli* were nearly 7.0 and 8.0 mg/mL, respectively [50].

Feng *et al*., produced PVA electrospun nanofibers by the incorporation of lysozyme (LYS) and CEO. Compared to the PVA/LYS nanofibers and PVA/CEO nanofibers containing an individual antibacterial substance, the PVA/CEO/LYS nanofibers displayed stronger antibacterial properties. The MIC and MBC values for *Listeria monocytogenes were* 0.8 and 6 (mg.ml-1) and for Staphylococcus enteritidis were 1 and 7 (mg.ml-1), respectively [51].

Sadri *et al*., prepared electrospun chitosan / PEO nanofibers and entrapped two types of thyme essential oils in this nanofiber. The broad-leaf and narrow-leaf thyme essential oils-loaded into chitosan / POE fibers were tested against *Pseudomonas aeruginosa* and, *S. aureus*: after 24 h of contacting, fibers showed inhibition of board-life: 10 and 19 mm and narrow-life: 8 and 15 mm for *P. aeruginosa* and *S. aureus*, respectively. Agreeing to these results broad-leaved showed more antibacterial activity than narrow-leaf spices in the presence of bacteria [52].

3.2. Electrospun Nanofibers Containing Herbal Bioactive Components: Herbal bioactive components obtained from plants have powerful antibacterial activities against a variety of foodborne pathogens. The use of bioactive chemical compounds as antibacterial agents such as gingerol, curcumin, asiaticoside, and shikonin have been researched widely [53, 54, 55, 56]. Curcumin (Cur) which is derived from the rhizome of Curcuma longa L. is renowned for its beneficial properties, including antioxidant, anti-inflammatory, and anticancer features.

Several works have been published on the electrospinning of curcumin-based fibers for wound healing and drug delivery applications. For instance, PCL-poly (ethylene glycol) fibers loaded with 0.5% wt. of Cur have been examined on wounds. The composite fibers showed the efficacy of 99% and 70% in inhibiting the rise of *S. aureus* after 12 and 24 h, respectively [57]. Karimi *et al*., perpetrated electrospun Poly (e-caprolactone), Poly (lactic acid), and their hybrid (50/50) nanofibrous mats containing thymol and evaluated the antibacterial properties of the electrospun mats against *Staphylococcus aureus* and *Escherichia coli*. Shikonin is another active herbal component which has antibacterial activity. It is obtained from the root of *Lithospermum erythrorhizon* [58].

The shikonin-loaded PCL/PTMC fibers were tested against *E. coli* and *S. aureus*: after 24 h of contacting, fibers containing 5% wt. of shikonin showed inhibition of 16.9 and 21.3 mm for *E. coli* and *S. aureus*, respectively [59]. Alta *et al*., produced electrospun nanofibers based on zein and poly (lactic acid) (PLA) and loaded carvacrol into this nanofiber for food packaging. The inhibition of aerobic mesophilic bacteria, yeast, and mold growth in bread samples increased when the carvacrol level increased in the zein and PLA nanofibers. Zein nanofibers at 20% carvacrol content showed 87.6% and 99.6% inhibition against aerobic bacteria, mold, and yeast whereas the rate of inhibition was 87.0% and 91.3% for PLA nanofibers at 20% carvacrol content, respectively [60].

3.3. Electrospun Nanofibers Containing Metals: The antibacterial activity of silver, zinc, and copper has been widely tested against some species. The inhibiting mechanism of metal against microorganisms is based on the consequence of its interactions with the cellular membrane, enzymes, and nucleic acids. For example, concerning Ag, it has been suggested that the mode of action of silver nanoparticles includes the damage to the bacterial cell membranes and the decay of the activity of certain enzymes. Silver is toxic to bacteria due to its affinity to nucleic acids and proteins [61, 62, 63]. Nanoparticles entrapped in electrospun nanofibers would be an efficient means of transportation for the delivery of metals to bacteria of interest [63].

3.3.1. Electrospun Nanofiber Containing Silver: In the last years, silver-containing electrospun nanofibers have attracted attention as a novel form of antibacterial substance. Ag is a known biocidal agent that is effective against various types of bacteria, fungi, and viruses; however, it is non-toxic to human cells [64, 65, 66]. Two important properties of electrospun nanofibers, including the mild conditions applied during electrospinning and high specific surface area of nanofibers, result in increasing the activity of such these nanofibers [67, 68]. The simplest and most commonly used technique for combining Ag nanoparticles with electrospun nanofibers is the suspension of Ag nanoparticles directly into the electrospinning polymer solutions [69, 70]. The presence of silver nanoparticles was proved by UV-vis spectroscopy at 420 nm [71].

The addition of Ag nanoparticles affects the morphologies of the electrospun nanofiber. The incorporation of Ag improves the production of more smooth and continuous electrospun nano-fibers and the electrospun nanofibers average diameter commonly decreases as the Ag contents increase [66]. This is because the conductivity of electrospinning suspensions promotes with the contents of Ag. The addition of Ag enhances the charge density of precursor suspensions, and therefore stronger elongation forces are applied on the ejected jets under the electric field, causing producing finer and attenuated electrospun nanofibers. Also, the antimicrobial activity of these nanofibers is highly dependent on the concentration and size of the Ag nanoparticles [72].

Nanofibers prepared using nanoparticle technique have proved diminished antibacterial efficiency due to Ag nanoparticle aggregation and subsequently reduced bioavailability. However, reduction of silver ions in pre-electrospinning solutions results in a more uniform dispersion of Ag nanoparticles, partially as a result of the stabilizing activity of polymer molecules [73, 74]. Reduction of silver nitrate in polymer solutions or polymer matrices by hydrogen gas [75], hydrazinium hydroxide [76], citrate [77], borohydride [78], and ascorbate [79] has been reported. To avoid the use of environmentally dangerous or hazardous chemical agents, a series of environmentally ‘‘green synthesis’’ techniques are under examinations [80]. These methods include the polysaccharide technique (synthesis of Ag nanoparticles through nanoscopic starch templates) [81], biological technique (synthesis of Ag nanoparticles via special plant extracts, bio-organism extracts, or even some microorganisms) [82], and irradiation technique (radiolysis of silver nanoparticles by means of microwave, pulse, and gamma irradiation) [83], However, these ‘‘green’’ methods generally require longer treatment times and additional processes to incorporate silver nanoparticles into polymer matrixes [84, 85].

Kalwar *et al*., prepared cellulose acetate (CA) nanofibers by the electrospinning method. Alkaline hydrolysis was introduced for conversion of CA nanofibers to cellulose nanofiber. Furthermore, Ag nanoparticles were incorporated on the cellulose nanofiber. Antibacterial activity of this fiber against *E. coli* and *S. aureus* after 18 h of contacting with the fiber was examined via the inhibition zone in LB medium. Results showed 16 and 14.4 mm of inhibition zone diameters at 1% Ag nanoparticles content against *E. coli* and *S. aureus*, respectively. Also, it was proven that increasing of Ag nanoparticle contents increased antibacterial activities [86].

3.3.2. Electrospun Nanofiber Containing Copper and Zinc: Copper (Cu) is a powerful natural biocidal metal being used since ancient times for the aim of manufacturing water drinking utensils. From the era of Hypocrites until now, Cu has been extensively used as an agent to reduce the microbial populations. When copper contacts with bacteria, it leads to distortion of cell walls causing the death of bacteria. Supporting of a polymeric matrix with Cu by electrospinning is an advanced method exploited by many researchers to deal with bacteria [87, 88]. Ahire *et al*. incorporated copper nanoparticles into nanofibers through the electrospinning of PEO and Poly-D, L-lactide. After 48 h at the presence of Cu-nanofibers, *S. aureus* and *P. aeruginosa* were reduced by 50% and 41%, respectively [89].

In recent years, Zinc oxide (ZnO) as a wide band-gap semiconductor, has received increased attention concerning potential electronic applications owing to its unique chemical, electrical, and optical properties [90]. Moreover, ZnO appears to inhibit the growth of strongly resistant bacteria [91]. There are some reports about the considerable antibacterial activity of ZnO, which is attributed to the production of reactive oxygen species, causing the generation of oxide substances. The advantage of these inorganic oxides as bactericidal agents is that they have mineral elements essential to human cells and exhibit strong activity even at small amounts [91].

P. Gopinath produced PVA-based nanofibers incorporated with CuO-ZnO (CZ). Then, they explored the antibacterial activities of CZ nanofibers. The growth of *S. aureus* and *E. coli* was investigated in the presence of CZ nanofibers. Visual turbidity analysis exhibited that 300 and 450 μg/mL concentrations of CZ nanofibers inhibited the growth of *S. aureus* and *E. coli*, respectively [92].

3.4. Electrospun Nanofibers Containing Antibacterial Drugs: Antimicrobial nanofibers containing antibacterial drugs have become one of the most encouraging nano-scaled materials used in wound health, filtration, and active packaging systems. A wide variety of antibacterial drugs have been chemically or physically formulated within electrospun nanofibers or on their surfaces, such as antibiotics and peptides. However, the release mechanism of antibacterial drugs is related to the polymer degradation, the release pathway, and the release profile of antibacterial drugs from electrospun nanofibers which may be tuned *via* the fiber morphology and polymer composition [93]. So far, some different antibacterial drug loading techniques have been developed to produce drug-loaded nanofibers [94, 95, 96, 97, 98, 99, 100, 101]. The conventional single fluid electrospinning technique allows direct entrapment of drug molecules within electrospun nanofibers by simply electrospinning the drug/polymer solution or post-adsorption of drugs onto/within the electrospun nanofibers. However, a burst release often occurs, which is not wanted in most of the cases. The techniques of coaxial electrospinning and emulsion used for drug delivery applications can diminish the burst release of the encapsulated/incorporated drug. In both methods, the drugs can be incorporated into the core section of the electrospun nanofibers to form a "core-shell" structure, in which the external polymer shell can act as an extra barrier to control the drug release profile [96, 97, 98]. Antibacterial drugs encapsulated in electrospun nanofibers have been proved to maintain the antibacterial property over a longer period compared to the unencapsulated form [93, 102].

Liu *et al.*, prepared ciprofloxacin loaded into the sodium alginate/poly (lactic-co-glycolic acid) electrospun nanofibers and examined the antibacterial activity of these fibers against *S. aureus*. The MIC of ciprofloxacin for *S. aureus* was determined to be 0.125 μg/mL using the broth microdilution method [103]. Han *et al.*, produced and indicated the antibacterial activity of cellulose acetate/nisin nanofibers. These nanofibers showed 99.99% decrease of *S. aureus* [104]. Finally, some examples, representing the antibacterial electrospun nano-fibers are summarized in **Table 1**.

TABLE 1: INSTANCES OF ANTIBACTERIAL ELECTROSPUN NANOFIBER PRODUCED IN DIFFERENT CONDITIONS

Polymer	Antibacterial agent	Inhibited bacteria	Electrospinning conditions		
			Voltage	Flow rate	Distance to collector
Chitosan/PEO	Lauric arginate [105]	*E. coli*, *S. aureus*	20 kv	1 ml/h	15 cm
Chitosan/PEO	Glucose oxidase, Hydrogen peroxide [106]	*E. coli* *S. aureus*	12 kv	10 μl/min	12 cm
PVA	Cinnamon essential oil, Lysozyme [51]	*L. monocytogenes* *S. enteritidis*	17-19 kv	0.5 ml/h	14 cm

Zein	Eucalyptus essential oil [107]	*L. monocytogenes* *S. aureus*	18 kv	1 ml/h	15 cm
Cellulose acetate	Rosemary oregano oils [108]	*E. coli*, *S. aureus*	12 kv	2 ml/h	15 cm
Silk	Olive leaf extract [109]	*E. coli*, *S. aureus*	15-20 kv	2 µl/min	10 cm
Poly lactic acid	Tea polyphenol [110]	*E. coli*, *S. aureus*	20 kv	20 ml/h	15 cm
Zein	Curcumin [111]	*E. coli*, *S. aureus*	15 kv	-	12 cm
Poly-(ε-caprolactone)	Carvacrol [112]	*Listeria innocua*	12 kv	1.2 ml/h	20 cm
Polyvinylpyrrolidone	Silver, Zinc, copper [61]	*E. coli* *S. aureus*	15 kv	0.8 ml/h	15 cm
PVA	Phytoncide [113]	*E. coli*, *S. aureus*	21-29 kv	0.8-3.6 ml/h	14-19.5 cm
Cellulose acetate	Silver [86]	*E. coli*, *S. arenus* *A. flavus*	18 kv	1 ml /h	16 cm
Nylon–6	Silver [114]	*E. coli*, *S. aureus*	22 kv	0.15 ml /h	15 cm
Sodium alginate	Ciprofloxacin [103]	*S. aureus*	20 kv	10 µl/min	10 cm
Poly(ε-caprolactone)	Amoxicillin [115]	*S. typhimurium* *S. aureus*	25 kv	5-6 ml/h	12 cm
PVA	Benzyl triethylammonium chloride [116]	*E. coli*, *S. aureus*	15 kv	1 ml /h	15 cm
Cellulose acetate	Nisin [104]	*S. aureus*	12-14 kv	0.2-1.2 ml/h	20 cm
Fish gelatin	Bovine lactoferrin [117]	*E. coli*, *S. aureus*	25 kv	0.2 ml/h	15 cm
Poly(ester urea)	Bacteriophages [118]	*S. aureus* *Streptococci pyogenes*	10-30 kv	0.5-5 ml/h	10-25 cm

CONCLUSION: In this review, recent researches of antimicrobial electrospun nanofibers incorporated with several antibacterial agents, *i.e.*, metallic nanoparticles, drugs, essential oils, and herbal bioactive components are presented. Electrospun nanofibers incorporated with antimicrobial substances show a wide spectrum of applications in drug delivery, food, packaging, filtration, and wound healing. Among the antimicrobial agents, metallic nanoparticles such as silver nanoparticles and essential oils are the most widely used antimicrobial agents incorporated into the electrospun nanofibers. The antibacterial performance of metallic nanoparticles incorporated into electrospun nanofibers depends on the size, concentration, and distribution of these nanoparticles.

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