Review

Electrospinning of Chitosan for Antibacterial Applications—Current Trends

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Abstract: Chitosan is a natural biopolymer that can be suitable for a wide range of applications due to its biocompatibility, rigid structure, and biodegradability. Moreover, it has been proven to have an antibacterial effect against several bacteria strains by incorporating the advantages of the electrospinning technique, with which tailored nanofibrous scaffolds can be produced. A literature search is conducted in this review regarding the antibacterial effectiveness of chitosan-based nanofibers in the filtration, biomedicine, and food protection industries. The results are promising in terms of research into sustainable materials. This review focuses on the electrospinning of chitosan for antibacterial applications and shows current trends in this field. In addition, various aspects such as the parameters affecting the antibacterial properties of chitosan are presented, and the application areas of electrospun chitosan nanofibers in the fields of air and water filtration, food storage, wound treatment, and tissue engineering are discussed in more detail.

Keywords: electrospinning; chitosan; natural biodegradable polymers; *Pleurotus ostreatus*; antibacterial; water filtration; medical textiles; nanocomposites; food storage; tissue engineering; sustainability

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1. Introduction

In light of the advancements made in the fields of textile material production and finishing chemistry, which contaminate our water and soil, the topic of sustainability is gaining popularity in the textile world. At the same time, traditional techniques requiring that fibers are coated with mineral oils, which represent a notable portion of the problem, are being tackled by scientists and replaced by greener alternatives. In order to use natural fibers in technical and industrial applications, such as filtration, geotextiles, and biomedicine, it is of major importance for the lifespan of the material to resist biodegradability through the attacks of bacteria and fungi in humid environments [1]. For this reason, several natural polymers have been evaluated as potential candidates having antibacterial effects to be either applied in coating solutions on the substrate instead of oils or to be electrospun as nanofibers in composite materials. Chitosan, a product of the natural polysaccharide chitin extracted from various arthropods, algae, and mushrooms, is one of the most abundant biopolymers possessing bacteriostatic and bactericidal properties [1,2]. Electrospinning technology is cost-effective and easy to handle, and allows the production of an almost unlimited number of nanofiber types from biobased and synthetic polymers [2,3]. Due to the extremely high internal surface areas, nanofibers can be used in many biomedical applications for tissue engineering, cell cultivation, and filtration, among others [4–8].

This paper evaluates the latest findings regarding the electrospinning of chitosan and the antibacterial activity levels of electrospun chitosan nanofibers, whether alone or in

combination with other polymers in composites. Finally, this review focuses on the various possible applications, depending on their compatibility and appropriateness.

2. Electrospinning

2.1. Definition and Technical Setup

The electrospinning method was introduced by Cooley in 1900 [9]. Electrospinning has the distinct advantage of being able to create nanofibers with diameters ranging from micrometers to nanometers with ease and flexibility. Nanofibers are desirable because they have a high surface-area-to-volume ratio, providing more binding sites to electrostatically connect with other particles. Nanofibers may also offer great tensile strength and flexibility in their surface properties [10]. Furthermore, compared to conventional materials, the effective porosity of electrospun nanofibers with numerous interconnected tiny holes increases the infiltration rate and contaminant rejection ratio [11]. All of these advantages make nanofibers an ideal option for technical, medical, and biological applications [12–16].

The needle-based and needle-free electrospinning methods can be roughly distinguished as follows. The needle-based electrospinning machine mainly consists of four components—a high voltage source, a calibrated tube with a needle, a syringe pump and a metal collector where nanofibers are deposited [17] (see Figure 1a). The needleless methods, as an alternative to needle-based methods, make use of different techniques, such as the wire-based technique (see Figure 1b). In this electrospinning method, a wire is coated with a polymer solution or melt and nanofibers are formed through a strong electric field and deposited on a substrate such as polypropylene (PP), which covers a second wire [18].

Figure 1. Electrospinning setup for (a) needle-based and (b) needle-free electrospinning methods. Adapted from reference [18], originally published under a CC-BY 4.0 license.

In all electrospinning methods, a surface charge density is generated from a polymer solution by applying an electric field between the needle and the collector, resulting in a stretched, suspended droplet forming a cone, the Taylor cone, on the plane of the spinneret [19]. The charged liquid jet is ejected from the tip of the cone. As soon as the electric field overcomes the surface tension of the liquid, a fine fiber is formed that is drawn to the collector with the opposite polarity. With continuous feeding and replacement of the polymer solution by the syringe pump, endless filaments are formed. The instability of the jet as a consequence of its surface charge interaction with the external field favors its bending in a whipping mode, rather than axial propagation, accompanied by solvent evaporation, in a way that the obtained mat comprises finer, extremely stretched, dry nanofibers [20,21].

A significant influence on the fiber orientation, fiber diameters, texture, morphology and surface geometry is exerted by many operating parameters, such as the surface tension, viscosity, and crystallinity of the electrospinning solution; the solvents used; the electrical conductivity and voltage; the temperature and humidity in the chamber; the distance between spinneret and collector; and the molecular weight of the polymer [22,23].

To obtain fibers through electrospinning, a critical concentration is required. Below the threshold, there are not enough chain entanglements to overcome Coulombic repulsion

inside the ejected jet, resulting in the production of sprayed droplets and beads due to the high surface tension of the polymer solution. The decrease in the surface tension of the solution promotes electrospinning and the formation of uniform and homogeneous fibers at lower electric fields [23].

The fiber diameter can be influenced by operating parameters as well. The applied voltage allows charge transfer from the needle tip to the collector, and raising it may alter the fiber diameter and pore size of the nanofibers. The flow velocity of the polymer solution may affect both the size and form of the fiber. Accelerating the flow rate, for example, increases the pore size by increasing the fiber diameter. Only constant replenishment of the polymer solution has been demonstrated to preserve a stable form of the Taylor cone at the tip of the needle [24]. As there is not enough time for the fiber to dry before it reaches the collector, a fast flow rate increases the diameter and promotes the production of ribbon-like fibers [25]. Finally, the diameter may be raised by increasing the concentration of the polymer solution and utilizing a greater molecular weight polymer.

2.2. Industrial Electrospinning Techniques

The needle-based electrospinning technique is not particularly suitable for industrial applications due to its low production rate and its lack of economic and productive potential for scale-up, such as with the needle-free electrospinning technique. In general, three additional techniques for scaling-up nanofiber manufacturing include multi-jet electrospinning, multi-needle electrospinning and needle-free electrospinning. In the multi-jet electrospinning technique, a uniform web of nanofiber is not generated due to the repulsion effect between jets. Using an array of syringes as spinnerets increases the pace of nanofiber production while also allowing for the mixing of various polymers at the right ratio [26]. However, needle-free electrospinning can achieve the highest mass production rate of nanofibers. This method is considered to be a novel electrospinning mode that originates from an open liquid surface. Unlike needle-like nozzles, many jets are produced from the surface of the polymer solution using a metal electrode and without the impact of the capillary effect. Using this needle-free electrospinning technique, it is possible to produce large quantities of nanofiber mats with consistent quality [27,28]. Electrospun membranes with interconnected porous morphology and homogeneously distributed nanopores are ideal for pressure-operated liquid filtration processes, which may be achieved by using various spinneret shapes [29]. A schematic representation of the needle-based (see Figure 2a) and needle-free electrospinning (see Figure 2b) machines is shown in Figure 2.

Figure 2. Needle-based (a) and needle-free (b) electrospinning setups. Reprinted from [30], originally published under a CC-BY 4.0 license.

3. Chitin

Chitin is a polysaccharide that can be found in a variety of living organisms. After cellulose, chitin is one of the most widely distributed natural polymers [31]. Chitin is found in the exoskeletons of arthropods and the cell walls of fungi as structured crystalline microfibrils. Crab and shrimp shells are the most common commercial sources of chitin, and the extraction process is tailored to the chitin source to ensure excellent quality. Chitin is extracted in industrial processing by first dissolving calcium carbonate in an acidic solution, then dissolving proteins in an alkaline solution. Chitin by nature is not colorless, and to obtain colorless pure chitin, a decolorization process is often used [32]. Chitin is a natural, biocompatible, and biodegradable polymer that is extensively utilized in biomedical and pharmaceutical applications. However, it has additional uses when partially deacetylated to chitosan under alkaline settings [33,34].

3.1. Extraction Procedure of Chitin from Crustaceans

One of the main advantages of chitin recovery from crustacean shells is that this unused resource can provide an additional source of chitin [35].

The shells from which chitin is extracted have a significant influence on the final quality of the isolated chitin. For example, shrimp have a thinner shell wall, which makes the isolation of chitin easier. To obtain chitin from shellfish, the shells are first cleaned, dried, and crushed into small pieces at the end of the process. Then, the two main components of the shell are removed—proteins by deproteinization and inorganic calcium carbonate by demineralization. Furthermore, chemical or enzymatic treatments are provided to remove colorants and lipids. In addition to deproteinization by NaOH, a partial deacetylation of chitin will take place, as well as hydrolysis of the biopolymer, resulting in a reduction in molecular weight [35].

Acid treatment in dilute hydrochloric acid is often used to demineralize calcium carbonate, which involves its breakdown into water-soluble calcium salts and the release of carbon dioxide. Salts may then be readily removed by filtering solid chitin followed by washing with deionized water [32]. Chitin has excellent film-forming characteristics as well as good stability, which is supported by the formation of a hydrogen bond network between the chains. Chitin imparts biocompatibility, biodegradability, non-toxicity, and antibacterial activity to the subsequently processed materials [33,34].

3.2. Chitin Resources In Several Types of Mushrooms

As a structural component, chitin is commonly included in the skeletons of invertebrates such as insects, sponges, squid, and octopus, as well as in shellfish derived from the waste of processing marine products, such as crabs, shrimp, and krill [36]. However, chitin also exists in the structure of the mushroom cell wall and determines its shape and rigidity [36,37].

The mushroom *Ganoderma Lucidum* was dried for 9 days, then the dried biomass was pulverized and washed with deionized water, subjected to ultrasonic vibration for 40 min to break cell membranes and facilitate further reactions, and finally centrifuged [36]. The obtained powder was washed with ethanol for 24 h to form a polysaccharide precipitate and to obtain purer chitin. Afterward, it was repeatedly deproteinized with alkaline solution NaOH at 100 °C for 2 h, 3 times in total, then neutralized. The extracted crude chitin was decolorized with potassium permanganate (KMnO₄) for 1 h, then treated with oxalic acid (C₂H₂O₄) for 1 h leading to an increased crystallinity. In the end, the suspension was centrifuged, washed, and neutralized with water, then dried at 50 °C. The yield of chitin was 14.42 mg per gram of dry biomass and the examined morphology revealed typical characteristics of chitin obtained from different sources, which shows that *Ganoderma Lucidum* can be a suitable raw material for chitin [36].

Several advantages can be attributed to the extraction of chitin from the cell walls of mushrooms. Figure 3 shows the fungal mycelium of the fungus *Pleurotus ostreatus*.

Figure 3. (a) Photographs of a fungal mycelium of the fungus *Pleurotus ostreatus* on agar malt extract and (b) confocal laser scanning microscope (CLSM) image of mycelium.

First, being a vegetal source lacking animal-based derivatives, the probability to cause an allergenic response to the human body is lowered. Second, fungi contain a low level of mineral impurities; therefore, the demineralization process is omitted and the extraction method of chitin has to undergo milder treatments, preserving its chemical structure, reducing treatment costs, and promoting a more sustainable process compared to exoskeleton sources [38]. This sustainable advantage was described in a study [39] that demonstrated acid-free isolation of chitin from a fungal substrate with minimal energy consumption based on hot water, alkaline extraction, and gentle mechanical agitation. This contrasts with the conventional method of extracting chitin from crustaceans, which always requires an acid extraction step to remove minerals. Third, the physicochemical properties of chitin are better controlled, as they usually vary between the different crustacean species and their harvesting periods [40]. This is due to the automation and synchronization of mycelia production. While crustacean shell wastes are limited by seasonal supply, mycelia can be grown all year round in a rapid, automated, and controlled process by fermentation under submerged culture in bioreactors, so that each batch produces a homogeneous quality and quantity of mushroom biomass [41,42]. Moreover, raw chitin from mushrooms has inhibitory effects on bacterial growth compared to chitin from crab shells, which does not have this effect [43].

Agaricus bisporus belongs to the most consumed mushrooms worldwide due to its simple and fast growth cycle, meaning it is a good alternative for the large-scale production of chitin [44].

In a previous experiment for chitin extraction from *Agaricus bisporus*, the highest dry weight chitin yield of 7.4% was found in the stripes, which are normally wasted during mushroom production [45]. By exploiting this resource generated from mushroom waste, an interesting opportunity can be foreseen for the production of high-purity chitin using a mild alkaline treatment with NaOH at 80 °C for 2 h to limit its degradation, followed by a lyophilization (freeze-drying) process. In order to preserve the physicochemical properties of chitin that influence its biological character, chemical extraction with strong acids and alkali was replaced by natural deep eutectic solvents. The procedure consisted of depigmentation of *A. bisporus* mushrooms with H₂O₂, followed by demineralization and deproteinization with mild acids such as ascorbic or citric acid, and ending with the heated dispersion of mushrooms in a homogeneous solution of choline chloride, betaine chloride, guanidine, urea, and sorbitol. After the reaction, the centrifuged chitin was rinsed and lyophilized and revealed good quality upon examination by different techniques [46].

Isolation of a class IV chitin synthase gene from the edible basidiomycete *Pleurotus ostreatus* was conducted in the study by Nishihara et al. [47].

Higher dry weight yields of chitin were obtained from two other mushroom species [48]. *Lactarius Vellerius* is a commonly consumed mushroom in Turkey, known for its antioxidant and antimicrobial performance [49]. Its obtained dry weight chitin content was 11.4%, of

which 73.1% generated chitosan, whereas the wild mushroom *Phyllophora Ribis*' dry weight chitin yield was 7.9%, 75.3% of which turned to chitosan [48].

4. Chitosan

The term chitosan belongs to the class of polymers formed by varying degrees of deacetylation of chitin, resulting in a random copolymer with a molar fraction of N-acetyl-D-glucosamine and D-glucosamine repeating units (Figure 4). The molar percentage of N-acetylated units distinguishes chitin from chitosan and is used to calculate the deacetylation degree (DDA), which indicates the balance between the two kinds of residues. The product is called chitosan when the DDA amount is greater than 50% [50]. Acetyl groups are removed during deacetylation, where depolymerization also occurs resulting in variations in chitosan molecular weight (Mw) [50].

The majority of chitosan types are not soluble in water and most organic solvents [51,52]. However, chitosan can be easily dissolved in acidic aqueous solutions below pH 6.3, whereas if the concentrations are higher than 2 wt%, this leads to extremely viscous behavior of the solutions [53]. Either enzymatic preparations or chemical processes would convert chitin to chitosan [54–56]. However, as glycosidic bonds are very sensitive to acids, alkaline hydrolysis is employed more often to convert the acetamide group to the amino group in chemical processes [56,57].

The distribution of N-acetyl-D-glucosamine and D-glucosamine residues along polymeric chains is influenced by heterogeneous or homogeneous N-deacetylation of chitin [58–60]. Variations in chitosan preparation, such as alkali concentration, incubation duration, chitin ratio, chitin source, temperature, deacetylation method, and single or multiple processes, may alter the degree of acetylation (DA), acetyl group distribution, Mw, and viscosity in the solution, thereby influencing chitosan's properties [61–63].

Figure 4. Chemical structures of chitin and chitosan. Reprinted from [64], originally published under a CC-BY 4.0 license.

4.1. Parameters Influencing the Antibacterial Properties of Chitosan

4.1.1. pH of the Solution

CS has a low solubility above pH 6.5, meaning the antibacterial effect is only developed at an acidic pH. This is due to the protonation of NH_2 groups, where CS becomes polycationic and interacts with negatively charged microbial cell membrane components such as phospholipids, proteins, anionic polysaccharides, fatty acids, and bile acids [65–67].

The positive charge enhances the adsorption of chitosan on bacterial cells and increases the antibacterial activity at lower pH values [68]. At higher pH values (>6), chitosan precipitates out of solution as it loses its charge due to deprotonation of amino groups [69–72].

The antibacterial activity of chitosan against *Klebsiella pneumoniae* depends on the degree of protonation of the chitosan amino groups, which depends on the degree of polymerization as well as the pH of the medium [73]. CS is not soluble in water. For the production of nanofibers, CS can be dissolved in acidic solutions with a pKa value of 6.3 or below, because at this pH value the glucosamine units will convert to the protonated soluble form (-NH_3^+). The solubility of CS depends on its precursor source, its molecular weight (MW), and the degree of deacetylation (DDA) [74].

4.1.2. Concentration

According to several studies, the antibacterial activity of chitosan increases with increasing concentration [75–78]. However, according to the study of El-Tahlawy et al., when comparing the antimicrobial activity of different concentrations of chitosan coated on cotton fabrics, it has been found that smaller concentrations of 0.5–0.75% achieved the highest antimicrobial activity, whereas the concentration of 1% led to decreased antibacterial effects [79]. It has been found that at low concentrations, chitosan binds to the negatively charged cell surface and destroys the cell membrane, which ultimately kills the cell. At higher concentrations of chitosan, mutual repulsion of positively charged bacterial cells occurs and agglutination is avoided [80].

Very low concentrations, on the other hand, do not destroy bacteria. According to the findings of another research, all chitosan samples exhibited excellent antibacterial activity against *Escherichia coli* (*E. coli*) at concentrations above 0.02%, while all samples at lower concentrations of 0.002% may encourage *E. coli* development. They proposed that chitosan might cause bacteria to flocculate and kill them, although at low concentrations of 0.002%, it could not flocculate and kill all of them, meaning their survival would continue via reproduction [81].

4.1.3. Molecular Weight

High-molecular-weight chitosan, low-molecular-weight chitosan, and oligochitosan (short-chain chitosan) are three distinguished types of chitosan [81]. Younes et al. [82] recently showed that lowering MW increased antibacterial efficacy for Gram-negative bacteria, while the reverse impact was found for Gram-positive bacteria. Several researchers observed the discrepancy in antibacterial activity, and it was postulated that high-molecular-weight chitosan cannot pass through the microbial membrane, meaning it accumulates on the cell surface, which alters the cell permeability by blocking nutrient transport and results in cell lysis [83–86]. Dissociated chitosan molecules in solution with a smaller molecular weight may, on the other hand, bind to DNA and suppress mRNA production by penetrating the nucleus of bacteria [87–90].

4.1.4. Degree of Deacetylation

Through the influences on the amount of free amino groups in the polysaccharides, the deacetylation degree of CS influences the solubility as well as the charge formation of the functional groups and is dependent on the positive charge density. The antibacterial effect of CS with a higher positive charge density is more effective [91].

5. Electrospinning of Chitosan

The D-glucosamine repeat unit in chitosan polymers is based of two monomers: N-acetyl-D-glucosamine and N-amino-D-glucosamine [92]. To achieve the desired properties, chemical reactions often target functional amino groups. The N-amino-D-glucosamine/N-acetyl-D-glucosamine ratio, called the degree of deacetylation (DDA), is a key indication that distinguishes chitosan from chitin, resulting in chitosan's unique characteristics. However, during the deacetylation process, acetyl groups are eliminated, influencing the

molecular weight (Mw) that must be considered when electrospinning a chitosan-based solution [93]. Several parameters, such as the voltage, flow rate, distance between electrodes, ambient temperature and humidity, electrospinning solution properties, pH, viscosity, molecular weight, and mixing ratios of polymers and solvents play crucial roles in the final nanofibers [94–98].

5.1. Voltage

Voltage plays an important role in the electrospinning process and has an influence on the nanofiber diameter and the surface morphology of the nanofiber mat. If the electrostatic force outweighs the surface tension of the electrospinning solution, a thin thread in the nano-range is produced. By regulating the voltage, different morphologies can be produced depending on the final requirement, from nanofibers to membranes [99,100].

5.2. Feed Rate

With needle-based electrospinning technology, a solution feed rate, located in a syringe, has an influence on the final nanofiber mats. As the solution feed rates increase, spun fibers have larger diameters compared to lower solution feed rates. With the needle-free technique, the wire is coated with an electrospinning solution. Therefore, there is no solution feed rate in this technique [101,102].

5.3. Distance

The variations in the jet flight duration and the electrical voltage, as well as in the distances between the electrodes, influence the morphology of the nanofibers produced. The nanofibers are formed, the solvent has enough time to dissipate, and dry nanofibers are deposited on the substrate. If the distance between the electrodes is too small and the solvent does not manage to dissolve completely from the nanofibers, this results in a membrane-like morphology or the nanofiber diameter increases [103].

5.4. Viscosity

The solution viscosity of chitosan is the main element that hinders continuous fiber formation and is normally affected by chitosan's molecular weight, concentration, and solvent characteristics. Rigid D-glucosamine repeating units in chitosan are responsible for the formation of inter- or intramolecular hydrogen bonds and the increase in crystallinity. This leads to low solubility in pure water as well as in other organic solvents. A high concentration of CS leads to high viscosity, because due to repeating polar groups on the polymer, the increase in hydrogen bonding is affected. The protonation of primary amines in low pH using an acetic acid solution in water improves the water solubility of chitosan [92]. This is because in aqueous acidic conditions intrachain hydrogen bonding is prevented by the electrostatic repulsive interactions between positive ammonium groups, while the enhanced creation of interchain hydrogen bonds with water molecules improves the chitosan solubility. Solubilizing the polymer in 90% acetic acid is one way to electrospin pure chitosan at a 7 wt% concentration, as it reduces the viscosity by slowing down the coagulation rate [97]. If the viscosity is too high, the solution will become excessively viscous and fiber production will be hindered. In this case, the electrical voltage will not be sufficient to overcome the viscosity of the solution. If the viscosity is too low, the production of nanofibers will either not be possible, because polymer chains are not intertwined enough, or instead polymer beads will often be formed [20,104].

5.5. Molecular Weight

In some cases, the lower molecular weight (9.5–10.5%) of the chitosan solution leads to the formation of large beads in nanofibers. For chitosan solutions with higher molecular weights (2.5–3%), coarser and finer nanofibers with fewer pearl defects are formed [20].

It can be seen from observations that very high molecular weights create significant entanglement interactions between long polymer chains and repulsive forces between

ionic groups [105,106]. This has a negative effect on electrospinning and the production of continuous fibers is inhibited [107,108]. For the optimal production of nanofibers with homogeneous nanofibers without beads, it is recommended to use a chitosan solution with a medium molecular weight.

5.6. Concentration

Chitosan is a cationic biopolymer and influences the hydrodynamics of the solution. Even low concentrations of chitosan in electrospinning solution become too viscous and impair the formation of nanofibers due to the difficulty of overcoming the electrical field. In summary, it can be said that the imbalance between the electrical forces and the surface tension leads to the elongation of the jet as well as the change in the charge density. In addition, evaporation of the solvent also has an influence on the unstable shape of the jet and the formation of secondary rays during the electrospinning process [109,110].

5.7. Auxiliary Solvents and Composites

Molecular modification of chitosan was also proven to improve its spinnability. For example, the addition of carboxymethyl to the chitosan structure significantly influences its water solubility [98]. The degradation temperature of chitosan below its melting point limits its processability as a casted film and its application development on a wide scale. On the other hand, the advantage of electrospinning chitosan nanofibers is their high surface area to weight ratio, biocompatibility, and physicochemical properties, and their particularly small diameter, which makes them functional for several biomedical applications, such as in wound dressings, sensors, water filtration, and food protection. However, since CS is a cationic polyelectrolyte, it only becomes positively charged when dissolved in water, resulting in poor electrospinning capabilities [111,112]. By tweaking the electrospinning process and solution parameters, the anticipated and final geometry of the processed solution may be estimated and controlled. It was shown that the molecular adhesion between chitosan chains is poor at low polymer concentrations, resulting in bead formation. Uniform and beadless nanofibers were effectively produced when the polymer content or Mw increased. The longer polymer chains allow sufficient chain entanglement and result in a stable filament with avoidance of fragmentation. However, high Mw chitosan produces extremely viscous and rigid systems that are difficult, if not impossible, to electrospin.

Similarly, stronger chitosan content solutions have higher repulsive forces, which results in more stretching and elongation, leading to nanofibers with a narrower fiber diameter distribution [111,112]. As the chitosan concentration increases, the average fiber diameter becomes smaller due to the increase in electrical conductivity. However, a very high content will increase viscosity. This critical variation of factors makes the electrospinning process of chitosan discouraging.

Researchers have experimented with a variety of techniques to enhance the electrospinnability of chitosan. To dissolve chitosan, some researchers utilized trifluoroacetic acid (TFA) for one-step electrospinning [113–115]. The use of anionic polyelectrolyte hyaluronic acid is known for neutralizing cations in chitosan solution [112–117]. However, hazardous solvents should not be used, particularly in biomedical applications [118]. TFA is cytotoxic, caustic, and ecologically damaging, making it unsuitable for uses such as food packaging [94].

Auxiliary solvents such as PEO and PVA have been presumed to interact with chitosan through hydrogen bonding [119,120], resulting in reductions in electrostatic repulsion and surface tension, lowering the system's viscosity while increasing its flexibility and promoting fiber production. PVA (polyvinyl alcohol) is a water-soluble polymerized alcohol that can improve the capability of the prepared nanofibers. The areas of application of PLA are numerous and this polymer is used in solvents, dispersions, paper manufacture, electronics, cosmetics, medicine, textile impregnation, as well as aerospace [121,122].

PVA is also widely used in biomedical fields due to its non-toxic, hydrophilic, and biocompatible properties [123]. Related research studies [124–126] have described some

cases of nanofibers made by electrospinning PVA and chitosan, whereby the aqueous PVA solution also removes potential organic solvent residues. As a result, antibacterial materials made of PVA and CS are considered harmless, are biocompatible, and offer easy adaptation for defined applications.

With increasing CS content ratio in the mix, the fiber diameter decreased progressively. However, no fiber was produced when the CS content was more than 30% [127]. This means that a very high content of chitosan in a copolymer solution induces repulsive interaction between ionic groups inside the polymer backbone and obstructs the electrospinning process. As for the solution concentration, it was found to simultaneously increase with the average diameter of the fibers with a minimal and maximal limit. Below 3 wt% of CS/PVA solution, it was impossible to obtain bead-free continuous nanofibers, while above 10 wt%, the solution became too viscous and the electrospinning process proved difficult to maintain. As a result, the electrospinning blend's optimal concentration should be between 7 and 9 weight percent [127].

The combination of PVA and CS composite materials leads to increased toughness, controlled biodegradability, and chemical functionality. As CS may interact with AgNO_3 and decrease the repulsive force between ionic groups inside the polymer backbones, adding a small quantity of AgNO_3 to a PLA/CS blend solution could optimize its electrospinning ability, resulting in a shift from a bead-on-fiber structure to uniform fibers [128]. In addition, the combination of CS with Ag nanoparticles sustained antibacterial activity and was greater than that of the individual components [129].

Derivatives of chitosan, such as quaternary ammonium salts, are anticipated to improve chitosan's water solubility and antibacterial activity. The newly synthesized polymer N-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride (HTCC) became water-soluble and electrospinnable when polyvinyl alcohol (PVA) was added. It was discovered that increasing HTCC concentration in blends improves the electrospinnability and decreases the fiber diameter. Nanofibrous PVA-HTCC mats showed good antibacterial activity against *E. coli* and *S. aureus* [130].

5.8. Discussions Regarding the Formation of Chitosan-Based Nanofibers for Defined Purposes

The production of chitosan-based nanofibers is challenging, and variations in the different electrospinning parameters influencing the antibacterial properties of chitosan have to be taken into account, as discussed in Sections 4 and 5. Starting from the surface morphology of the nanofibers, the mechanical properties, the defined application for which the nanofibers are intended, and many other aspects must be considered and balanced. Little information can be found regarding the obstacles faced in achieving the desired fiber properties, such as size and diameter properties. During the electrospinning process, many parameters such as the concentration, solvent, viscosity, electrode-to-electrode distance, environmental conditions, and the electrospinning technology used (needle-based, needle-free, etc.) affect the final morphology and mechanical properties of the nanofibers. Obviously, the studies in the field of chitosan-based nanofiber production are supportive, although in order to produce defined nanofibers, experimental studies are required.

In summary, it can be stated that an optimal solution for defined nanofibers with desired morphology and antibacterial properties is difficult because the variations are manifold. Therefore, a balance should be struck between different aspects to produce optimal nanofibers with defined properties.

6. Antibacterial Effect of Chitosan Nanofibers

The flexibility of the chitosan application depends on its physical, chemical, and biological properties. These properties are a function of its structural characteristics and production method, such as the extraction source and procedure, molecular weight, pH dependency, concentration, and electrospinning technique. In acidic environments, the protonation of its functional amine groups gives it a polycationic nature, while at higher

pH, intermolecular interactions via hydrogen bonds and van der Waals forces facilitate the formation of fibers, films, porous structures, and gels [131].

Chemical modifications to its molecular structure, including crosslinking, may affect the material properties such as the tensile strength, biodegradability, and microbial cell affinity, which are crucial for several application fields prone to the attack of different bacteria strains.

6.1. Mechanism of Antibacterial Activity of Chitosan

Studying the antimicrobial mechanism of chitosan, several theories have been proposed according to its characterized nature and electrostatic attraction. The first theory suggests that positive charges carried by the chitosan polymeric chain interact with negatively charged cell wall components such as phospholipids and lipopolysaccharides [132] in the case of Gram-negative bacteria, and with teichoic acids linked to peptidoglycan present in the cell wall in Gram-positive bacteria, creating cavities and leading to increased perforation and altered permeability of the membrane that results in the leakage of intracellular components, ending up in bacterial lysis (breakdown) [133]. Figure 5 shows the mechanism of attack of chitosan on the bacterial cell wall. Furthermore, the amine groups responsible for the chelating effect of chitosan will cause displacement of metal ions such as Ca^{++} from anionic sites of the membrane, eventually destabilizing these trace metals that are essential for the bacterial growth, leading to their efflux from the cytoplasm to the external medium. Simultaneously, high-molecular-weight chitosan surrounds and encloses the bacterial cell and blocks the cell exchange with the extracellular matrix and absorption of essential nutrients, preventing cellular metabolism, while electronegative sites of the membrane compete for the remaining Ca^{++} , leading to subsequent leakage of the intracellular material [134,135] (see Figure 5a,b). In contrast, low-molecular-weight chitosan can penetrate the cell, causing impairment of the physiological activities of the bacteria and preventing the replication of DNA, which leads to cell death.

Figure 5. (a) Mechanism of attack of chitosan on the bacterial cell wall of Gram-positive and (b) Gram-negative bacteria. Adapted from [136], originally published under a CC-BY 4.0 license.

The antibacterial properties of chitosan nanofibers may be based on the action mechanism of partial solubilization of chitosan in the medium. Short chitosan chains can penetrate the plasma membrane and the cell wall, while longer chains can encapsulate bacteria and prevent the exchange of cells with the extracellular medium [136]. Further forcing the antibacterial activity of chitosan nanofibers, the surface characteristics and cell stages were considered by another research group in response to the treatment with chitosan nanofibers. An impermeable envelope of chitosan around the bacteria could be observed, which may have blocked the absorption of vital components into the cells, and the cytoplasmic membranes of *E. coli* and *S. aureus* cells completely collapsed after 30 min contact time. It was hypothesized that chitosan nanofibers' bactericidal action is the

consequence of membrane permeability and perforation, involving a complicated mix of various bactericidal effects. First, bacterial growth is inhibited by membrane capture and perforation. Then, partially solubilized chitosan chains restrict cell exchange and nutrient absorption. Finally, chitosan particles induce osmotic shock by chelating trace elements required for bacterial survival. However, no indication of membrane penetration could be deduced. Since bacteria require adhesion to surfaces to grow and multiply better, the researchers concluded that the bacteria migrate to the surfaces of the nanofibers [94,137].

The capacity of chitosan to attract and capture bacteria is, therefore, the initial stage in the nanofibers' mode of action, which is followed by membrane perforation, cytoplasmic compound leakage, and ultimately cell lysis and disintegration.

6.2. Susceptibility of Different Bacterial Strains to Chitosan

Conflicting opinions regarding the greater or lower sensitivity of chitosan towards Gram-positive and Gram-negative bacteria are found in the literature.

The different strain behaviors are attributed to the variance in the membrane structures of Gram-positive and Gram-negative bacteria and the electrostatic charges involved. Most bacterial cell surfaces have a net negative charge due to proton dissociation of carboxyl, phosphate, and amino groups on bacterial cell surfaces [138,139].

The cell wall of Gram-positive bacteria consists of two layers. The first layer consists of a thick peptidoglycan mesh layer (Murein), which is located over the plasma membrane. The other layer is a lipid bilayer made of interconnected sugars and peptides that are functionalized with teichoic acids and are mainly responsible for the negative charge on the bacterial membrane. Gram-negative bacteria exhibit a sandwich membrane structure with an outer wall composed of phospholipids, lipopolysaccharides (endotoxins), and lipoproteins, as well as a thin peptidoglycan layer and an inner cytoplasmic membrane. The phosphate groups of LPS on the outer cell wall make the cell membrane more hydrophilic and highly polar, with a negative surface charge density [140]. The cell wall compositions of Gram-positive (see Figure 6a) and Gram-negative (see Figure 6b) bacteria are shown in Figure 6.

Figure 6. Cell wall compositions of (a) Gram-negative and (b) Gram-positive bacteria. Preprinted from [141], originally published under a CC-BY 4.0 license.

Some researchers discovered that chitosan showed bactericidal effect against the Gram-positive bacteria *L. monocytogenes*, two *Bacillus* strains, *S. aureus*, and three *Lactobacillus* strains. However, it was only able to stop further growth of Gram-negative bacteria such as *Escherichia coli* (*E. coli*), *P. fluorescens*, and *V. parahaemolyticus*, without killing them. Meanwhile, *S. typhimurium* was totally resistant to chitosan [142].

One research group theorized that chitosan acts on Gram-positive bacteria due to a polymer barrier on the cell surface that prevents food penetration [143]. Another research group suggested that low-molecular-weight chitosan penetrates the cell wall, interacts with

the electronegative lipopolysaccharides, and upsets cell metabolism, which consequently inhibits the growth of *E. coli* [144].

An investigation was conducted to analyze the bacterial susceptibility or resistance to chitosan nanofibers with regards to the membrane hydrophilicity, surface charge density, and pathogenicity of each bacteria strain. In typical conditions, bacterial growth phases were clearly noticed. Per contra, the presence of CNFs in a bacterial culture entirely suppressed *E. coli* growth, while *S. typhimurium* development was substantially affected. Moreover, there was no growth recovery after deprotonation of chitosan when the pH of the suspension was neutralized with NaOH, indicating that CNFs possess a bactericidal effect that is irreversible and permanent. In another attempt to find out the main factor behind this effect, CNFs were treated with the anionic surfactant sodium dodecyl sulfate (SDS) in order to conceal their positive charges. The slight decrease in bacteria growth can be either attributed to the cell lysis typically caused by SDS solution at high concentrations or to chitosan chains forming a barrier layer that prevents cell exchange, as well as to the fact that certain amino groups of CNFs tend to be protonated after the addition of salt. To rule out other possibilities, NaCl was also applied to screen the charges of NH_3^+ at concentrations lower than that which can normally cause the cell lysis of *E. coli*, although the same growth with a decreasing effect was reached. This contradicts the fact that SDS caused the lysis. Furthermore, the slight decrease in OD was insignificant compared to the antibacterial effect that could be achieved by the positively charged groups. The results suggest that the protonated amino groups in CNFs were dominantly responsible for the inhibition of *E. coli* [144].

With the aim of reproducing real food systems, the mechanism of action of CNFs against food spoilage bacteria such as *Escherichia coli* (*E. coli*) and *Listeria innocua*, as well as pathogenic *Staphylococcus aureus* (*S. aureus*) and *Salmonella typhimurium*, were investigated under the same environmental conditions [145]. After 60 min of exposure, 99.9% of Gram-negative *E. coli* were inactivated compared to 180 min for Gram-positive *L. innocua* and 240 min for *S. aureus*. As for *S. typhimurium*, only a decrease of 99% could be marked. The presence of lipopolysaccharides containing phosphate and carboxylic groups is considered the major reason for the increased hydrophilicity and negative surface charge density of Gram-negative bacteria [145], giving the cell wall a highly polar character and leading to a stronger affinity for chitosan than Gram-positive bacteria, which is the main responsible factor of the bactericidal effect. However, the results revealed that the antibacterial activity of CNFs is not Gram-dependent but rather strain-specific. Accordingly, as shown in Figure 4, chitosan's strain sensitivity can be classified in the following order: *E. coli* > *L. innocua* > *S. aureus* > *S. typhimurium*. Nevertheless, the results (Figure 7) show that Gram-positive *L. innocua*, which is supposed to be less hydrophilic, was more susceptible than Gram-negative *S. typhimurium*. The bacteria were then classified between pathogenic and innocuous (harmless). It was observed that the most susceptible strains *E. coli* and *L. innocua* were both innocuous, while the most resistant *S. aureus* and *S. typhimurium* were pathogenic. The conclusion was that chitosan nanofibers have little influence on pathogenic bacteria regardless of whether they are Gram-positive or Gram-negative. However, they can inhibit the growth of innocuous bacteria depending on the hydrophilicity and surface charge density, so the greater influence, in this case, would be on the more hydrophilic Gram-negative innocuous bacteria *E. coli*, followed by the Gram-positive innocuous bacteria *L. innocua* [146].

Figure 7. Antibacterial activity of electrospun chitosan/PEO (80:20) nanofibers with different molecular weight against *E. coli*, *S. Typhimurium*, *L. innocua*, and *S. aureus*. Reprinted from [146], originally published under a CC-BY 4.0 license.

6.3. Effect of Surface Topography on the Antibacterial Activity

Using electrospinning and film casting techniques, two samples of chitosan/PVA with the same volume ratio and concentration were created [147]. For the film formation, a solution of 4 wt% hydrolyzed chitosan in acetic acid at 60 °C was produced. The 10 wt% PVA solution was then produced using a chitosan/PVA ratio of 70:30 [147].

To enhance chitosan electrospinning, the crude powder was hydrolyzed in a 50% NaOH solution to increase its DDA and decrease its molecular weight. Because of its non-toxicity, biocompatibility, biodegradability, solubility in acetic acid, and ability to form hydrogen bonds with chitosan through amino or hydroxyl groups, PVA was added to chitosan to improve the solution mixing and formation of stable jets. A voltage of 21 kV and a distance of 15 cm were the optimum electrospinning parameters for the generation of a beadless structure. The goal of the performed antibacterial assay was to determine the effects of surface nanotopography on the antibacterial activity of materials using two distinct bacterium strains, *E. coli* and *S. aureus* [147].

The initial step of colonization and biofilm development is bacterial adhesion to surfaces. Thus, the impact of the morphology on the bacterial adhesion was tested using an identical composition for a film-cast sample (8a) and electrospun sample (8b). Images of the samples are shown in Figure 8. For both bacterium strains, sample (8b) had greater antibacterial activity than sample (8a), indicating that bacteria adhere better to the sample (8b).

The amount of bacterial attachment has been demonstrated to be influenced by surface roughness, as bacterial cells may detect roughness changes of one micrometer or less [148]. The nanofibrous membrane's enhanced roughness relative to the film (Figure 8) seems to have a favorable impact on antibacterial activity, which is substantially higher in both Gram-positive and Gram-negative strains for sample (8b). Given the micron size of bacteria, it is known that the attachment of rod-shaped bacteria such as *E. coli* to nanofiber structures with diameters less than the bacterial length causes conformational changes in bacteria as they wrap around each fiber [149]. Consequently, the surface's overall antibacterial action may be enhanced.

Figure 8. Comparison of the surface topography between a film (a) and nanofibers (b), both made of chitosan. The nanofibers show a rough structure favorable to the attachment of bacteria. Adapted from [147], originally published under a CC-BY 3.0 license.

7. Application Fields of Electrospun Chitosan Nanofibers and Chitosan Derivatives

In the realm of materials research, the integration of nanofibers in product innovation and development is a fast-expanding subject. Chitosan's inherent characteristics, such as its non-toxicity, biodegradability, and antibacterial activity, as well as its ability to be processed into high-surface-area nanofibers, make it suitable for a large selection of future-oriented research and commercial purposes. Because of its cationic character and high crystallinity, processing pure chitosan into nanofibers can be difficult; consequently, it is frequently treated or mixed with various additives to increase its processability and customize its properties according to specific requirements. In addition, bioactive inorganic compounds can be incorporated into these nanofibers to render them more functional.

7.1. Water Filtration

Pathogenic contamination is considered a significant worldwide water concern. Traditional techniques for water purification and disinfection, such as treatment with chemicals or conventional membrane filtration, are not flawless.

Conventional water contaminant removal includes the use of chemical disinfectants and biocides to inactivate pathogens found in water, such as chlorine gas and dioxide. These methods, however, have proven to require a long reaction time and chemical agents are required in high concentrations, which are difficult to prepare because of their fast degradation and capacity to react with other substances in the water, leading to carcinogenic by-products [150]. Moreover, due to the regular use of chemical disinfectants, new variants of pathogens have emerged and adapted to the existence of these agents to the point of resisting them. In addition to chemical pollution and cytotoxicity concerns, the continual addition of bactericide may result in bacterial resistance when bacteria are subjected to subinhibitory biocide doses [8].

Therefore, physical processing methods such as membrane filtration and distillation are growing in importance because of their low cost, simple application, and efficiency. Con-

ventional membrane filtration of microorganisms, on the other hand, results in the development of biofilms that considerably reduce the quality of the filtered water [151]. Because of the difficulties with traditional water purification and filtration systems, the improvement of water filtration systems is an appealing research topic. Functional nanofibers are increasingly acknowledged as effective materials for environmental remediation. Nanofiltration is a technique for purifying, desalinating, and disinfecting water. Electrospun nanofibers have unique properties such as a large surface area and a high aspect ratio combined with a high mechanical stability. It is also possible to incorporate nanoparticles with different functionalities into the fibers. These properties make the functionalized fibers an excellent filter medium for water, other liquids, and air [152,153]. Reducing the fiber diameter while preserving the mechanical properties, fiber functionalization, and increased throughput are the major challenges [154].

7.1.1. Filtration Types and Membrane Criteria

Usually, the most often used characteristics to assess membrane performance are high filtration capacity, high water flux and hydrophilicity, low transmembrane pressure, high porosity, small pore size, mechanical strength, antifouling and self-cleaning ability, and space-saving.

Water filtration can be performed in different ways and a distinction between the 4 types of water filtration systems is possible [155]:

1. Microfiltration (MF) is extensively used in particle removal and degreasing processes;
2. Ultrafiltration (UF) is a technique used for separating ions from oil, water, and emulsions without the use of chemicals. With UF, paints can be additionally recovered, and fats, oils, and greases from the food sector can be separated, which MF cannot remove. It can remove high-molecular-weight compounds, particles, colloids, and inorganic and organic polymers, but not low-molecular-weight ions such as magnesium chloride, calcium, or sodium [155];
3. Reverse osmosis (RO) membranes are used as a popular filtering technique that removes large amounts of pollutants from water. A semipermeable membrane is used to separate dissolved solutes in this system. The distinction between RO membranes and other filtration membranes is that they do not need actual pores. These membranes are hydrophilic, which means that water may readily pass through them. They are easy to set up and provide for a high production capacity [156], but their limiting factors are biofouling and high transmembrane pressure requirements. The biological fouling increases as the number of eliminated microorganisms and other particles in water increases [157], which is one of the drawbacks of using RO membranes. Several studies have shown that at greater working pressures bacterial cells shrink in size, resulting in increased penetration through the filter. Therefore, to maintain antibacterial action during long-lasting application of the membrane, lower transmembrane pressure (TMP) is generally sought [8];
4. Electrospun nanofibers provide the possibility of producing membranes of nanocomposites. The pore size and porosity as well as the antibacterial properties are adjustable by use of antibacterial components. Therefore, the easiest method to eliminate microorganisms, metal ions, and dyes while maintaining minimal pressure drops and high water flux is by nanofiber adsorption.

In particular, nanofiltration membranes are capable of separating particles with sizes ranging from 0.001 to 0.01 μm because of their high surface-area-to-volume ratio, their pores being in the nano-meter range, the high porosity, and their high air and water permeability. The large surface area of the electrospun nanofiber membranes offer an abundance of adsorption sites for metal ions and dyes, and the higher porosity results in lower driving forces to drive water through the membrane, making the process less energy-demanding and simpler [158].

With less fouling and lower transmembrane pressure, electrospun nylon nanofibers were able to eliminate bacteria via size exclusion as well as conventional membranes. This

showed that nanofibers can enhance membrane function while keeping bacteria filtration levels high [159].

The right choice of water filtration system depends on the application and can save costs and resources. Table 1 gives an overview of the advantages and disadvantages of the different water filtration systems.

Table 1. Comparison of different water filtration systems.

Water Filtration System	Technology	Species size	Filtered species (examples)	Advantages	Disadvantages
Microfiltration	Membrane	0.1–5 μm [160]	Particles, large bacteria and pathogens, grease	Low-pressure filtration, low energy consumption, no chemicals required, low production costs [160]	(Bio)fouling reduces efficiency, pre-treatment and chemical cleaning are necessary [156,160]
Ultrafiltration	Membrane	0.01–0.1 μm [160]	High-molecular weight material, colloids, particles, endotoxins, some viruses, oil	No chemicals required, low-pressure filtration, low energy consumption, low production costs [155,156]	(Bio)fouling limits performance, pre-desinfection or membrane modification is necessary [155]
Reverse Osmosis	Membrane	0.0001–0.001 μm [160]	Salts, metal ions, solids, colloids, organics	Most important potable system, ultra-pure water [157]	(Bio)fouling limits performance, pre-treatment is necessary, eg. by UV-irradiation [157]
Nanofiltration	Nanofibers, nanofibrous membranes	0.001–0.01 μm [160]	Particles, endotoxins, microorganism, dyes, disinfection, desalination	High surface-to-area ratio, high porosity, small driving forces needed, high water flux, low energy consumption, usage of antimicrobial chitosan reduces biofouling, no further treatment necessary, improved hydrophilicity [158,161,162]	Bio(fouling) is possible

7.1.2. Electrospun Fibers from Chitosan–Polymer Mixtures for Water Filtration

Preparing electrospun nanofiber mats from pure chitosan is difficult due to its cationic character and high crystallinity. Therefore, various experiments were performed with mixtures with different synthetic polymers:

The addition of chitosan to the nylon-6 nanofiber membrane enhanced its hydrophilicity and mechanical strength. Due to the presence of functional groups such as primary amino and hydroxyl groups, increasing the chitosan concentration significantly improved the hydrophilicity of the membrane for pure nylon-6 nanofiber. Increasing the chitosan concentration resulted in an increase in the tensile strength of the chitosan–nylon-6 electrospun membrane. The inhibition zone of chitosan–nylon-6 nanofibers with a polymer ratio of 30:70 on *E. coli* bacteria measured up to 8 mm, while increasing the chitosan amount improved the antibacterial activity to 96%. As a result, the electrospun nanofibers were able to capture bacteria, which prevented them from polluting water. The nanofiber membrane's increased hydrophilicity can increase the antifouling effect, making the electrospun nanofibers a potential candidate for water filtration and improving the membrane performance at low pressures [161].

Due to its hydrophilic nature, the addition of chitosan enhances the membrane's hydrophilicity [158]. In addition, it is assumed that chitosan-modified membranes withstand biofouling for longer periods.

Chitosan and polycaprolactone (PCL) were electrospun from a formic acid–acetone solvent combination. In terms of the fiber morphology, samples with 25 wt% chitosan produced the best results. The electrospinning of chitosan-incorporated PCL nanofibers with a diameter range of 200 to 400 nm resulted in a 50% improved effect against *S. aureus* colonization compared to membranes made of pure PCL fibers. They also seemed promising as pre-filters for water filtration, since they allowed for a high water flux while removing all 300 nm particles [162].

The water transport process through the membrane involves three stages: sorption, diffusion, and desorption. The hydrophilicity of the membrane serves as a driving factor

for water sorption into the membrane. Usually, biofouling decreases as the hydrophilicity of the polymeric material increases, resulting in an increased flux ratio. According to the literature, hydrophobic membrane surfaces have a greater fouling propensity; therefore, hydrophilic membranes are less prone to develop biofouling because they are believed to strongly adsorb a layer of water molecules, preventing bacteria and other foulants from colonizing on their surfaces [163].

Due to the antibacterial potential of chitosan nanofibers, they have been applied to textile-based microfilters to reduce the pore size and to filter microorganisms from air and water based on size exclusion. A blended solution of chitosan–polyethylene oxide (PEO) was electrospun on a non-woven polypropylene substrate and the nanofibrous filter resulted in *E. coli* reduction after 6 h of contact [164].

7.1.3. Enhancing the Antibacterial Performance with Bactericidal Agents

Due to their distinctive polycationic character, chitosan and chitosan derivatives have been suggested for applications in food, gastronomy, agriculture, pharmaceutical products, biomedicine, and biotechnology [165,166]. The antibacterial activities of chitosan are restricted due to the amino groups on the chitosan backbone, which serve as relatively weak positive charge centers. One possibility of increasing the antibacterial activity of chitosan is to add positively charged groups [167].

The large number of hydroxyl and amino groups in chitosan allows it to form metal complexes depending on the type of metal ion, molar ratio, molecular weight, and degree of deacetylation of the chitosan [168]. Various kinds of metal ions and chitosan complexes have recently been developed to enhance the antibacterial action [169]. Silver is the most prominent representative of antibacterial additives. In comparison to unmodified chitosan, the inclusion of a modest proportion of 2.15 w% of silver nanoparticles was adequate to substantially improve *E. coli* inactivation [170]. Another research team found that the Ag–chitosan nanocomposite exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria [171]. Chitosan can additionally stabilize silver nanoparticles and prevents them from sticking together below a threshold concentration. Chitosan leads to a positive charge on the surfaces of nanoparticles, which helps them attach to the negative charged cell wall [172].

Bezir et al. studied the influence of silver doping of chitosan nanofibers on the antibacterial activity. For this, chitosan was dissolved in acetic acid (1 g/20 mL), followed by stirring for 24 h at room temperature, then stirring for 3 h at 80 °C until a homogeneous solution was reached. Afterward, 3 g of the chitosan solution was mixed with 14 mL of polyvinylalcohol (PVA) solubilized in distilled water. Five different silver concentrations, varying from 1% to 5%, were added stepwise and stirred with a magnetic stirrer. For each Ag concentration, Ag-doped chitosan nanofibers were electrospun at a collector distance of 8 cm, with 25 kV of applied voltage and a constant feeding rate of 0.1 mL/h. *S. aureus* bacteria were used to investigate their antibacterial properties. The results of the experiments showed that the antibacterial performance was enhanced as the Ag concentration rose [173]. Another group observed an intense disruption of the *S. aureus* cell wall incubated with chitosan-silver nanocomposites, resulting in a change in its morphology, and concluded that chitosan and silver nanoparticles work synergistically, displaying greater potency than either component interacting on its own. Furthermore, the chitosan–Ag nanocomposites' minimum inhibitory and bactericidal concentrations were reported to be lower than those of pure Ag nanoparticles (AgNPs) [174].

Adibzadeh et al. prepared chitosan/PVA membranes with AgNPs on the fiber surface by adding AgNO₃ to the electrospinning solution. Electrospun fibers were collected on a polyester substrate, resulting in improved mechanical properties that are needed for water filtration. Different contents of AgNO₃ were tested in static and dynamic antibacterial filtration assessments against *E. coli*. Nanofibers from chitosan/PVA with 2 wt% AgNO₃ aimed to kill all bacteria within 60 min exposure time. Doubling the AgNO₃ concentration in the electrospinning solution from 2 to 4 wt% halved the elimination times

for all bacteria. In the release experiments, differences were detected between the static and dynamic filtration assessments. Within 24 h, about 15% of the total Ag content was released in the static experiment and about 10% in the dynamic experiment. The findings showed that antibacterial water filtration and disinfection may be accomplished with the use of chitosan/PVA electrospun nanofibrous membranes containing AgNPs. Further research is needed to regulate the silver release content, which may restrict the application based on various nations' drinking water regulations, as well as to evaluate the long-term performance of the AgNP-containing electrospun nanofibrous membranes [175].

Many reports have suggested that incorporating non-toxic metallic nanoparticles, such as zinc oxide (ZnO) nanoparticles, into electrospun polymers improves the bacterial resistance, stability, and bacterial removal [176,177]. ZnO powder was added to the polyacrylonitrile (PAN) solution, which was electrospun on a rotating drum, and a chitosan/PEO solution in acetic acid at a ratio of 70:30 was electrospun on top of the non-dried PAN/ZnO membrane. It was observed that the smooth fibrous chitosan layer was strongly linked in between the PAN nanofibers, providing high physical interaction, rigidity, and stress absorption, which led to an increase in the tensile strength of 74% and in the elastic modulus of 32% for the dried nanocomposite membrane. As a result, the PAN/chitosan membrane had smaller pores on the surface, meaning the bacteria filtration performance through size exclusion killed up to 99.99999% of *E. coli* in 24 h. However, water flux during bacteria filtration was decreased due to the clogging of pores induced by the increased bacteria concentration. Incorporating ZnO nanoparticles disrupted the polymer chains of PAN and created nano-cavities, which improved the water permeability of PAN nanofibers by 71%. In addition to bacteria filtration, studying the antibacterial effect is important to prevent biofouling. A dramatic improvement in antibacterial performance was observed in PAN/ZnO–chitosan membranes, which killed all bacteria in 3 h compared to the PAN/chitosan membrane, reducing the bacterial concentration by 99.6% after 24 h. Similar to NH_3^+ ions of chitosan, Zn^{2+} ions may interact with the anionic bacteria membrane, harming its surface structure and breaking through it to prevent nucleic acids from replication. In addition, electron pairs in ZnO nanoparticles break under light, generating highly active free radicals capable of penetrating and destroying bacterial cells [178]. Consequently, combining the synergistic effects of chitosan nanofibers with ZnO nanoparticles into the PAN/ZnO–chitosan electrospun membrane increases the antibacterial efficacy upon bacteria filtration and fights the biofouling of the membrane, giving it a self-cleaning property.

7.2. Air Filtration

To protect humans from germs, bacteria, and viruses, ventilation filters are diversely used in buildings, vehicles, and airplanes. Despite the use of a ventilation system, several studies revealed that there were still significant amounts of bacteria in closed environments. For instance, airplane walls are padded with heat-insulating blankets, which might offer a dark environment with condensed moisture for bacteria to thrive and be discharged into the aircraft, threatening passengers' health [179].

The integration of electrospun fibers into air filtration systems has been demonstrated to overcome problems with conventional systems.

In a study by Sun et al. [179], the survival rates of typical airborne bacteria *E. coli* and *Bacillus subtilis* (*B. subtilis*) on blankets with hydrophilic or hydrophobic properties under simulated flight cabin climate conditions were assessed for a classic flight duration of 445 min, repeated in five cycles. The numbers of bacteria decreased tremendously during the first cycle because of the 30% relative humidity, which is unfavorable for bacteria survival, leading to cell dehydration and nutrient deficiency. Nevertheless, at the end of the fifth cycle, 0.4% of the initial *B. subtilis* load survived versus a much lower *E. coli* rate. The interpretation is that *B. subtilis* produces endospores capable of freezing in harsh conditions such as dehydration and radiation and of resuming their reproduction after re-stabilization of the environment. This reveals an alarming risk for human health in closed areas, where

home textiles such as blankets, curtains, and filters represent potential hosts for pathogenic bacteria and allow long-term proliferation if they are not completely killed and even a small fraction survives. In such situations, antibacterial filters integrated within indoor ventilation systems come in handy [179].

Airborne bacterial survival can be reduced using effective filtration systems. Thus, in this study, different filter media were prepared and the *E. coli* survival rates were assessed for 4 groups of positively charged and chitosan-modified filters. A substrate layer of melt-blown fibers covered with a PTFE membrane comprised the positively charged composite filter medium. The three filters were made of pure nylon-6, chitosan-dipped nylon-6, and nylon–chitosan electrospun nanofibrous membranes. In comparison to the *E. coli* decreasing rate on the uncharged control sample, the *E. coli* bacterial survival rate on the positively charged filter dropped to 17.8% after six hours, whereas the bacteria dropping rates were sharper on the nanofibrous filters, with 8.4% after two hours (pure nylon filter), 7.1% after two hours (chitosan/nylon composite filter), 2.8% after two hours, and 0% after four hours (chitosan-dipped nylon filter). These findings reveal that the chitosan on the filter's surface may significantly suppress microorganisms. Additionally, the filtration preparation method had an influence on its efficiency; filters with chitosan coatings were superior to filters containing blended nanofibers. This might be due to the possible formation of hydrogen bonds between the carbonyl groups in nylon-6 and the amino groups in chitosan, thereby reducing the generated amount of NH_3^+ groups in reaction with water molecules. Due to this cohesive mixture, fewer chitosan molecules were present at the fiber surface, resulting in less chitosan–bacteria interactions and influencing the antibacterial ability of the blended electrospun membrane in comparison to the dipped filter medium [180].

Decreasing the nanofiber diameter has several positive benefits, including an increased specific surface-area-to-volume ratio and decreased average pore size. Ultrafine chitosan/PVA hybrid nanofibers incorporating TiO_2 or Ag nanoparticles were produced by utilizing needle-free electrospinning at a large scale. The daily production may exceed 1.2 kg, allowing these ultrafine nanofibers to be used in air filtration and environmental cleaning. Fibers were produced at a rate of 50 g/h, which was much greater than the conventional needle electrospinning method's range of 0.02–1 g/h. Smooth and homogeneous nanofibers with diameters ranging between 25 and 60 nm were produced by adjusting the spinning parameters, and Ag nanoparticles were coated on the surfaces of the nanofibers. The chitosan hybrid nanofibers demonstrated a substantial improvement in filtering effectiveness (>99%). Thus, the nanoparticle aerosol removal was similar to commercial high-efficiency air filters for particles, but with less mess and a reduced pressure drop. They also demonstrated remarkable antibacterial activity while laying a thin coating of nanofibers, killing 99% of *S. aureus* and 97% of *E. coli* within two hours. Under high-humidity and water immersion conditions, the crosslinked nanofibers showed good water resilience and maintained a stable microstructure [181].

In summary, in air filtration, electrospun fibers offer a novel and efficient technique for removing contaminants with excellent filtering efficiency in the submicron domain and low air resistance. Thus, problems with conventional non-woven macro fibers are overcome. It is feasible to regulate air permeability by varying the thickness of nanofibers so that they can remove aerosol particles as tiny as 0.5 μm due to their high surface-area-to-volume ratio and associated strong surface cohesion [182]. The filtration efficiency may be improved by using smaller electrospun nanofibers, although their strength in this case as a filtering medium may be unfavorable due to their usage, which may be improved by crosslinking electrospun nanofibers.

7.3. Food Storage

Food preservatives comprising chitosan are environmentally friendly, developed from renewable resources, and intrinsically biocompatible. Chitosan also has unique characteristics, including high film-forming capabilities and barrier properties against aromas and in

the dry state, making it a good choice for packaging structures and food coatings [183,184]. Nevertheless, although chitosan films are ecologically safe and sustainable, they tend to have poor mechanical performance. Furthermore, edible coatings and packaging materials that come into direct touch with food may have an impact on the quality [185].

Nanofiber technology has the potential to create ultra-thin non-woven mats for food packaging. Because of their physicochemical characteristics allowing them to conserve the quality and safety of food supplies across distribution and storage, nanofibers with a high chitosan content are employed for antibacterial packaging [186]. The future implementation of chitosan nanofibers in food packaging materials and processing might promote food production efficiency, sustainability, and storage capacity, while also promising more ecologically friendly food delivery.

7.3.1. Fruit Freshness

Due to its proven antibacterial preserving properties and biocompatibility, chitosan has been used in food containers to extend the shelf life of fruits and meat and to keep them as fresh as possible during long transportation distances, in storage rooms, and on shelves. The disadvantage of coating fruit with a chitosan wet film is that it absorbs moisture from the air, providing a good environment for bacterial growth on the fruit's surface. Constant drying is, therefore, required through wet coating applications, which causes moisture loss and damage to the fruit. The alternative solution would be to produce a protective nanofibrous membrane via electrospinning of chitosan blend solutions.

For this purpose, a solution of a chitosan derivative, carboxymethylchitosan (CMCS), and PEO was prepared for electrospinning of a nanocomposite membrane to explore its effectiveness in maintaining strawberry freshness. A reference sample covered with a commercial wrap was tested for fruit weight loss and degree of rotting and compared to the nanofibrous package, whose antibacterial activity, porosity, hydrophilicity, and intermolecular structure were also assessed [187]. As it is difficult to form chitosan-derived fibers using electrostatic fields, in order to optimize the electrospinning process, water-soluble non-toxic PEO was added to the CMCS solution, as this facilitates polyol binding and the solubility of the CMCS. With concentrations in the range of 2.5–7.5 wt% for each CMCS and PEO solution, clear Taylor cone building was observed. The composite nanofibers had an average diameter of 290 nm. The packaging film consisted of an electrospun membrane with a ratio of 1:12 PEO/CMCS, because the viscosity of that mixture was the most suitable for the formation of a complete electrospun film with homogeneous nanofibers and uniform micropore size. This ensured even breathability, promoting the exchange of air through the packaging film with regulated concentrations of O₂ and CO₂ for an optimum fruit storage environment. With infrared spectroscopy, characteristic peaks indicated no change in the structure of electrospun pure CMCS powder compared to CMCS/PEO composite nanofibers. To test the antibacterial effect of the CMCS/PEO nanofibrous membrane against the most spoiling bacteria found in food, *E. coli* and *S. aureus* were placed on two control non-woven filters and two electrospun membranes separately. The antibacterial rings clearly formed around the two bacteria strains in each of the samples including the composite material and were absent in the control samples, which confirmed the inhibitory effect of CMCS/PEO. Afterward, to test its compatibility with the fruit storage industry, four groups of similar strawberries were prepared. The first control sample was completely exposed to air, the second one was wrapped with a conventional polyethylene (PE) film, the third group was coated with a protective layer of CMCS/PEO solution with the film being dried, and the last group was covered with the electrospun CMCS/PEO membrane. All groups were placed at the same room temperature and their weights were measured multiple times. After 6 days, the unprotected control group experienced a fast weight loss and degradation due to the increase in fruit metabolism. The second covered group did not exhibit any bacteriostatic effect, as mildew was spotted on the surface, although its weight loss was slower than the coated group 3, as the latter exhibited surface browning and shrinkage but without any rotting effect. This demonstrates that the PE cover was

very compact, not letting any air enter, seeing as it could control the fruit dehydration but not rotting, whereas the CMCS/PEO protective coating prevented the bacterial attack. As it was exposed to air moisture on the fruit's surface, however, it caused the oxidative degradation of ascorbic acid and internal water loss. Regarding the fruit covered with the composite membrane, the water retention and appearance were mostly maintained, without any traces of bacteria (Figure 9). These results validate the plausible prolonged freshness of transported fruits through the optimistic application of the biocompatible CMCS/PEO electrospun nanofibers, due to their breathable structure and antibacterial effects [187].

Figure 9. The results of the antibacterial assessment of five strawberry groups: (a) initial state; (b) no cover; (c) plastic cover; (d) coating of chitosan solution; (e) covered with chitosan-derivative electrospun nanofiber membrane. Adapted from [187], originally published under a CC-BY 4.0 license.

Variations in the electrospinning and solution parameters (concentration and ratio of chitosan and PEO) resulted in optimized nanofiber properties, which were adjustable for different requirements [187].

7.3.2. Meat Shelf Life Prolongation

The antibacterial ability of chitosan/PEO nanofibers to increase the shelf life and prevent *E. coli* infection of meat was evaluated by Arkoun et al. in situ under refrigeration at 4 °C [140]. For antibacterial experiments, a chitosan/PEO ratio of 80:20 (*w/w*) was selected as a compromise between the ratio of 90:10 (*w/w*), which had the maximum antibacterial effect but low electrospinning ability, and the 70:30 (*w/w*) ratio, which had less bactericidal impact but greater nanofiber production capacity. Fresh meat cubes were sliced, immersed in a suspension of *E. coli*, and enclosed in the electrospun mats. Additionally, samples were wrapped and sealed in a standard multilayer food packaging to support the weak barrier properties of chitosan-based nanofibers. Bacteria in the conventionally packaged sample lacking chitosan-based nanofibers grew in concentration as they were fed by the nutrients in the meat, but their increase was moderate compared to the uncovered sample. This proves that the commercial meat package has strong barrier properties for oxygen and moisture necessary for bacterial survival and can slow down the bacterial growth but cannot completely eradicate it. On the other hand, 92% of the bacterial population was eliminated when infected meat was wrapped in chitosan-based nanofiber mats plus commercial packaging, allowing the meat's microbiological quality and safety to be preserved while also extending its shelf life to 7 days at 4 °C [188].

7.4. Biomedicine

7.4.1. Wound Dressings

Wound dressings are topical therapeutic patches that are applied to wounds to help them recover naturally. A variety of natural and synthetic materials are already in use in different structures. Due to their nanofibrous structure, dressings of electrospun fibers can improve wound healing in several ways. They have the ability to absorb undesired fluids from the injured area but also to preserve helpful fluids and proteins inside the wound area. They can additionally guide cell proliferation due to their structure being similar to the composition of collagen fibrils in the extracellular matrix [189]. Their porosity in the size range of nanometers is also able to ensure their breathability and can protect the wound from bacterial penetration [7].

Chitosan-based products are advantageous for use in tissue engineering and wound healing because they are natural antimicrobial components [189]. Nanofibrous products, in particular, are considered for biomedical applications due to their low cost, biodegradability, non-toxicity, and antibacterial characteristics [190].

As mentioned above, the electrospinning of pure chitosan fibers is difficult for several reasons. Therefore, chitosan was combined with different polymers to obtain high-quality nanofiber mats that are suitable for biomedical applications. Further functionalization of the wound dressings is possible, transporting active substances into wounds and ulcers when these dressings come into contact with the body and supporting wound healing (via the addition of drugs) or preventing infections (via the addition of antibacterial compounds) [189,190]. A bicomponent nanofibrous mat comprising chitosan and poly(lactic acid) (PLA) was produced by electrospinning. In comparison to a casted chitosan–PLA film, this mat successfully prevented the development of *S. aureus* and *E. coli* bacteria in wounds [191]. The core–sheath structure has also been demonstrated to enhance fibroblast reproduction and tissue regeneration [192]. Incorporation of moxifloxacin hydrochloride, an antibiotic with intrinsic antibacterial activity against Gram-positive and Gram-negative bacteria, in electrospun nanofibers from chitosan and PVA significantly increased the antibacterial properties of the product. Unfunctionalized chitosan/PVA nanofiber mats did not show any inhibition zone on the tested agar plates, as chitosan was not able to diffuse in the agar diffusion test. Vibration methods are, therefore, recommended for proving the antibacterial properties of pure chitosan products [118,192].

It was found that polyaniline (PANI)/chitosan composite membranes help wounds heal by stimulating skin development. Synergistic effects were observed by promoting the growth of both fibroblasts and osteoblasts. [193]. In order to determine the antibacterial properties of this composite material, PANI/chitosan nanofibers were electrospun according to the proper blend ratio, relative humidity, and voltage that lead to stable jet formation. The fibers were then evaluated against two bacteria strains, *E. coli* and *B. subtilis*. Mats with a high PANI concentration exhibited enhanced bactericidal activity, making the composite membrane a potential candidate for wound dressing applications because of the biocompatibility, electrical charges, and antibacterial properties of the blended materials in use [194].

In another study, electrospinning was used to produce a membrane of chitosan and silk fibroin (SF). Silk fibroin was used to improve the mechanical characteristics. The membrane inhibited the development of *E. coli* according to turbidity measurements. The antibacterial activity of chitosan/SF nanofibrous membranes increased dramatically as the percentage of chitosan was raised, which was significantly beneficial to these membranes when employed as wound dressings [195].

Natural substances such as aloe vera have been utilized to enhance the hydrophilicity and antibacterial characteristics of chitosan/PCL nanofibers [196]. Electrospun scaffolds with several layers have also been developed for wound healing. The sophisticated structure of the first layer of PCL or PCL/cellulose acetate provided mechanical support, while the second layer of the chitosan/PEO blend came into direct touch with the wound surface. These scaffolds were biocompatible with fibroblast cells, had 80% porosity, strong vapor

permeability, and could expand by up to 370% without losing mechanical or structural features, making them ideal as resorbable dressings for thick exudative wounds [197].

The wound treatment and antibacterial performance of chitosan nanofibers can be improved by infusing inorganic nanoparticles within the fibers. The incorporation of metallic nanoparticles is also useful for the function of various medical textiles. Biosensors and drug carriers are examples of textiles used for diagnostic or therapeutic applications, where the metals included in nanofiber scaffolds can produce electric or magnetic fields essential for the functionality of wearable smart textiles or the direction of cell migration. As a matter of fact, hyperthermia therapy is only possible when the material can be heated, which can be achieved by loading chitosan nanofibers with magnetic nanoparticles, such as Fe_3O_4 [198].

Since the addition of bactericidal agents can substantially improve the antibacterial capacity of nanofibrous scaffolds, silver nanoparticles (AgNPs) on chitosan/PEO membranes showed substantial efficacy against *E. coli* [199]. Chitosan/gelatin nanofibers containing AgNPs also produced positive results [200].

Nanofibers used for passive antibacterial wound healing were prepared by encapsulation of delaminated $\text{Ti}_3\text{C}_2\text{T}_z$ (MXene) flakes within chitosan nanofibers via electrospinning. Antibacterial experiments on biocompatible crosslinked $\text{Ti}_3\text{C}_2\text{T}_z$ /chitosan composite fibers against *S. aureus* and *E. coli* reduced the bacterial colonization by 62% and 95%, respectively, after 4 h, revealing the electrospun $\text{Ti}_3\text{C}_2\text{T}_z$ /chitosan nanofibers as viable non-toxic candidate materials for wound healing applications [201].

Burns are among the most frequent and serious injuries that may cause skin function damage. The protective properties of the skin are lost in high-degree burn injuries, causing heat-induced skin degradation and the formation of scarring. The scar area is a wet, alkaline, protein-rich, non-vascular environment devoid of immune cells. As a result, it is thought to be an ideal habitat for the development of bacteria, resulting in infections that spread on the wound surface. Although topical antibiotic therapy with medicines does not sterilize wounds, it does decrease the number of germs in the tight tissue underneath the scar to a level where the host immune system can manage them [201].

Debridement is one of the therapy options for scar removal. To minimize moisture loss, prevent microbial infection, and improve wound healing, the skin's surface must be covered with an appropriate membrane after surgical excision of infected tissue. Nanofibrous films are one of the most effective wound dressing options.

A number of antibacterial potential films were electrospun, including pure PVA, chitosan/PVA, and drug-loaded chitosan/PVA at different concentrations. Abbaspour et al. [202] examined the capacity to prevent microbial penetration of the different nanofiber materials by using an open test tube containing nutritional broth without any covering material as the positive control, while another tube, which was closed with a densely packed cotton ball, served as negative control. The positive control tube's intense turbidity showed that the nutrient broth medium was appropriate for microbial growth, whereas the negative control tube's absence of turbidity indicated that sterile conditions were maintained throughout the experiment. Microorganisms penetrating through the film into the test tube induced microbial growth and increased the turbidity of the medium throughout the tests. After 4 h, microorganisms in the open control sample had grown to an uncountable number of colonies. Drug-free chitosan/PVA nanofibrous films showed antimicrobial effects when compared to pure PVA films, indicating that they could limit the growth of microorganisms, which is attributable to chitosan's antibacterial characteristics. When comparing drug-free and drug-loaded formulations, it was observed that with higher drug quantities in the film formulation, the drug release resulted in a stronger and faster reduction in viable bacterial count. For *Pseudomonas aeruginosa* (*P. aeruginosa*), nanofibers containing 40% mafenide acetate, the most efficient antibiotic for scarring, resulted in a significant decrease in the bacterial count after 4 h and the eradication of live bacteria after 8 h. As a consequence, the medium's transmittance was comparable to or near that of the negative control tube.

Overall, the findings showed that in spite of the porous and non-compact structure of the films, both drug-loaded and drug-free nanofibrous chitosan/PVA membranes have core power to protect wounds from secondary bacterial infections, thanks to their sufficiently small pore size, which only allows water vapor and air to pass through the membrane, while airborne microorganisms are trapped. Moreover, chitosan's NH_3^+ groups may easily bind to negatively charged bacteria, preventing them from penetrating. Films containing mafenide acetate had a stronger antibacterial impact on the microorganisms tested, indicating that they could be useful as a protective, healing, and antimicrobial wound dressing in the future [202].

7.4.2. Tissue Repair by Controlled Drug Release via Coaxial Electrospinning

When medicines are electrospun from biodegradable polymers, an effective drug release mechanism may be achieved. Additionally, it is feasible to alter the drug release kinetics and concentration by changing the parameters of the electrospinning process. More precisely, by regulating the diameter of the fibers, biodegradable polymers in a fiber structure allow for smooth and controlled medication release [203].

The technique of coaxial electrospinning offers the possibility of preparing fibers with a core-shell structure and allows the combination of the advantageous properties of two different materials within one fiber (see Figure 10). Nanofibrous scaffolds produced with the coaxial electrospinning technique can be effectively used for the construction and implementation of biodegradable antimicrobial layers because they are lightweight and can incorporate antimicrobial agents inside their large surface area.

Figure 10. Coaxial electrospinning. Adapted from [204], originally published under a CC-BY 4.0 license.

An example of the advantages of utilizing core-shell fibers was published by Keirouz et al. [204]. Here, nylon-6 (PA6) in the core provides mechanical stability and the mixture of chitosan and PEO in the shell provides bacteriostatic action and hydrophilicity. The addition of 5-chloro-8-hydroxyquinoline (5CLO8Q) and poly(hexamethylene biguanide) (PHMB) to the chitosan or to PA-6, respectively, resulted in the preparation of a binary antimicrobial system that was tested against *S. aureus* and *P. aeruginosa*. These are two of the most prevalent pathogenic bacteria, which are often found in surgical infection sites. PHMB is a well-known polycationic disinfectant of mucous membranes and wounds that can permeabilize and destroy bacterial membranes and is used as an antiseptic in wound dressings [205]. Furthermore, 5CLO8Q is an aromatic nitrogen molecule that is slightly soluble in water with antibacterial, fungal, and amoebic activities. The antibacterial

effect of chitosan, which may permeabilize the cell membrane of prokaryotes by creating ionic compounds with its negative charges, was significantly increased by 5CLO8Q. Pure PA6 did not have an effect on bacterial growth. The chitosan in the structure's shell was used to regulate and guide the drug release model. With this technique, it was possible to protect sensitive substances from the outside environment by placing the drug within the fibers, provide programmable release kinetics for defined concentrations of drugs [206], and improve the mechanical behavior by incorporating a mechanically strong polymer into the core. For homogeneous fiber production, PEO was added to the chitosan solution as a carrier polymer [204].

The encapsulated composite nanofibers seemed to totally resist the development of both bacteria strains, demonstrating that an anti-adhesive surface may be obtained in addition to the biocompatibility of chitosan and the synergistic antibacterial characteristics of these two substances. Meanwhile, the control electrospun mat chitosan/PA6 only delayed *S. aureus* growth but had no effect on the more robust strain *P. aeruginosa* [204]. The findings showed that this electrospun composite material may be used to avoid bacterial infections on surgical mesh surfaces in the future.

For biomedical applications, nanofibers have been shown to be superior to films in the areas of wound coverage and tissue engineering [207]. The nanofibrous structure is similar to that of collagen fibrils and can guide cell proliferation. Additionally, the porosity ensures breathability and fluid transport, but also protects the wound from bacterial penetration. The inclusion of chitosan supports these effects via its antimicrobial properties.

7.5. Application Fields Summary

This study highlights the new advancement of nanofibers made of chitosan, chitosan derivatives or blends, and composites in filtration, food, and biomedical applications and discusses the challenges and opportunities arising from their antibacterial performance, with the goal of underlining the long-term potential of these natural polymers and their conceivable use in bacteria-prone products. An overview of relevant research papers is given in Table 2.

Table 2. Literature overview of electrospun chitosan composites used for different applications.

Material	Method	Applications, Antibacterial Tests	Reference
Filtration Systems			
Chitosan (MW: 190–310 kDa) + PEO (MW: 600 kDa)	Needle-free, Nanospider Lab	Filtration	Grimmelsmann et al. 2017 [10]
Water Filtration			
Chitosan (DDA: 85%) + PCL	Needle-based electrospinning	Antibacterial efficiency of nanofibrous membranes tested with <i>S. aureus</i> for water filtration	Cooper et al. 2013 [162]
Chitosan (MW: 338 kDa, DDA $\geq$ 90%) + Nylon-6 (MW: 25 kDa) (100:0, 80:20, 70:30)	Needle-based electrospinning	Removal of metal ions ($\text{Pb}(\text{NO}_3)_2$, NaCl) and antibacterial efficiency against <i>E. coli</i> for water filtration	Jabur et al. 2016 [161]
Chitosan solution + PVA solution (70:30) $\pm$ AgNO_3 solution (1, 2, 3, 4, 5%) (Ag-doped chitosan nanofibers)	Home-made needle-based electrospinning setup	Antibacterial efficiency against <i>S. aureus</i>	Bezir et al. 2019 [173]
Chitosan (DDA: 75–85%) + PVA (MW: 72 kDa) $\pm$ AgNO_3 solution (0, 2, 4%) (Ag-doped chitosan nanofibers)	Needle-based electrospinning, rotating steel collector, covered with polyester substrate	Antibacterial efficiency tested with <i>E. coli</i> for static and dynamic water filtration	Adibzadeh et al. 2014 [175]
Polyacrylonitril (PAN, MW: 150 kDa) $\pm$ ZnO-nanoparticles (Zano [®] 20) $\pm$ [chitosan (MW: 50 kDa, DDA: 75–85%) + PEO (MW: 900 kDa)]	Needle-based electrospinning	Antibacterial efficiency of PAN/ZnO-Cs membranes tested with <i>E. coli</i> and <i>Enterococcus faecalis</i> for water filtration	Makaremi et al. 2016 [178]

Table 2. Cont.

Material	Method	Applications, Antibacterial Tests	Reference
Air Filtration			
Nylon-6 + chitosan (80:20) solution or pure nylon-6 solution for electrospinning; compared with nylon-6 nanofibrous filter media, dipped in chitosan solution	Multi-jet electrospinning with three spinnerets, rotating drum collector	Survival rate of <i>E. coli</i> and <i>B. subtilis</i> on hydrophilic and hydrophobic blankets and on air filtration media	Sun et al. 2020 [179]
Chitosan (DDA: 80–95%, MW: 590 kDa) + PVA (86–90% hydrolysed, MW: 118–124 kDa) (1:2–3:1) ± AgNO ₃ (0.04–1.0%) ± TiO ₂ (0.04%)	Home-made needle-less electrospinning	Filtration efficiency tested for removal of nanoparticles aerosols for air filtration, antibacterial efficiency tested with <i>E. coli</i> and <i>S. aureus</i>	Wang et al. 2016 [154]
Food Storage			
Carboxymethyl chitosan (MW: 80–250 kDa, DDA: 95%) + PEO (MW: 5000 kDa)	Needle-based electrospinning	Nanofiber membranes for fruit freshness	Yue et al. 2018 [187]
Chitosan (DDA: 95%, MW: 4, 10, 50 kDa) + PEO (MW: 600 kDa) (50:50, 60:40, 70:30, 80:20, and 90:10)	Home-made needle-based electrospinning	Antibacterial efficiency against <i>E. coli</i> , <i>Salmonella enterica</i> serovar Typhimurium, <i>S. aureus</i> , <i>Listeria innocua</i> for food protection	Arkoun et al. 2017 [188]
Wound Dressing/Tissue Engineering			
Chitosan (deacetylation degree 80–90%) + PVA (polymerization degree 1700, alcoholysis degree 88%) + Moxifloxacin hydrchloride	Needle-based electrospinning	Drug encapsulation, antibacterial material	Liu et al. 2021 [118]
Chitosan (50 kDa, DDA: 95%) + PEO (MW: 600 kDa) (80:20)	Horizontal needle-based electrospinning	Antibacterial efficiency tested with <i>E. coli</i> , <i>S. aureus</i> , <i>L. innocua</i> , and <i>S. Typhimurium</i> (food contamination and skin infections)	Arkoun et al. 2017 [94]
Chitosan (MW: 600–800 kDa, DDA: 90%) ± Polyaniline emeraldine base (PANI, MW: 50 kDa)	Needle-based electrospinning	Antibacterial efficiency tested with <i>E. coli</i> , <i>B. subtilis</i> for wound dressing	Moutsatsou et al. 2019 [194]
Chitosan (MW: 190–310 kDa, DDA: 77%) ± MXene (Ti ₃ C ₂ T _z flakes)	Needle-based electrospinning; fibers crosslinked with glutaraldehyde	Antibacterial efficiency tested with <i>E. coli</i> , <i>S. aureus</i> for wound dressing	Mayerberger et al. 2018 [201]
Chitosan (viscosity < 500 cp) + PVA (MW: 72 kDa) ± mafenide acetate	Needle-based electrospinning	Antibacterial efficiency tested with <i>S. aureus</i> and <i>Pseudomonas aeruginosa</i> for wound healing	Abbaspour et al. 2015 [202]
Single fibers: PA6, chitosan/PEO (80:20), PA6-PHBM, chitosan-5CLO8Q core-shell fibers with core: PA6-PHMB shell: chitosan-5CLO8Q + PEO	Single needle-based electropinning, co-axial needle-based electrospinning	Antimicrobial efficiency tested in vitro against <i>S. aureus</i> and <i>P. aeruginosa</i> in surgical site infections	Keirouz et al. 2020 [204]
Chitosan (medium MW, DDA: 75–85%) + silk generated from cocoons of <i>Bombyx mori</i> silkworm	Needle-based electrospinning	Antibacterial efficiency tested with <i>Escherichia coli</i> and <i>Staphylococcus aureus</i> for wound dressing	Cai et al. 2010 [195]
Double layer scaffold 1. layer: PCL ± cellulose acetate (10%) 2. chitosan + PEO	Needle-based electrospinning	Dressing for skin lesions, low cytotoxicity to L929 fibroblasts, promotion of cell proliferation	Trinca et al. 2017 [197]

DDA: degree of deacetylation; MW: molecular weight; 5CLO8Q: 5-Chloro-8-hydroxyquinoline, PHMB: Poly (hexamethylene biguanide).

8. Conclusions

Material science research addressing the use of sustainable polymers to replace plastic materials has grown in prominence in recent years. Chitosan is a polycationic natural biopolymer that is ideal for an extensive range of applications, including biomedicine, filtration, and food protection, due to its biocompatibility, biodegradability, and sustainability. In acidic media, chitosan's amino groups are protonated, allowing it to interact with anionic microorganisms or to form metal complexes.

In contrast to conventional coatings with chitosan-based films, the flexible parameters of the electrospinning technique, which involves the application of an electric field between a charged polymer solution and a collector, aid in tailoring the final properties of chitosan nanofibers, such as their high porosity, high surface adhesion to bacteria, small diameter, narrow pores, hydrophilicity, and rough morphology that rod-shaped bacteria can wrap themselves around. Due to the high crystallinity resulting from H-bonds between chitosan monomers, which provides high viscosity and surface tension, it is difficult to expel a steady and stable jet out of the spinneret and electrospin homogeneous continuous filaments from

pure chitosan; thus, it is frequently diluted with non-toxic auxiliary solvents to lower its surface tension. The second benefit of blending is that it allows the combination of the copolymer's various characteristics, such as by producing a hydrophobic core and a hydrophilic surface with enhanced tensile strength and e-modulus. Since different bacterial strains are engaged in various application areas, it is critical to identify those that are sensitive to chitosan. Pathogenic bacteria, regardless of the membrane structure or classification, were found to be the least susceptible because chitosan's effect on them is not bactericidal, allowing them to reproduce and pose a threat to human health or form a biological film on filter media and reduce water permeability by clogging the pores. The use of bactericidal agents such as metallic nanoparticles in the electrospinning process improved the antibacterial activity of chitosan, in addition to improving the water flow; however, the leaching of dissociated metal ions was another issue that needed to be addressed. In food storage, chitosan nanofibers were more capable of preserving fruits and prolonging their shelf life than chitosan films because of their air permeability and ability to shield the fruit surfaces from contact with moisture. In biomedicine, the resemblance of these nanofibers to collagen fibrils in the extracellular matrix makes them an appealing alternative for drug release mechanisms. In a regulated procedure, electrospun nanofibers are packed with antibiotics and released within the tissue, with the diameter size influencing the drug release kinetics and concentration. Additionally, chitosan-based nanofibers not only protect wounds from bacteria proliferation, but their hydrophilicity also enables them to absorb excess fluids from thick wounds and aid in wound healing without leaving scars. Finally, chitosan electrospun nanofibers have great potential in a variety of application areas where antibacterial performance is crucial, although further research on this subject is needed, particularly in cases when chitosan is unable to completely kill specific bacteria strains on its own.

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