Review

Electrospun biodegradable polymers loaded with bactericide agents

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Abstract: Development of materials with an antimicrobial activity is fundamental for different sectors, including medicine and health care, water and air treatment, and food packaging. Electrospinning is a versatile and economic technique that allows the incorporation of different natural, industrial, and clinical agents into a wide variety of polymers and blends in the form of micro/nanofibers. Furthermore, the technique is versatile since different constructs (e.g. those derived from single electrospinning, co-electrospinning, coaxial electrospinning, and miniemulsion electrospinning) can be obtained to influence the ability to load agents with different characteristics and stability and to modify the release behaviour. Furthermore, antimicrobial agents can be loaded during the electrospinning process or by a subsequent coating process. In order to mitigate burst release effect, it is possible to encapsulate the selected drug into inorganic nanotubes and nanoparticles, as well as in organic cyclodextrine polysaccharides. In the same way, processes that involve covalent linkage of bactericide agents during surface treatment of electrospun samples may also be considered.

The present review is focused on more recent works concerning the electrospinning of antimicrobial polymers. These include chitosan and common biodegradable polymers with activity caused by the specific load of agents such as metal and metal oxide particles, quaternary ammonium compounds, hydantoin compounds, antibiotics, common organic bactericides, and bacteriophages.

Keywords: Electrospinning; drug encapsulation; metal particles; chitosan; hydantoin compounds; antibiotics; quaternary ammonium compounds; bactericides; bacteriophages
1. Introduction

Severe health and environmental problems are caused by the adhesion and proliferation of bacteria on the surface of materials. Bacterial biofilms are constituted of microcolonies where bacteria are organized in communities with functional heterogeneity [1]. Microorganisms can be transferred into material surfaces and survive for long periods of time, especially in hospital environments, due to their ability to develop biofilms through several growth steps (Figure 1) [2,3].

Figure 1. Scheme showing the different steps involved in the formation of Pseudomonas aeruginosa strain PAO1 biofilms on the surface of materials: a) reversible attachment, b) irreversible attachment, c-d) maturation, and e) dispersion. Reproduced with permission from [2] © 1994, Nature Publishing Group.

The structure of biofilms is characterized by the presence of channels that facilitate the circulation of nutrients, thus allowing bacteria to easily multiply and disperse. In fact, most chronic bacterial infections are related to biofilm formation. In this way, demand for bacteriostatic, antiseptic, and bactericide agents has increased in order to prevent bacterial survival on the surface of materials [1,4]. Staphylococcus aureus is the bacteria mostly associated with hospital infections, as its drug-resistant strain is the most dangerous [5]. To prevent infections from natural agents, amoebas and phages may be considered, as well as industrial and clinical agents, such as silver [6], quaternary ammonium groups [7], hydantoin compounds [8], and tetracycline antibiotics [9]. In general, efforts are focused on the prevention of biofilm formation by means of typical bactericide agents and antibiofilm agents that inhibit the microbial attachment process [10].

The conventionally accepted approach in the preparation of antimicrobial polymeric materials is usually based on the incorporation of the appropriate agent into the polymer matrix. High functionality is hindered by the low compatibility between the antimicrobial agent and the polymer, which leads to unfavorable aggregation [11]. Furthermore, materials usually have a low surface-to-mass ratio that hinders the contact between the agent and the microorganism [12].
These problems can be solved by means of the electrospinning technique, which is an easy process that can lead to the production of porous mats with a tunable porosity. These mats are constituted of micro/nanofibers which can be loaded with active agents for the required application when necessary. In a typical electrospinning process, a high electrical field is applied to a liquid droplet held at the end of a capillary tube. The drop becomes charged and when the electrostatic repulsion counteracts the surface tension, it is stretched and a jet ejects towards a grounded target (collector) (Figure 2) [13-15]. The morphology of the fibers is highly dependent on the solution characteristics (e.g. type of solvent, vapor diffusivity, surface tension, solution conductivity, polymer concentration, and viscosity) and operational parameters (e.g. distance between the needle to the collector, flow rate, and applied voltage). The deposition of the electrospun fibers leads to a consistent mat, membrane, or scaffold.

Figure 2. Schematic diagram showing the electrospinning process. Reproduced with permission from [97] © 2011, Springer.

Electrospun membranes can also play important role for microfiltration purposes since water permeability is high and consequently a high flux operation can be achieved. Nanofiber membranes functionalized with biocide can be used as a cost-effective alternative to chlorine [16] and obviously could also act as anti-biofouling membranes [17].

Development of antimicrobial layers is of high interest for food packaging materials in order to inhibit the growth of pathogens on the surface of meat. Electrospun mats with antimicrobial properties are seriously considered to provide an appropriate inner packaging surface for food preservation. Furthermore, and unlike conventional drugs, a simple spraying of bacteriophages on the meat surface may cause early inoculation and problematic deactivation [18]. Encapsulation of phages again becomes essential to provide sustained release.

Different methods have been developed to incorporate biocide agents into electrospun fibers and have been well summarized by Gao et al. (Figure 3) [19]. In general, the small size of nanofibers leads to a considerably high burst effect. This can be avoided by considering various strategies such as the generation of a core-shell structure, promoting physical absorption of the drug onto the fiber.
surface, establishment of covalent bonds between the polymer and the bactericide agent, or previous encapsulation of the agent in nanostructures that have been electrospun with the polymer. Antibiotics, like amoxicillin, have been encapsulated in laponite nanoparticles and then dispersed in poly(lactic-co-glycolic acid) for electrospinning [20]. This organic/inorganic hybrid system shows a sustained drug release profile that is of interest for applications in tissue engineering and pharmaceutical science.

Figure 3. Methods developed for the incorporating of pharmacological agents into electrospun nanofibers. Reproduced with permission from [19] ©2014, John Wiley & Sons.

2. Incorporation of metal and metal oxide nanoparticles into electrospun scaffolds

Silver (Ag) is likely the most toxic element for microorganisms and thus is used in different devices, such as antimicrobial filters and wound dressing materials. Metallic silver, silver salts, and silver complexes are of great interest in the biomedical field due to their relatively low human toxicity, high antimicrobial properties (including aerobic and anaerobic bacteria), antifungal, antiviral, and also potential anticancer activity [21-24]. It has been indicated that Ag interferes with the respiratory process of the cytochromes, affects the electron transport system, and can be bound to DNA and inhibit its replication [25-28]. The use of Ag nanoparticles leads to remarkable antimicrobial activity due to the large surface activity of the nanoparticles with respect to conventional Ag based compounds.

Silk fibroin (SF) is also of potential interest in the area of biomedical science due to its biocompatibility, biodegradability, and minimum inflammatory response [25,29,30]. Thus, it has been
considered as scaffolding material for skin regeneration and has potential as a burn wound dressing when combined with Ag compounds. SF nanofibrous matrices containing silver sulfadiazine (SSD) have been prepared by electrospinning in a 98% formic acid solution and have been demonstrated to be a wound dressing that maximizes antimicrobial activity and minimizes cellular toxicity [5].

Nanofibers of different polymers such as poly(vinyl alcohol) [31], poly(L-lactide) [32], acetylcellulose [33], poly(vinyl chloride) [34], polyacrylonitrile [35], and polyurethane [36] have been loaded with silver nitrate, as an effective antimicrobial agent [37], through electrospinning. Cautions should be taken into account as Ag has shown some cytotoxicity and may cause problems when it is in contact with skin [38].

Silver complexes have the advantage due to the fact that antimicrobial activity can be controlled by changing the type of ligand to which they are bound (e.g. the Ag(I) imidazole complex has high antibacterial activity [39], whereas the phosphine adduct of Ag(I) imidazole has no antimicrobial effect [40]). Silver(I) N-heterocyclic carbene complexes have been effectively encapsulated in electrospun fibers of a hydrophilic polyether-based thermoplastic aliphatic polyurethane from an ethanol solution [6]. The hydrophilic character of the polymer is essential to facilitate the release of silver cations from the encapsulated complex and also to provide an appropriate moist environment for optimal wound healing [21]. The encapsulation of the silver complex increased the bioavailability of active silver species, reduced the amount of silver used and increased the bactericidal activity over a longer period of time when compared to aqueous silver. The electrospun silver mats were demonstrated to be effective against E. coli, P. aeruginosa, S. aureus, C. albicans, A. niger, and S. cerevisiae.

Bactericidal activity of Ag nanoparticles can be improved through the assistance of an electric field [42]. In this context, Tijing et al. [43] incorporated tourmaline (TM) nanoparticles into a polyurethane (PU) matrix by electrospinning and subsequently silver nanoparticles were positioned onto the mat by photoreduction under ultraviolet light irradiation. This combination of Ag and TM provided a synergistic effect on the antibacterial functionality due to the spontaneous surface electric field of TM.

Incorporation of copper (Cu) can also give rise to polymeric materials with antibacterial and antifungal properties [44,45]. Basically, Cu causes a distortion of the cell wall of microorganisms that may provoke cell death. PU nanofibers containing nanoparticles of Cu have been prepared by the electrospinning technique and the antimicrobial activity determined for both Gram positive and Gram negative bacteria. Results indicated the suitability of these nanofibers for many biological applications, such as antimicrobial wound dressings and as internal aid for filtration processes [46]. The indicated nanofibers were obtained from an initial mixture of a colloidal suspension of Cu nanoparticles in dimethylformamide (DMF) and PU dissolved in tetrahydrofuran (THF)/DMF. Highly crystalline Cu nanoparticles were attached to the surface of the amorphous PU fibers (Figure 4).

Different metal oxide particles with a bactericide activity should be mentioned, with zinc oxide, copper oxide, and titanium dioxide (TiO₂) being the most relevant. The latter may lead to a photocatalytic inactivation of viruses and Gram-positive and Gram-negative bacteria. Nevertheless, TiO₂ has inherent drawbacks as an efficient biocidal agent due to the large bandgap energy and fast recombination rate of photogenerated electron–hole pairs.

ZnO/TiO₂ nanofibers were produced after calcination (500 °C for 3 h) of electrospun poly(methyl methacrylate) (PMMA) nanofibers prepared from DMF/acetic acid solutions containing
PMMA, zinc acetate, and titanium isopropoxide [47]. These ZnO/TiO$_2$ composite nanofibers have high antibacterial activity without light irradiation and an enhanced activity under UV irradiation with respect to pristine TiO$_2$ (Figure 5), due to the increased recombination time of photogenerated electron–hole pairs.

![Image of nanofibers](image)

**Figure 4.** Transmission electron micrographs of PU nanofibers containing different amounts of copper: (A) 5%, (B) 7%, (C) 10%. A high magnification of Cu particles and the corresponding electron diffraction pattern is shown in (D). Reproduced with permission from [46] © 2011, Elsevier.

![Graph showing survival rates](image)

**Figure 5.** % Survival of *S. aureus* after treatment with control, TiO$_2$ nanofibers, and ZnO/TiO$_2$ nanofibers in the absence and the presence of UV light irradiation at 312 nm for 30 seconds. Reproduced with permission from [47] ©2015, Royal Society of Chemistry.
Functional metal oxide fibers can be easily prepared by electrospinning a polymer solution containing metal oxide nanoparticles. For example, electrospun PU nanofibers containing zinc oxide nanoparticles were prepared from a DMF solution and were found to have over a 98% reduction in the growth of both *Staphylococcus aureus* and *Klebsiella pneumoniae* [48]. Titanium dioxide/PVA nanocomposite fibers were also easily prepared by electrospinning. In this case, a heat treatment (160 °C for 3 min) stabilized the PVA nanofibers against dissolution in water without the requirement for toxic chemicals [49] (Figure 6).

**Figure 6.** Electron micrographs of TiO$_2$/PVA nanofiber webs (left) untreated TiO$_2$/PVA nanocomposite fibers after immersion in water, heat-treated TiO$_2$/PVA nanocomposite fibers after immersion in water (center) and heat-treated TiO$_2$/PVA nanocomposite fibers after agitation in water (right). Reproduced with permission from [49] ©2008, Wiley&Sons.

Another method to incorporate metal oxide nanoparticles on the surface of electrospun fibers, which can be achieved by liquid phase deposition and also by a simple coating of the electrospun fibers [50,51]. A new and versatile approach has recently been proposed by Horzum et al., which is based on the in situ formation of metal oxide nanoparticles on surface-functionalized polymer fibers [52]. Poly(styrene-co-vinylphosphonic acid) fibers were produced by electrospinning and were used as templates for the in situ formation of metal oxide nanoparticles (Figure 7). Metal oxide nanoparticle formation could be effectively enhanced by the introduction of functional phosphonate groups in the copolymer, demonstrating the role of vinylphosphonic acid as nucleation centers along the fiber.

Nanoparticles of ionic metal oxides are highly interesting because they can be prepared according to morphologies that have numerous edges, corners, and other reactive surface sites [53,54]. It is also interesting to point out that MgO nanoparticles can adsorb a large amount of chlorine or bromine (close to 20 wt%) with respect to commercial MgO (ca. 4 wt%). Thus, MgO/Cl$_2$ formulations can be more active biocides than free Cl$_2$, MgO nanoparticles, or commercial MgO microcrystals [55]. In fact, nanoparticles were able to cover bacterial cells and release halogens in a high concentration and in proximity to the cell [55]. Also, excellent activity against *E. coli*, *B. megaterium*, and *B. subtilis* spores [55] was demonstrated.
3. Incorporation of quaternary ammonium compounds (QACs)

QACs are well-known antiseptics and disinfectants [56] which are also employed to prepare antimicrobial electrospun scaffolds due to their broad spectrum of antibacterial activity caused by a cationic interaction with cell membranes [57], interaction with K\(^+\) cations, loss of cytoplasm, and changes in DNA and RNA structure [58,59]. Quaternary ammonium microbiocides are recommended for use as agents to inhibit the growth of microorganisms in textile materials and paints [58]. The use of QACs have some advantages over Ag nanoparticles since technologies ensuring an uniform particle distribution are not necessary and, furthermore, QACs can be easily degraded after use in contrast to the harmful effect of Ag in the environment. Selection of appropriate QACs may be problematic due to constraints related to the materials applications (e.g. appearance, stability, adhesion, and workability) [60]. Water soluble polymers like poly(vinyl alcohol) may be the better matrices to incorporate the indicated cationic surfactants [61].

Nevertheless, QACs such as benzyl triethylammonium chloride (BTEAC) have also been employed to increase spinnability of non-water soluble polymers like poly(lactic-co-glycolic acid) (PLGA) [62] and poly(hydroxybutyrate-co-hydroxyvalerate) [63] during electrospinning due to the increase of electrical conductivity and the decrease of the surface tension of the solution. Kim et al. studied the effect of increasing concentrations of BTEAC in PVA electrospun nanofibers. Logically, the increase of electrical conductivity lead to higher average diameters (i.e., from 181 to 465 nm when the concentration varied from 0 to 2.6%). Antibacterial test with BTEAC-PVA nanofibers demonstrated the successful growth inhibition of *S. aureus* and *K. pneumonia*, whereas antiviral test also demonstrated the inactivation of both MS2 and PhiX174 viruses (Figure 8) [64]. BTEAC has also been incorporated into electrospun polycarbonate (PC) fibers giving rise to a clear improvement of antimicrobial activity [65]. Electrospinning was performed in chloroform solutions and it was
observed that the addition of small amount of BTEAC to the solution caused a significant decrease (i.e. from 8.1. to 1.0 μm) in the average fiber diameter and favored the formation of continuous and uniform fibers.

Biodegradable filters based on polylactide/polyhydroxybutyrate nanofibers were prepared by electrospinning and functionalized with ammonium-based ionic liquid (IL) belonging to QACs. A “multi-layering” method was adopted to fabricate highly efficient nanofiber filters with a greatly reduced pressure drop and antimicrobial properties [65]. Non-woven nanofiber mats for use in antimicrobial nanofilter applications were also obtained by electrospinning of polyurethane cationomers (PUCs) [66]. These could be easily obtained by a chain extension reaction of the base polymer with compounds having a tertiary amino group and subsequent quaternization of the tertiary nitrogen atoms with various acids or alkyl halides [67]. The average fiber diameters of PUCs decreased with increasing quaternary ammonium group content. The PUC nanofibers had a diameter that decreased with increasing quaternary ammonium content as a consequence of the stronger elongation forces produced by the increased charge density. It was also observed that fibers were adhered due to a slow evaporation of the solvent, yielding a film-like character. Interestingly, a very strong antimicrobial activity against *S. aureus* and *E. coli* was observed.

![Figure 8. Antimicrobial and antiviral test results of 2.6% BTEAC-PVA nanofibers: (a) S. aureus; (b) K. pneumonia; (c) PhiX174; (d) MS2. Reproduced with permission from [64] © 2015, Elsevier.](image)

High surface area material can be obtained by incorporating surface segregating biocides into the electrospinning solution. The study of the segregation capability of QACs has recently been evaluated [60] in conjunction with the derived biocidal activity. Results indicated that both QACs surface concentration and biocidal activity could be significantly improved by increasing the amphiphilic character of QAC.
Other examples corresponding to electrospun scaffolds loaded with QACs are the followings:

a) Polyvinylpyrrolidone/cetyltrimethylammonium bromide system revealed a significant reduction in bacterial activity of *Klebsiella pneumonia*, *S. aureus*, and *E. coli* when the content of the quaternary amino compound was at a minimum of 2.5 wt% [68].

b) Polyacrylonitrile electrospun fibers loaded with *N,N*-didecyl-*N,N*-dimethylammonium chloride and bis-(3-aminopropyl)-dodecylamine [37] were highly effective against *E. coli* and *S. aureus* since they were eliminated to levels higher than 99.8% after 6 h of exposure.

c) The electrospun self-quaternized block copolymer constituted by 4-vinyl pyridine (4VP) and pentachlorophenyl acrylate (PCPA) segments exhibited high bactericide activity against *E. coli* and *S. aureus* cultures. QASs were generated by *N*-alkylation of pyridine groups of P4VP block and chloroaromatic groups of PPCPA block [69].

Gemini surfactants (also named dimeric surfactant) like *N,N*-didodecyl-*N,N*,*N*,*N*-tetramethyl-,*N*,*N*-ethanediyl diammonium dibromide are highly interesting amphiphilic compounds that have two hydrophobic tails and two hydrophilic head groups joined by a spacer (Figure 9). Digemini surfactants with short spacers tend to pack in cylindrical geometries. These gemini surfactants can be electrospun from water/methanol mixtures due to the high viscosity that can be attained and gives rise to hydrophilic continuous microfibers with diameters from 0.9 to 7 μm [70] (Figure 10). The new membranes may provide charged hydrophilic surfaces with potential applications for tissue engineering and coatings for biocompatible devices. In fact, current electrospinning of low-molecular-weight polymers is an innovative approach for the development of biomedical membranes [71].

![Figure 9](image-url)  
*Figure 9. Chemical structure of *N,N*-didodecyl-*N*,*N*,*N*,*N*-ethanediyl diammonium dibromide gemini surfactant.*

![Figure 10](image-url)  
*Figure 10. SEM micrographs of electrospun fibers from 42 wt% (left) and 44 wt% (right) gemini *N,N*-didodecyl-*N*,*N*,*N*,*N*-ethanediyl diammonium dibromide surfactant solutions in water/methanol. Reproduced with permission from [70] © 2010, American Chemical Society.*
4. Incorporation of halamine compounds

An N-halamine is a compound containing one or more nitrogen-halogen covalent bonds that can be obtained by the simple halogenation of imide, amide, or amine groups (Figure 11). The antimicrobial activity of the halogenated compounds follows the order of imide > amide > amine halamines, which is the opposite order of their stability [72]. A halogen exchange reaction can be produced when microorganisms come into contact with N-halamines, leading to the death of the cell. These compounds have some advantages when compared to the use of inorganic halogens (e.g., chlorine or bromine), since are more stable and less corrosive. Monomeric N-halamines such as 1,3-dichloro-5,5-dimethyl hydantoin and 3-bromo-1-chloro-5,5-dimethylhydantoin have been employed as disinfectants due to their instantaneous and complete sterilization of a wide range of microbes [73]. Halamines have well demonstrated advantages, such as stability in both wide temperature and humidity ranges, durability, and regenerability [74].

In addition, efforts have also been focused to graft N-halamine structures into polymers in order to provide them with antimicrobial characteristics (Figure 12). Nevertheless, surface treated polymers showed limited activity due to the small amount of the N-halamine compound that could be incorporated, as well as the reduction of activity during time mainly caused by friction.

A covalent binding is not always possible and consequently the use of N-halamine-based antimicrobial agents and also hydantoin derivatives as effective precursors of N-halamine have been considered for additivation of polymers to provide antimicrobial functions [75,76].

Figure 11. Examples of chemical structures of halamine containing polymers.

Figure 12. Examples of grafting reaction and subsequent activation by spraying chlorine bleach solution.

The antibacterial performance of N-halamines strongly depends upon their activated surface area and, consequently, the bactericide effect can be enhanced by means of the electrospinning...
technique because it allows for the development of smaller sized \( N \)-halamines with enlarged specific surfaces [7]. Kang et al. [78] reported an interesting strategy based on a combined copolymerization-electrospinning-chlorination process. Specifically, 3-allyl-5,5-dimethylhydantoin (ADMH) as \( N \)-halamine monomer was first copolymerized with methyl methacrylate. Subsequently, electrospinning and chlorination processes were performed. Figure 13 shows that the roughness of fiber surface depends on the chlorination time and on the order in which electrospinning and chlorination were performed.

Fibers with a controlled morphology can be obtained based on the chlorination order and period, and usual parameters like comonomer ratio or solution concentration. \( N \)-halamine fibers showed an unexpected activity enhancement with respect to spherical samples toward \textit{E. coli} and \textit{S. aureus} pathogens. It was reported that fiber morphology, roughness of fiber surface, and positive zeta potential had a great influence on the improvement of antibacterial properties.

![Figure 13. SEM images of fibers prepared by: copolymerization-electrospinning chlorination (a), copolymerization-chlorination-electrospinning (b) techniques. Effect of chlorination time on the morphology of \( N \)-halamine fibers: 6 (c) and 12 h (d). Reproduced with permission from [78] © 2015, American Chemical Society.](image)

The hindered amine-based bis(\( N \)-chloro-2,2,6,6-tetramethyl-4-piperidinyl) sebacate (Cl-BTMP) was electrospun with cellulose acetate (CA) to obtain nanofiber fabrics containing the antimicrobial agent. The selected amine-compound had low solubility/compatibility in CA and consequently conventional films showed low efficacy against microorganisms. However, activity was clearly enhanced when electrospun nanofibers with the same amount ratio of Cl-BTMP were employed because of the greater increase in the specific surface [79].

Nanofibrous nylon 6 membranes were prepared via electrospinning, with three structurally
different N-halamines introduced [80]. In all cases, N-halamines were uniformly distributed on the electrospun membrane surface. Total kill was achieved in less than 1 h for these electrospun nylon 6 membranes. Nevertheless, it was also found that with the same active chlorine contents, chlorinated 5,5-dimethylhydantoin (CDMH), which has both an imide and amide halamine group, gave quicker activity than both chlorinated 2,2,5,5-tetramethyl-imidozalidin-4-one (CTMIO), which has both an amide and amine halamine group, and chlorinated 3-dodecyl-5,5-dimethylhydantoin (CDDMH), which has an amide halamine group. The slower reaction of chloroamide group and the attached long alkyl chain were likely the main reasons for this slower activity.

Dickerson et al. [81] prepared halamine derivatives by chlorination of nitrogen-bearing moieties of keratin. Thus, antimicrobial functionalization of keratin-based films and nanofibers could be easily performed. Halamine-charged materials exhibited rapid and potent bactericidal activity against several species of bacteria and induced up to a complete reduction in the colony forming units of Bacillus thuringiensis within 10 min. Electrospun core/shell nanofibers could be engineered to maximize keratin-Cl surface and gain a higher bactericide activity against bacteria (e.g. S. aureus) than films composed of the same materials. The halamine-based antimicrobial functionalization methods were demonstrated for keratin-based materials.

Antibacterial fibrous membranes of polyhydroxybutyrate with poly[5,5-dimethyl-3-(3’-triethoxysilylpropyl)hydantoin] (PSPH) were prepared by electrospinning and subsequently exposed to chlorine bleach. Compared with control samples, the chlorinated membranes with chlorine loading of 0.31% could inactivate 92.10% S. aureus and 85.04% E. coli within 30 min of contact time [82]. It was also observed that the chlorinated samples were more powerful for inactivation of S. aureus than E. coli O157:H7 within each contact time. This is likely due to the existence of an extra lipid layer on the outer cell membrane of the Gram-negative bacteria, which does not exist in Gram-positive bacteria. After chlorination, the hydrophobicity was enhanced, as could be deduced from contact angle measurements. N-H bonds in N-halamines were converted into N-Cl bonds, which also might affect the antimicrobial behavior.

5. Incorporation of antibiotics

Antibiotics, such as tetracycline hydrochloride (TCH), amoxicillin (AMX), ciprofloxacin, levofloxacin, and moxifloxacin have been satisfactorily encapsulated in electrospun nanofibers based on polymers like PLA, PLGA and PCL for wound-dressing [83]. Logically, drug release is highly influenced by the nature of polymeric carrier and drug content. Electrospun fiber mats can be applied as a postsurgical anti-adhesion barrier [84,85] in order to avoid severe clinical complications. Adhesion can be a consequence of the wound healing process that is often associated with tissue inflammation. The electrospun nanofibers can physically separate the wound site from an adjacent organ or tissue and release locally therapeutic agents, such as antibiotics.

Electrospun PLGA/PEG-PLA membranes impregnated with 5 wt% of cefoxitin sodium were demonstrated to be highly efficient to prevent postsurgery-induced abdominal adhesions. The membrane constitutes a physical barrier able to release antibiotic and has clear advantages over other conventional and less efficient materials. In particular, it was claimed to possess the possibility to adjust composition, the drug-loading capability, and the easy placement handling due to the relatively hydrophobic character of the membrane [86].

Two-stream electrospinning approaches are currently being considered to combine the different
properties given by independent fiber populations. Thus, composites based on biodegradable poly(ester urethane) urea (PEUU) with elastomeric properties and PLGA loaded with the antibiotic tetracycline were developed for abdominal wall closure applications [87].

PLA matrices with a dual function were prepared by co-electrospinning in such a way that an immediate release of lidocaine HCl for pain alleviation was attained, whereas a sustained release of the antibiotic mupirocin over several days for the prevention bacterial infection was achieved [88].

A PLGA biodegradable localized delivery system for the combinatorial release of fusidic acid (FA) and rifampicin (RIF) was also developed using electrospinning. A good antibiotic encapsulation (i.e. between 75% and 100%) and a biphasic drug release profile were determined, as well as an in vitro activity against S. epidermidis and methicillin-resistant S. aureus [89]. This system is of interest to solve clinical problems related to implant-associated infections which may develop after invasive orthopedic surgery. Strategies based on perioperative antibiotics have resulted in various systemic toxicities and the promotion of antibiotic resistant microorganisms.

Wang et al. [20] and Qi et al. [90] attempted to reduce the typical burst effect of antibiotics like TCH and AMX into electrospun fibers. Emulsion and coaxial electrospinning have been developed to mitigate the burst release since drugs can be embedded into the core region of fibers, forming the polymer shell a barrier for the diffusion of the drug. Nevertheless, co-axial electrospinning has difficulties associated with the optimization of operational parameters, whereas the second process may have problems based upon the emulsifier if it decreases the biocompatibility of the nanofiber [91]. New processes are based on the efficient encapsulation of drugs into nanotubes and nanoparticles. Specifically, TCH was encapsulated on halloysite nanotubes (HNTs) and then electrospun with PLGA [90]. Other natural or synthetic clay materials can be used to encapsulate drugs (e.g. AMX) and dispersed into the electrospun polymer solution (Figure 14) [20].

Figure 14. Scheme showing the loading of free (a) and encapsulated AMX within PLGA nanofibers (b). Reproduced with permission from [20] ©2012, American Chemical Society

Laponite (LAP) is a good drug carrier since the interlayer spacing is appropriate to provide a
high drug retention capability (Figure 14b) [92]. It was demonstrated [20] that AMX could be loaded into LAP nanodisks with an efficiency of 9.76% and then incorporated into PLGA nanofibers by electrospinning without causing a significant morphological change. Furthermore, the release profile of AMX from PLGA/LAP/AMX nanofibers was significantly improved with a biphasic and sustained manner (Figure 15). These loaded nanofibers displayed effective antibacterial activity and non-compromised cytocompatibility in comparison with pure PLGA nanofibers (Figure 15).

![Figure 15](image-url)

**Figure 15.** In vitro release in phosphate buffered saline (PBS) solution of AMX from LAP/AMX nanodisks, PLGA/AMX electrospun nanofibers, and electrospun nanofibers of PLGA containing LAP/AMX nanodisks (left). SEM images (right) of porcine iliac artery endothelial cells (PIEC) cultured onto PLGA (up) and PLGA/LAP/AMX nanofibers (down) after 3 days of culture. Reproduced with permission from [20] ©2012, American Chemical Society.

6. Incorporation of other common organic biocides

Triclosan (TCS), chlorhexidine (CHX) and polybiguanide (PHMB) are biocides with a high consumption since all may be employed in both home and hospital settings for disinfection. The incorporation of such compounds into electrospun fibers are also discussed in the present work since are examples of hydrophobic (TCS) and hydrophilic (CHX) compounds, as well as low (CHX) and high (PHMB) molecular weight compounds (Figure 16).

TCS (2,4,4’-trichloro-2’-hydroxydiphenyl ether) has a high bactericide activity since it is a competitive inhibitor of enoylacyl carrier protein reductase, a component of the lipid biosynthesis pathway [93]. CHX (1,1’-hexamethylene-bis-5-(4-chlorophenyl)biguanide) has a high activity towards microorganisms [94] as a consequence of the presence of secondary amines that can be protonated, and therefore positively charged under normal pH conditions [95]. PHMB is a cationic oligomer having an average of 7–11 biguanide groups spaced by flexible hexamethylene segments. The high number of biguanide groups lead to it being highly effective against microorganisms [96].

Scaffolds constituted by blends of hydrophilic and hydrophobic polymers are of interest because
their hydrophilicity/hydrophobicity may gradually vary according to the final composition. In this way, electrospun microfibers constituted by different ratios of polylactide and poly(ε-caprolactone) were prepared, where it was observed that the release of TCS exemplified that the release of a hydrophobic drug is dependent on the composition [97] (Figure 17). Furthermore, a certain tunable biocide effect was detected when samples were assayed with \textit{E. coli} and \textit{S. epidermidis} bacteria. This effect, as well as the CHX release, was favored when samples were enriched on the PLA component.

![Figure 16. Scheme showing the chemical structure of TCS, CHX and PHMB hydrochloride.](image)

![Figure 17. Cumulative triclosan release profiles of PCL/PLA-x electrospun samples (x indicates the wt% of PCL in the electrospun mat) in a Sörensen medium containing 70% and 10% volume percentages of ethanol. Final released percentages are indicated for each sample. Reproduced with permission from [97] ©2011, Springer.](image)
Electrospun PLA micro/nanofibers loaded with TCS were also used as reinforcing fibers of differing polyester matrices. Large differences in the release behavior were detected depending on the loading process, fiber diameter size, and hydrophobicity of the polyester matrix. Interestingly, a sustained release was observed when only TCS was loaded into the electrospun fibers, resulting in longer lasting antimicrobial activity [98].

Scaffolds comprising different ratios of PEG and PLA electrospun fibers were prepared by co-electrospinning using a single rotary collector (Figure 18). PEG was considered as a sacrificial polymer due to its high solubility in water. The proposed methodology provided for scaffolds with tuned porosity (from 40 to 80%) by water immersion of the dual fiber samples. Drugs, such as TCS and PHMB, which have different hydrophilic/hydrophobic character and molecular size, were loaded into PLA microfibers, with the release and the bactericide effect being dependent on the porosity of the sample [99].

![Figure 18. Scheme showing the co-electrospinning set up to prepare scaffolds constituted by a mixture of PCL and PLA fibers. The fibers may be easily solubilized in water giving rise to materials with different porosity depending on the initial PLA/PEG ratio. Reproduced with permission from [99] © 2014, Springer.](image)

Coaxial electrospun microfibers having different core-shell distributions and compositions of PEG and biodegradable poly(butylene succinate) (PBS) were also studied [100]. The PEG component of electrospun fibers could be solubilized, as explained above, by immersion of the scaffolds in an aqueous medium, giving rise to high porosity and hydrophobic samples. Scaffolds can be effectively loaded with hydrophobic drugs having antibacterial and anticarcinogenic activities, such as TCS and curcumin, respectively. The coaxial design would offer the opportunity of differentiated release of the two drugs (e.g. a fast effect for the bactericide and a sustained release for the anticarcinogenic compound) [100].

PLA electrospun microfibers loaded with TCS, ketoprofen (KTP), or their combination were prepared to obtain multifunctional scaffolds with bactericide and anti-inflammatory properties. Dual drug-loaded scaffolds showed a peculiar behavior, as a delayed release of TCS and KTP was detected as a consequence of the establishment of intermolecular interactions. Antimicrobial activity of all TCS-loaded samples was verified against *E. coli* and *M. luteus* bacteria. Furthermore, KTP-loaded samples showed a slight bactericide activity. Crystallinity of the polymer matrix influenced the
release behavior, as deduced from scaffolds prepared using PLAs with different ratios between L- and D-lactide units [101].

There are several types of naturally occurring cyclodextrins (CDs) that differ in the number of glucopyranose units that form a cyclic motif, which is organized giving rise to a truncated cone-shaped molecular structure. CDs have a cavity that allows the formation of intrusion complexes with a wide variety of compounds, including typical biocides, such as triclosan. In this case, the formation of complexes is highly important because the antibacterial activity of triclosan (TCS), which is nearly water-insoluble, can be significantly enhanced by increasing its solubility [102]. Kayaci et al. [103] studied the incorporation of triclosan/cyclodextrin complexes in PLA nanofibers via electrospinning (Figure 19). Results were successful when larger cycles (7 or 8 units) were used. PLA nanofibers incorporating TCS/CDs showed better antibacterial activity against *S. aureus* and *E. coli* bacteria compared to PLA nanofibers. It has recently been demonstrated that antibacterial electrospun nanofibers could also be obtained from highly concentrated aqueous solutions of the TCS/CD complex alone [104].

**Figure 19.** Schematic representation of naturally occurring α-CD, β-CD, and γ-CD showing approximate dimensions and the electrospinning of PLA incorporating TCS/CD complexes. Reproduced with permission from [103] © 2013, American Chemical Society.
Electrospun nanofibers were prepared from DMF solutions with cellulose acetate (CA) as a polymer base, chlorhexidine (CHX) as a bactericidal agent, and organic titanate as a cross-linker. A small amount of high-molecular-weight poly(ethylene oxide) (PEO) was also incorporated into the blends to promote the electrospinning [105]. Bactericide activity was caused by both a gradual release of unbound CHX from the fibers and contact with CHX bound on the fibers. Post-spin treatment of CA-PEO fibers to immobilize CHX on the fibers via titanate linkers gave rise to a similar efficiency compared to that of the CA-CHX fibers electrospun from the blends, even with a much lower CHX content.

Microfibrous electrospun mats from styrene/maleic anhydride copolymers were prepared and subsequently CHX was subsequently attached covalently through a reaction between maleic and amine units to provide high antimicrobial activity [106]. Fiber meshes of poly(hydroxybutyrate) (PHB) and poly(hydroxybutyrate)/poly(ethylene oxide) (PHB/PEO) containing CHX have also been evaluated as a polymer based drug delivery system with high bactericidal potential [107].

Electrospun micro/nanofibers of a biodegradable poly(ester amide) (PEA) constituted by L-alanine, 1,12-dodecanediol, and sebacic acid loaded with antimicrobial agents, such as silver and CHX, have been prepared. The scaffolds supported cell adhesion and proliferation and gave rise to a clear and well differentiated antimicrobial effect against both Gram-positive (e.g. *M. luteus*) and Gram-negative (e.g. *E. coli*) bacteria. Specifically, bacterial adhesion was significantly inhibited when samples were loaded with weight percentages equal or greater than 0.05% and 1.2% of silver nitrate and chlorhexidine, respectively [108].

A poly(ester amide) constituted by L-phenylalanine, adipic acid, and 1,4-butanediol has also been considered due to the increasing applications of PEAs in the biomedical field [109,110]. Furthermore, the high solubility of the indicated poly(ester amide) provided for the appropriate electrospinning conditions to incorporate bactericide agents and also degrading enzymes, such as α-chymotrypsin, without a significant denaturation. A specific delay in the release of the polymeric biguanide was observed with respect to CHX, although PHMB still showed clearly enhanced activity [111].

Amino acid containing poly(ester urea) (PEU) have been developed as promising materials in the biomedical field and specifically in tissue engineering applications. The polymer having L-leucine, 1,6-hexanediol, and carbonic acid units is highly soluble in most organic solvents, an interesting feature that facilitated the electrospinning process and the effective incorporation of drugs with bactericidal activity (e.g. biguanide derivatives such as CHX and PHMB) and enzymes (e.g. α-chymotrypsin) that accelerated the degradation process. It was found that PHMB led to narrow fibers and had an increased antibacterial effect against Gram-positive and Gram-negative bacteria with respect to CHX loaded samples [112].

Polyurethane was electrospun with a montmorillonite clay loaded with antibacterial chlorhexidine acetate. The fibrous mat had broad-spectrum activity against both Gram-positive and Gram-negative bacteria. Sustained release activity was observed, indicating the new mats may be useful in topical drug delivery and in wound healing with long-term activity [113].

The antimicrobial PHMB agent was incorporated into electrospun PLA micro/nanofibers to evaluate the potential application of new materials as temporary and medicated scaffolds. PHMB release was found to be highly dependent on the hydrophilicity of the medium and differed from that which was determined for CHX. PHMB-loaded PLA scaffolds inhibited adhesion and bacterial growth, in addition to exhibiting biocompatible characteristics for the adhesion and proliferation of both fibroblast and epithelial cell lines. Cells were more sensitive to the oligomeric drug (PHMB).
when compared to CHX when tissue (i.e. a monolayer) was formed and less sensitive in suspension (e.g. during colonization of material or cell division). Several simultaneous cell interactions on a cell monolayer enhanced cell death compared to cells in suspension (Figure 20) [114].

Figure 20. Scheme of the interaction of oligomeric PHMB and monomeric CHX with cells forming a suspension (left) and a monolayer (right). Reproduced with permission from [114] © 2015, Elsevier.

7. **Chitosan based scaffolds for tissue engineering applications**

Chitosan (CS) and its derivatives are currently investigated for biomedical applications due to their excellent biocompatibility, non-toxicity, biodegradability, antibacterial, antifungal, and antitumoral activities [115]. In addition, these polymers may activate crucial processes in wound healing (e.g. fibroblast activation; stimulation of the formation of cytokines) and may even have a hemostatic effect due to their capability to bind red blood cells [116].

CS is soluble in organic acids (e.g. acetic, formic, and lactic acids), as well as in water mixtures with methanol, ethanol, and acetone if a limited amount of the organic acid is added. Nevertheless, the presence of free amino groups in the molecules (Figure 21) leads to a positively charged polyelectrolyte at low pHs, which causes a viscosity increase of the solution and hinders the electrospinning process [117]. Furthermore, formation of strong hydrogen bonds hinder the movement of molecular chains exposed to the electric field [118]. It has been postulated that electrospinning of polyelectrolytes can be performed when a second, non-charged polymer is added. In this way, mixtures of chitosan and polyethylene oxide (PEO) have been successfully processed with a general requirement of a weight ratio of chitosan lower than 1 [119-121]. Nevertheless, chitosan has been electrospun in 1,1,6,6,6-hexafluoroisopropanol (HFIP) [122], formic acid [118], trifluoroacetic acid [123], and acetic acid [124,125].

Figure 21. Chemical structure of chitosan.
Interestingly, hydrolyzed chitosan was found to be electrospinnable in low concentrations of acetic acid (e.g. 80–70%), which is the least toxic solvent of all which were assayed, leading to continuous nanofibers with an average diameter around 260 nm. Hydrolysis was performed in aqueous 50% NaOH during 48 h that caused a decrease from $1.1 \times 10^6$ g/mol to $2.9 \times 10^5$ g/mol. It was proposed that this lower molecular weight facilitated an effective alignment of the molecular chains within in the electric field.

PLA has been used with CS to prepare composite materials with improved toughness, controllable biodegradability, and chemical functionalities. The addition of a small amount of AgNO$_3$ to a PLA/CS blend solution may improve its electrospinning ability since AgNO$_3$ has a high influence on the morphology and therefore a change from bead-on-fiber structure to uniform fibers was observed [126]. In fact, CS can interact with AgNO$_3$ and reduce the repulsive force between ionic groups within the polymer backbones. Furthermore, heat annealing of the produced electrospun fibers can lead to Ag nanoparticles whose dimensions and ratio differ from those formed from a simple reaction of CS with Ag$^+$ cations during the electrospinning process. Logically, the antibacterial activity of CS incorporating Ag nanoparticles becomes higher than that of each component [127].

Different mixtures of poly($\varepsilon$-caprolactone) (PCL) and CS have been electrospun from formic acid/acetone solvent mixture. The hydrophobicity of PCL and the hydrophilicity of CS caused difficulty when using as a single solvent [128]. The best results in terms of fiber morphology were obtained with samples having 25 wt% of CS. The derived membranes significantly reduced *Staphylococcus aureus* adhesion when compared to PCL fibrous membranes. Furthermore, new membranes appeared interesting as pre-filters for water filtration, as they supported a high water flux (e.g. $\sim 7000\ L/(h\cdot m^2)$) with 100% removal of 300-nm particles [129].

Electrospinning of CS and poly(vinyl alcohol) (PVA) appears to be more easily performed because both polymers have an hydrophilic character. It should also be noted that PVA strongly interacts with chitosan through hydrogen bonding on a molecular level [130]. In addition, it has been demonstrated that PVA/CS blends have potential as biomedical materials as cell cultures becomes enhanced with respect to pure PVA [131]. A series of PVA/CS membranes were prepared from water/acetic acid mixtures with the morphology, diameter, and structure of the nanofibers being mainly dependent on the concentration of the blend solution and the weight ratio of the blend [132]. Nanofibrous membranes based on a series of nylon-6/chitosan blends with different compositions were also fabricated by electrospinning [133]. This system is again interesting due to the establishment of hydrogen bonding between the two polymers, which could give rise to networks that have influence on both mechanical and biological properties [134].

Poly(ethylene terephthalate) (PET) nanofibers have been proposed to be used as blood vessels [135], so it would be of interest to introduce antibacterial activity on their surfaces, a feature that can be easily achieved through the preparation of PET/CS nanofibrous scaffolds [136]. CS based nanofibers have also been considered for wound healing applications. Thus, CS/PEO membranes containing silver nanoparticles (AgNPs) exhibited significant activity against *E. coli* [137]. Good results were also achieved with CS/gelatin nanofibers containing AgNPs [138]. For the same purpose, membranes of CS and silk fibroin (SF) were succesfully prepared by employing an electrospun mixture of HFIP and 2,2,2-trifluoroethanol [139].

Logically, quaternary ammonium salts of CS should gave rise to an improved antibacterial activity with respect to CS alone. These kind of derivatives, for example
N-[(2-hydroxy-3-trimethylammonium)propyl] chitosan chloride) (HTCC), could be easily synthesized by the reaction of CS with glycidyl-trimethylammonium chloride (Figure 22). The new polymer (HTCC) is water-soluble and could be electrospinable when a high-molecular-weight polymer like polyvinyl alcohol (PVA) was added. It was found that increasing HTCC content enhances electrospinnability of the blends and reduces the electrospun fiber diameter. Electrospun nanofibrous PVA–HTCC mats of the combination of the two polymers showed a good antibacterial activity against the Gram-negative bacteria *E. coli* and Gram-positive bacteria *S. aureus* [240].

Lok et al. [141] prepared block copolymers of poly[(2-dimethylamino)ethyl methacrylate)-co-(glycidyl methacrylate)] (P(DMAEMA-c-GMA)) and poly(pentachlorophenyl acrylate) (PPCPA) (P(DMAEMA-c-GMA)-b-PPCPA) by atom transfer radical polymerization (ATRP) as a method to provide monodispersed polymers with a controlled molecular weight. These copolymers could be electrospun from THF/DMF solution mixtures, giving rise to microfibers with diameters in the range of 300 nm to 1.3 μm. Insoluble nanofibers were subsequently prepared by treatment with 1,6-hexanediامine to crosslink the epoxy groups, whereas quaternary ammonium salts (QASs) were generated via *N*-alkylation of tertiary amine groups of the P(DMAEMA-c-GMA) block by the chloroaromatic compounds of the PPCPA block (self-quaternization). The combination of the hydrophobic interaction of the PPCPA segments and the electrostatic interaction of QACs resulted in microfibers exhibiting high antibacterial activity.

![Figure 22. Synthesis scheme to get a quaternary ammonium salt of CS by reaction with glycidyl-trimethylammonium chloride.](image)

8. Incorporation of bacteriophages

Bacteriophages or phages are bacterial viruses that invade bacterial cells and, in the case of lytic phages, disrupt bacterial metabolism and cause the bacterial lysis. The history of bacteriophage discovery has been the subject of lengthy debates, including a controversy over claims for priority [142-144]. Ernest Hankin, a British bacteriologist, reported in 1896 on the presence of marked antibacterial activity against *Vibrio cholera*, which he observed in the waters of the Ganges and Jumna rivers in India, and he suggested that an unidentified substance (which passed through fine porcelain filters and was heat labile) was responsible for this phenomenon. Two years later, the Russian bacteriologist Gamaleya observed a similar phenomenon while working with *Bacillus subtilis*; the observations of several other investigators are also thought to have been related to the bacteriophage phenomenon. However, none of these investigators further explored their findings until Frederick Twort, a medically trained bacteriologist from England, reintroduced the subject almost 20 years after Hankin’s observation by reporting a similar phenomenon and advancing the
hypothesis that it may have been due to, among other possibilities, a virus. However, for various reasons Twort did not pursue this finding, and it was another two years before bacteriophages were “officially” discovered by Felix d’Herelle, a French-Canadian microbiologist at the Institut Pasteur in Paris. In contrast to Hankin and Twort, d’Herelle had little doubt about the nature of the phenomenon, and he proposed that it was caused by a virus capable of parasitizing bacteria. The name “bacteriophage” was also proposed by d’Herelle. The name was formed from “bacteria” and “phagein” (Greek; to eat), and was meant to imply that phages “eat” bacteria.

Phage therapy is the therapeutic use of bacteriophages to treat pathogenic bacterial infections. Bacteriophages have many potential applications in human medicine as well as dentistry, veterinary, agriculture, and food processing. If the target host of a phage therapy treatment is not an animal, the “biocontrol” of bacteria is a more exact term. Because phages replicate in vivo, a smaller effective dose can be used. On the other hand, this specificity could be considered as a limitation: a phage will only kill a bacterium if it is a match to the specific strain. Accordingly, phage mixtures (phage cocktails) are often applied to improve the therapeutic effectiveness. Bacteriophages tend to be more successful than antibiotics where there is a biofilm covered by a polysaccharide layer, which antibiotics typically cannot penetrate.

Bacteriophages are much more specific than antibiotics or synthetic antimicrobials, so they could hypothetically be chosen to be less harmful, not only to the host organism (human, animal, or plant), but also to other beneficial bacteria, such as gastrointestinal flora. Phages are currently being used therapeutically to treat bacterial infections that do not respond to conventional antibiotics, mostly in Georgia, Poland, and Russia [142-145]. In the West (U.S.) during the 1940s, commercialization of phage therapy was undertaken by the large pharmaceutical company Eli Lilly [144,145]. However, when antibiotics were discovered in 1941 and marketed widely in the U.S. and Europe, Western scientists lost interest in further use and study of phage therapy for some time. Currently, no therapies are approved for use on humans in the West, although phages are in use for killing food poisoning bacteria [146].

Bacteriophages are especially effective in treatment of infected, poorly healing wounds, such as those seen in diabetic patients with foot ulcers, bedridden patients with pressure sores, and patients with venous stasis ulcers, which are some of the major problems in modern medicine [147]. A key element in the management of chronically infected wounds is the suppression of potentially pathogenic bacteria by high local concentrations of effective drugs at the injured site. Bacteriophages are used as liquid preparations (i.e. water solutions). The retention of high local concentrations of phages in the wound site using liquid bacteriophage preparations needs frequent dressing changes, a treatment that often increases in-patient cost dramatically.

For applications in food processing, it was suggested that phages could be added by dipping, spraying, or as a liquid addition to a large volume of food [148]. These methods may not be ideal, as they could be wasteful and lead to the potential inactivation of the phage particles as a consequence of the inclusion of other materials within the wash fluid. Moreover, if the phage-containing fluids themselves contain nutrients that support bacterial growth, then the potential for the bacterial evolution of phage resistance exists. Thus, when phages are added directly to a batch of food, the following problems may be encountered: the dilution and inactivation of phages and the evolution of bacterial resistance. The problems which can arise during the application of liquid bacteriophages in both therapy and food processing may be overcome by combination of phages with polymers via either their incorporation (encapsulation) into a polymeric matrix or immobilization onto a polymeric
surface. This approach is relatively new, therefore, there is scarce data in literature regarding bacteriophage/polymer conjugates.

**Bacteriophage encapsulation.** The first reported bacteriophage-polymer combination was PhagoBioDerm, a polymeric wound dressing (bandage) elaborated on in Georgia and reported in 2002 [149]. PhagoBioDerm represents a biodegradable polymeric matrix into which phages and other active ingredients are incorporated during manufacturing so that the phages and other medications can be released slowly and continuously over a period of time following application [150]. PhagoBioDerm can be applied to wounds or infections as sheets, or it can be cut into small pieces or ground into powder and placed directly into wounds. Amino acid based poly(ester amide) [109,150,151] was used as a biodegradable polymeric matrix for the deposition of bacteriophages and other medications. PhagoBioDerm showed promise in management of infected venous stasis ulcers and other poorly healing wounds [149]. The preparation was also effective in the complex treatment of multidrug-resistant *Staphylococcus aureus*-infected local radiation injuries caused by exposure to Sr90 [152].

Puapermpoonsiri et al. of the University of Strathclyde (Glasgow, UK) [153] encapsulated bacteriophages in a biodegradable polymer with the purpose to develop further bacteriophage formulations in anticipation of their emerging clinical use. This team showed that bacteriophages selective for *Staphylococcus aureus* or *Pseudomonas aeruginosa* can be encapsulated into biodegradable polyester (50:50 poly(DL-lactide-co-glycolide) microspheres via a modified w/o/w double emulsion-solvent extraction method and a subsequent freeze-drying with only a partial loss of lytic activity. Loss of lytic activity was attributed to the exposure of the bacteriophages to the water-dichloromethane interface, while the freeze-drying process had a little effect. The microspheres were engineered to have an appropriate size and density to facilitate inhalation via a dry-powder inhaler and fluorescently labeled bacteriophages were distributed entirely within the internal porous matrix. The release profile showed a burst release phase (55–63% release within 30 min), followed by a sustained release until approximately 6 h, as appropriate for pulmonary delivery. Despite the poor shelf-life of the formulation (presumably due to phage inactivation by lactic and glycolic acids released upon hydrolysis of the polyester), the work is proof-of-concept for the formulation and controlled delivery of bacteriophages, as suitable for the treatment of bacterial lung infections.

**Bacteriophage immobilization.** Phages were immobilized to various surfaces with the purpose to solve different tasks. For example, bacteriophages (wild-type phages or biotinylated) were immobilized in various ways onto gold surfaces to develop biosensors [154-156]. The bacteriophage specific to *Salmonella enteritidis* was biotinylated and were then coated onto streptavidin-labeled magnetic beads and were used to capture the bioluminescent *S. enteritidis* cells [157]. The number of cells captured by the constructed biosorbent was five times higher than that of the control, magnetic beads coated with nonbiotinylated phage, indicating the capture was specific.

According to Cademartiri et al. [158], the immobilization of phages could be particularly useful to create antimicrobial surfaces. The authors noted that current immobilization strategies based on chemical bioconjugation to surfaces or more difficult processes involving modification of their head proteins to express specific binding moieties are both time and money intensive. Different bacteriophages, active against a variety of food-borne bacteria, *E. coli, Salmonella enterica, Listeria monocytogenes, and Shigella boydii*, were effectively physisorbed to silica particles and prepared by silica surface modification with poly(ethylene glycol), carboxylic acid groups, or amines. The phages
remain infective to their host bacteria while adsorbed on the surface of the silica particles. The number of infective phage bound to the silica is enhanced by the presence of ionic surfaces, with greater surface charge (to a maximum) correlating with greater concentration of adsorbed phage. At concentrations above the maximum charge concentration, the number of active phages drop.

Bennet et al. [159] carried out passive immobilization of bacteriophages onto a polystyrene surface by simple immersing polystyrene strips in a Salmonella-specific bacteriophage suspension. The authors used this novel biosorbent for the separation and concentration of Salmonella from food materials. Anany et al. [148] used the polyelectrolyte nature of bacteriophages for their oriented immobilization via electrostatic forces to cellulose membrane and modified using them cationic polymer (polyvinylamine). The method is based on charge differences between the bacteriophage head, which exhibits an overall net negative charge, and the tail fibers, which possess an overall net positive charge. Hence, the head would be more likely to attach to positively charged surface, leaving the tails free to capture and lyse bacteria. It was established that the number of infective phages immobilized on the positively charged cellulose membrane was significantly higher than that on unmodified membranes. Cocktails of phages active against Listeria or E. coli immobilized on these membrane were shown to effectively control the growth of L. monocytogenes and E. coli O157:H7 in both ready-to-eat and raw meat, respectively, under different storage temperatures and packaging conditions. The phage storage stability was investigated to further extend their industrial applications. It was shown that freeze-drying can be used as a phage-drying method to maintain their infectivity on the newly developed bioactive materials. The utilization of the charge difference between phage heads and tails provided a simple technique for oriented immobilization which is applicable to a wide range of phages and allowed the retention of infectivity.

Pearson et al. [160] covalently attached T1 and Φ11 bacteriophages to inert polymeric surfaces while maintaining the bacteriophage’s biological activities capable of killing deadly human pathogens. The first step involved the formation of acid (COOH) groups on polyethylene (PE) and polytetrafluoroethylene (PTFE) surfaces using microwave plasma reactions in the presence of maleic anhydride. The covalent attachment (covalent anchoring) of the phages to the modified surfaces was carried out via acid-amine reaction leading to amide linkages using primary amine groups of phage capsid. The phages effectively retained their biological activity as manifested by a rapid infection with their own DNA and effective destruction of E. coli and S. aureus human pathogens. These studies showed that simultaneous covalent attachment of two biologically active phages effectively destroy both bacterial colonies and eliminate biofilm formation, thus offering an opportunity for an effective combat against multibacterial colonies, as well as surface detections of other pathogens.

Dai et al. [161] studied the ability of bacteriophage T7 to be encapsulated and preserved in water soluble polyvinylpyrrolidone by means of electrospinning. Loss of activity was evaluated after the electrospinning process and during subsequent storage. Addition of magnesium salts in the electrospinning solution was revealed to effectively protect phages from the high applied electrical field. Unfortunately, the added salts were not useful as a protectant during storage of the dried sample, a problem that could be minimized by the simultaneous addition of trehalose. Despite the use of aqueous media seeming appropriate for the electrospinning of phages, it should also be considered that the subsequent evaporation of water and dehydration of the phage could lead to a complete loss of activity. In order to overcome this problem, an emulsion electrospinning process wherein the phage is pre-encapsulated in an alginate reservoir has been evaluated with promising results [162].
Polyvinyl pyrrolidone electrospun fibers were effectively loaded with M13 bacteriophage [163], being observed to display an instantaneous release of the M13 bacteriophage and a sufficient activity to infect the bacterial host. Encapsulation of T4, T7, and λ bacteriophages in polyvinyl alcohol (PVA) fibers were also performed, with a significant loss of activity (i.e. between 99 and 94%) being reported after release, a feature that was attributed to phage dehydration and solvent evaporation during electrospinning [164]. To overcome the sensitivity of bacteriophages to the electrospinning process Korehei and Kadla [165] investigated emulsion and coaxial electrospinning processes to encapsulate the T4 bacteriophage into a poly(ethylene oxide) (PEO) fibers. In the first case, the bacteriophage was pre-encapsulated in an alginate reservoir, giving a higher activity compared with a simple electrospinning process; however, the drop of activity was still significant (two orders of magnitude). On the contrary, full activity was maintained by the coaxial process that clearly improved protection by allocating the bacteriophage into the core of a core/shell fiber structure. Full activity was even preserved after storage for several weeks at +4 °C. In this case, coaxial electrospinning was performed using a PEO solution in chloroform to form the shell and T4 bacteriophage/buffer suspension for the core (Figure 23).

![Figure 23. Electrospun fibers from a T4 bacteriophage/calcium-alginate/polyethylene oxide emulsion (left). Inset shows T4 bacteriophage/calcium-alginate capsules. A core/shell electrospun PEO fiber middle) and PEO fibers with encapsulated T4 bacteriophages (right). Inset shows a typical T4 bacteriophage. Reproduced with permission from [165] ©2013, John Wiley&Sons.](image)

10. Conclusions

At the present time, the preparation of materials with bactericide properties is fundamental to avoid severe health and environmental problems. Incorporation of industrial and clinical bactericide agents such as silver, copper, compounds with quaternary ammonium groups, hydantoin compounds, and antibiotics, as well as the use of antimicrobial polymers, is a usual practice to suppress biofilm formation and gain the appropriate materials for both specialty and commodity applications. Biomedical and food packaging sectors are clear examples where these materials have a growing demand.

As has been reviewed in the present work, electrospinning has emerged as an economic and versatile process to obtain micro/nanofibers of antimicrobial polymers and synthetic polymers that incorporate typical bactericidal compounds. Different systems have been successfully developed, but in general, the effectiveness has only been evaluated through in vitro experiments. Therefore, it is highly necessary to contrast the performance of the biocide materials by in vivo experiments, where samples become exposed to large volumes of dynamic fluid and incorporated biocides are highly

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diluted.

Efforts are also focused to assure scalability and cost-effectiveness of electrospun fiber platforms for use as a microbicide and clinical applications. Nevertheless, production of nanofibers in high volume and low in cost and the difficulty to efficiently integrate nanofibers into high speed manufacturing processes are the main limitations faced. Several companies are currently involved in electrospinning technology (Elmarco, Finetex, eSpin Technologies, Donaldson, Dienes Apparatebau GmbH, SNS Nanofiber Technology, Ahlstrom, TopTec, etc.), with the market of filtration products likely being the most active (e.g Ultra-Web™ from Donaldson, NanoWave™ from Hollingsworth & Vose, Technoweb™ from Finetex, and ProTura™ from United Air Specialists).

New needle-free technologies are being developed to create Taylor cones, and the subsequent flow of material, giving rise to higher fiber packing density, results in increased productivity and better fiber homogeneity. These technologies may be problematic for incorporating ionic biocides (e.g. PHMB and quaternary ammonium compounds), since they could have a negative influence on the process.

Single electrospinning, co-electrospinning, coaxial electrospinning, and miniemulsion electrospinning are available processes that may be selected according to the hydrophilicity/hydrophobicity of loaded compounds and the control of the release. In fact, several strategies have been proposed to mitigate the typical burst effect caused by the small size of fibers. Generation of a core-shell structure, enhancement of physical absorption, establishment of covalent bonds, and previous encapsulation are alternatives that are being studied. Again, problems may be related to the employment of needleless industrial machines, as well as the cost associated with scaling up the process. The development of specialized equipment is still a clear need for the increased rate of production of materials coupled with a minimum burst effect.

Finally, the incorporation of bacteriophages in electrospun mats appears a promising alternative to the use of antibiotics as long as problems related to the influence of electric field, the use of organic solvents, and dehydration could be properly solved.

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Conflict of interest

Authors declare that there is not conflict of interest.

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