Incorporation of Inorganic Antimicrobial Agents into Nanofibers

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Nanofibrous materials represent a very promising form of advanced carrier systems that can be used industrially, especially in regenerative medicine as highly functional bandages, or advanced wound dressings. By incorporation of antimicrobial additives directly into the structure of the nanofiber carrier, the functionality of the layer is upgraded, depending on the final requirement—bactericidal, bacteriostatic, antiseptic, or a generally antimicrobial effect. Such highly functional nanofibrous layers can be prepared mostly by electrospinning technology from both synthetic and natural polymers. The presence of a natural polymer in the composition is very advantageous. Especially in medical applications where, due to the presence of the material close to the human body, the healing process is more efficient and without the occurrence of an unwanted inflammatory response.

Keywords: nanofibers ; antimicrobial agents ; silver nanoparticles ; Zinc oxide nanoparticles ; metal ; polymer

1. Introduction

Nanofibrous materials have experienced a significant evolution in the past decade, eliciting considerable interest from various research institutions and technology companies, where they are processed into final products. Primarily prepared from polymers, these materials hold significant potential, especially within the textile, healthcare, and food industries. These materials consist of fibers with a diameter smaller than 1 μm, which create a porous network in the form of nonwoven textile. The electrospinning method is prevalent in the production of nanofibrous layers. Two factors contribute to this fact: learning the principles of electrospinning is relatively simple, and the electrospinning process could be upgraded to allow mass production. Using this technology, it is possible to prepare homogeneous nanofibrous layers with sufficient weight, which could be further processed or modified for specific applications [1][2].

Nanofibers can be prepared from a wide range of polymeric materials, provided they satisfy certain requirements pivotal to creating fibers with a defect-free morphology. The parameters of polymer composition are chosen based on the intended characteristics of the polymer solution, including an appropriate molecular weight and the associated viscosity of the solution, high solubility in the solvent system (ideally resulting in the creation of a “true solution”), and a surface tension conducive to the formation of the Taylor cone and stability of the fiber-forming jet. Electrospinning is suitable for use with both natural and synthetic polymers, with synthetic polymers displaying generally better spinnability (the polymer chains are more homogeneous). Often, a combination of both polymer types is preferred, with the synthetic material acting as a “fiber-forming polymer” [3]. This synergistic approach frequently results in enhanced mechanical properties attributed to the synthetic component, while the natural component promotes biocompatibility, non-toxicity, and improves overall biodegradability or resorption capacity. The fiber-forming polymer can also protect natural materials that are labile in high-voltage environments.

The transformation of polymer materials into (nano)fibrous meshes is the solution of the future. Current research and development in the field of polymer materials focus mostly on easily degradable materials based on natural polymers, which are produced with technologies and processes that do not burden the environment and whose decomposition does not result in the formation of toxic degradation products. Another benefit of natural polymer materials is that they could inherently possess other beneficial properties as well, e.g., antimicrobial activity, antioxidant, and anti-inflammatory properties, and they can induce hemostasis and support wound healing, cell adhesion, and proliferation [4][5]. At the same time, the nanofibrous structure mimics the extracellular matrix, with the small pore size facilitating gas exchange while impeding the passage of bacteria and viruses. Thus, nanofibrous materials based on natural polymers exhibit significant potential, particularly in biomedical applications (tissue engineering and wound healing), and their properties may far surpass those of conventional materials [6].

Antimicrobial activity is an especially important property of nanofibrous materials applied in the medical and even food sectors, where these materials face environments that are highly conducive to the development of microbial growth and the contraction of unwanted infections. A nanofibrous structure that does not have an antimicrobial effect by itself can be modified by adding an antimicrobial agent. There are many ways of incorporating an antimicrobial agent into a nanofibrous network—the simplest one is to add an antimicrobial additive to a spinning polymer solution. The other, increasingly complex methods consist of spraying an antimicrobial additive solution onto an already spun fibrous network, adsorbing the additive to the surface of the nanofibers by soaking the layer in a solution containing the additive, and preparing structurally more complex core–shell fiber structures, in which the antimicrobial additive is part of either the core or the shell. The incorporation of antimicrobials and other additives, which can be partially degraded by high voltage, could be potentially problematic. However, their degradation can be prevented, for example, by using the core–shell technology or polymeric and non-polymeric nanoparticles as a secondary transport drug delivery system for such labile components. Core–shell technology produces fibers in which the additive is protected by a polymer shell. In the latter case, the antimicrobial additive is incorporated into the nanofibrous structure together with the nanoparticle.

Antimicrobial agents themselves can be of both organic and inorganic origin. Their nature does not affect their effectiveness. They can be divided into groups according to the microorganisms they primarily act against—antibiotics (bacteria), antifungals (fungi, yeast), antivirals, and antiparasitics. Based on the specific type of antimicrobial effect, antimicrobial agents can be divided into microbiostatic agents, which inhibit the growth of microorganisms and microbicidal agents that can kill the microbes [7]. The nomenclature further differentiates disinfectants, which kill microbes on non-living surfaces, antiseptics, which are applied to living tissue (e.g., healthcare personnel handwashes, surgical scrubs, and irrigation solutions), and antibiotics, which are usually taken orally as drugs, but could also be administered topically. In practice, a wide range of antimicrobial agents are used. As each group has its own mechanism of action, specific types are carefully selected. The choice is influenced, among other things, by the intended application of the product, depending on which the product may be required to have either a broad-spectrum effect, in which case antimicrobial substances that are effective against the widest possible spectrum of microorganisms are chosen (e.g., broad-spectrum antibiotics), or, on the contrary, a highly specific effect, in which case one reagent is preferred.

The most widely used inorganic antimicrobial agents are undoubtedly nanoparticles (NPs) [8][9][10], with silver nanoparticles (Ag NPs) being the most common [8][11][12], but there are also studies that report using reduced graphene oxide [13] or modified clay minerals [14]. To incorporate reduced graphene oxide or carriers based on clay minerals into nanofibrous mats, the additive is mixed into the electrospinning solution, which is then often sonicated prior to electrospinning. One of three approaches is usually used to incorporate inorganic nanoparticles: (1) by blending the prepared NPs into the spinning solution (blend)—NPs are dispersed by vigorous stirring [15] or ultrasound [16], (2) by adding a precursor or precursors into the spinning solution—NP synthesis prior to or after electrospinning [17], and (3) by using NP immobilization or in situ synthesis on the surface of the nanofibers during post-processing [9][18][19][20]. Naturally, each approach has certain advantages and disadvantages. Blend electrospinning is the easiest method for incorporating NPs into nanofibrous mats. As stated above, mechanical stirring or sonication are used to achieve homogeneous dispersion of NPs in the spinning solution; nevertheless, NPs can still agglomerate or aggregate due to van der Waals interactions. A change in the viscosity and electrospinnability of the polymer solution can occur, resulting in a lower quality of nanofibrous mats. Furthermore, antimicrobial efficiency can be reduced because NPs are anchored inside the polymer fibers and enveloped by the polymer. This reduces their bioavailability and NPs, or metal ions might not be released to interact with pathogenic microorganisms [17][21][22][23]. In situ preparation of NPs in a spinning solution produces better results in terms of NPs' dispersion because components of the polymer solution can serve as stabilizing agents. However, some reagent residues may be toxic [21]. The spinning solution must be designed in such a way as to dissolve both the polymers and the NP precursor as well as produce smooth defect-free nanofibers when electrospun. Most procedures used for in situ synthesis of NPs on the surface of nanofibers during post-processing make use of the adsorption of precursors or metal ions onto nanofibers prepared from a spinning solution. This approach is, therefore, unsuitable for polymers that can dissolve or swell excessively in these solutions because the nanofibrous structure could be impaired.

2. Metal-Based Antimicrobial Agents

2.1. Silver Nanoparticles

As previously stated, the most commonly used antibacterial NPs are silver nanoparticles (Ag NPs). Silver vessels as well as remedies containing silver were used for water and food storage and in the field of medicine (treatment of wounds and ulcers, dental hygiene, surgical sutures, etc.) since the time of the Persian Empire until almost the middle of the 20th century. The popularity of preparations containing silver declined as the use of antibiotics rose at the verge of the 20th century. However, due to the continued increase in antimicrobial resistance in bacteria, silver is returning to prominence in

the field of antimicrobial applications [24][25]. It is generally known that materials in the form of nanoparticles often have different physical and chemical properties from those they display in their bulk form. Ag NPs possess strong antimicrobial properties, and at the same time, the risk of microbes developing resistance to their effects is low because the antimicrobial action they produce involves several mechanisms. Ag NPs have the ability to penetrate bacterial cell walls, change the structure of cell membranes, increase the permeability of cell membranes, produce reactive oxygen species (ROS), and interrupt the replication of deoxyribonucleic acid by releasing silver ions [26][27]. The toxicity of Ag NPs is still being discussed. Some studies claim that Ag NPs have low toxicity for human cells. Nevertheless, their toxicity depends on the manner of administration used, their size, shape, concentration, Ag^+ ions release, etc. [28][29][30].

Studies dealing with nanofibers with Ag NPs are summarized in **Table 1**. The studies are divided by the way Ag NPs were incorporated into nanofibrous mats made from natural polymers or a combination of natural and synthetic polymers. The NPs that have been reported the most in these studies are nanofibers made from cellulose acetate, which was in many cases subsequently regenerated in the post-processing stage to cellulose by NaOH treatment [8][31][32][33]. Synthetic polymers include the commonly used polyvinylalcohol (PVA), polyvinylpyrrolidone (PVP), and polycaprolactone (PCL). Synthetic polymers were added into the spinning solution to enhance its spinnability [15][17] or the mechanical properties of the produced nanofibers [34].

Only a few of the studies report the use of blending for the incorporation of Ag NPs into nanofibrous mats. To incorporate Ag NPs into nanofibrous mats made from hyaluronic acid (HA) and PVA, El-Aassar et al. (2020) [15] first reduced Ag NPs using polygalacturonic acid (PGA), which also served as a stabilizing agent. Even though Ag NPs were dispersed in the spinning solution merely by mechanical stirring, they were distributed homogeneously in the produced nanofibers. The prepared nanofibrous mats produced a stronger antibacterial effect and induced faster wound closure compared both to the control and a commercially available ointment. Similarly, Fereydouni et al. (2023) [35] prepared the Ag NPs with chitosan (CHIT) as a stabilizing agent. For electrospinning, a solution of poly(ethylene oxide) (PEO) was added to the mixture. Resulting nanofibers had good antimicrobial activity against both Gram-positive and Gram-negative bacteria. To neutralize the possible toxicity of Ag NPs, Jatoi et al. (2019) [36] immobilized Ag NPs on the surface of TiO_2 NPs using a polydopamine coating, which served simultaneously as an adhesive, reducing, and stabilizing agent. The Ag NPs/TiO_2 NPs nanocomposites were incorporated into the spinning solution by stirring and sonication and were, therefore, well dispersed in the produced nanofibers. The prepared nanofibrous mats displayed antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* for up to 72 h.

Another method, which was reported by only a handful of the reviewed studies, is the incorporation of Ag NPs into nanofibers by reducing an Ag precursor to NPs in the spinning solution. Maharjan et al. (2017) [11] mixed a precursor ($AgNO_3$) in a zein/polyurethane solution in dimethylformamide (DMF), which served as the reducing agent. They were able to control the size of the produced Ag NPs by adjusting reduction time—the longer the time, the bigger the NPs. Ag NPs of 5–30 nm in size were uniformly distributed on the surface of the fibers. Eghbalifam et al. (2020) [17] dissolved $AgNO_3$ in an aqueous solution of gum Arabic (GA), which was then added to a PVA/DMF solution and left overnight. Both GA and DMF acted as reducing agents, and GA also acted as a stabilizer. The produced nanofibrous mats inhibited the growth of bacteria but did not hinder the proliferation of fibroblasts, which makes them a promising material for the treatment of the infected wounds.

The most reported method of preparation of Ag NPs-coated biopolymer nanofibers is the reduction of silver ions to Ag NPs during post-processing. Nanofibrous mats that have been prepared in advance are immersed in an aqueous solution of silver nitrate ($AgNO_3$) for various periods of time (30 min to 24 h), and the reducing agents and procedures differ in every study. The most frequently used reducing agent is an aqueous solution of sodium borohydrate ($NaBH_4$). The $NaBH_4$ solution can be added directly to the $AgNO_3$ solution containing nanofibers [8] or nanofibrous mats can be immersed in the $NaBH_4$ solution as a second step [23][33], with washing possibly included in between the two steps [12]. Moreover, silver ions can be reduced to Ag NPs by applying thermal treatment and using DMF as the reducing agent [31] or by using a glycol solution of sodium hydroxide [37]. Srivastava et al. (2019) [38] synthesized Ag NPs on the surface of tasar silk fibroin nanofibers via the “green route” using the leaf extract of *Tridax procumbens* as the reducing agent.

Some collectives decided to combine Ag NPs with other additives. Depending on the nature of the substance used, these combinations can produce a synergistic antibacterial effect, encourage wound healing, etc. For instance, Rather et al. (2023) [23] investigated the combination of rosemary essential oil and Ag NPs, observing a synergistic effect on antibacterial properties. Yang et al. (2020) [39] used side-by-side electrospinning and a special spinneret to simultaneously spin blends of PVP/ciproflaxin (CIP) and ethyl cellulose/Ag NPs and produce Janus nanofibers that could be used in wound healing. By using a combination of the two antibacterial agents, the authors created a material that has both a strong initial antibacterial effect caused by the immediate release of CIP and a long-term antibacterial effect produced by

Ag NPs. Strong antibacterial and anti-inflammatory effects were also observed by Chen et al. (2022) [34], who prepared 3D nanofibrous sponges, decorated by a silver-metal organic framework (Ag-MOF) and curcumin. By incorporating dimethyloxallyl glycine into cellulose acetate nanofibers and synthesizing Ag NPs on their surface, the researchers of [37] produced a nanofibrous material that had antibacterial properties and promoted wound healing. Sofi et al. (2021) [33] prepared a nanofibrous scaffold with incorporated particles of nano-hydroxyapatite that acted as nucleation sites inducing apatite growth. The mineralization provided the physiological environment necessary for the adhesion, growth, and proliferation of cells. To avoid microbial infections near the area of application of this scaffold, the surface was covered by Ag NPs.

The presented studies claim that the prepared nanofibrous mats can be used in antibacterial applications in general or, more specifically, in the field of medicine as wound dressings, drug delivery vehicles, or for tissue engineering. However, only a few studies substantiated their claims by in vivo testing. In these studies, the prepared materials were tested on full-thickness wounds in pigs [12], rats [15], or on infected full-thickness wounds on mice [34]. The application of the new nanofibrous materials led to faster re-epithelization compared to commercially available dressings or creams. The 3D nanofibrous sponges made of gelatin/PCL with incorporated Ag-MOF and curcumin significantly promoted the healing of wounds infected with *S. aureus* without any adverse effects (redness, swelling, or suppuration), in comparison to the control.

Table 1. Nanofibrous materials with incorporated Ag NPs.

Polymers	Additives	Incorporation	Antimicrobial Activity	Application	References
Polygalacturonic acid, hyaluronic acid, PVA	Ag NPs	Blend	*E. coli*, *S. aureus*, *B. subtilis*	Wound dressing	El-Aassar et al. (2020) [15]
Chitosan, PEO	Ag NPs	Blend	*E. coli*, *P. aeruginosa*, *S. aureus*, *S. mutans*	Wound healing	Fereydouni et al. (2023) [35]
Ethyl cellulose, PVP	Ag NPs, ciproflaxin	Blend	*E. coli*, *S. aureus*	Wound dressing	Yang et al. (2020) [39]
Cellulose acetate	Ag NPs anchored on TiO_2 NPs	Blend	*E. coli*, *S. aureus*	Antibacterial applications	Jatoi et al. (2019) [36]
Polyurethane, zein	Ag NPs	Precursor reduced in the spinning solution	*E. coli*, *S. aureus*	Wound dressing	Maharjan et al. (2017) [11]
Gum Arabic, PVA, PCL (coating)	Ag NPs	Precursor reduced in the spinning solution	*S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans*	Wound dressing for infectious wounds	Eghbalifam et al. (2020) [17]
Cellulose acetate	Ag NPs	Post-process, in situ reduction	*E. coli*, *S. aureus*	Antibacterial applications	Kalwar et al. (2018) [8]
Cellulose acetate	Ag NPs	Post-process, in situ reduction	*E. coli*, *S. aureus*	Antibacterial applications	Jatoi et al. (2019) [31]
Cellulose acetate	Ag NPs	Post-process, in situ reduction	*E. coli*, *S. aureus*	Tissue engineering	Moon et al. (2021) [32]
Jellyfish biomass, PCL	Ag NPs	Post-process, in situ reduction	*E. coli*, *S. aureus*, *B. subtilis*	Wound healing	Nudelman et al. (2019) [12]
Tasar fibroin	Ag NPs	Post-process, in situ reduction	*E. coli*, *S. aureus*, *P. aeruginosa*, *S. epidermidis*	Drug delivery vehicle, tissue engineering	Srivastava et al. (2019) [38]
Cellulose acetate, polyurethane	Ag NPs, rosemary EO	Post-process, in situ reduction	*E. coli*, *S. aureus*	Wound dressing	Rather et al. (2023) [23]
Cellulose acetate	Ag NPs, hydroxyapatite	Post-process, in situ reduction	*E. coli*, *S. aureus*	Wound healing, bone tissue regeneration	Sofi et al. (2021) [33]

Polymers	Additives	Incorporation	Antimicrobial Activity	Application	References
Cellulose acetate	Ag NPs, dimethyloxallyl glycine	Post-process, in situ reduction	*E. coli*, *B. subtilis*	Diabetic wound healing	Li et al. (2022) [37]
Gelatin, PCL	Ag-MOF, curcumin	Post-process, in situ reduction	*E. coli*, *S. aureus*	Wound dressing	Chen et al. (2022) [34]

2.2. Zinc Oxide Nanoparticles

Zinc oxide nanoparticles (ZnO NPs) possess many attractive properties, which make them useful in many areas of human activity. Similar to silver, zinc oxide has been known for its antibacterial properties throughout history. Historical records show that ointments with ZnO were used to treat injuries and ulcers as early as 2000 BC. The exact mechanism behind the antimicrobial action of ZnO NPs is not yet known, but it is believed that ZnO NPs induce the production of excess reactive oxygen species, which can accumulate in the outer cell membrane or cytoplasm and release zinc ions. This would cause bacterial cell membrane disintegration, membrane protein damage, and genomic instability, resulting in the death of the affected bacterial cells. The antimicrobial activity of ZnO NPs is dependent on their size, shape, and photocatalytic activity. ZnO NPs have been approved by the FDA as a food additive, and they are generally recognized as safe and non-toxic in low concentrations. ZnO NPs can also absorb and block UV radiation and are, therefore, used as additives both in skin creams/sunscreens and textiles resistant to UV-VIS light [40][41][42].

Three methods of incorporation of ZnO NPs into nanofibrous mats have been reported, namely blend electrospinning, side-by-side electrospinning/electrospraying, and post-process immobilization of NPs on prepared fibers (**Table 2**). To disperse ZnO NPs in blend electrospinning solution, various researchers used either stirring [10][43][44][45][46] or sonication [16][46][47], but little to no attention was paid to the homogeneity of NP distribution in the nanofibrous mats. Chen et al. (2019) [48] made use of the variability of the electrospinning process by using the side-by-side electrospinning/electrospraying process, in which a gelatin solution was spun and, simultaneously, a dispersion of ZnO NPs was sprayed. An image analysis of the produced nanofibers confirmed that NPs were present only on the surface of the nanofibers and were uniformly distributed. The method of post-process immobilization uses NPs that have been prepared in advance. Hasannasab et al. (2021) [18] first modified the surface of silk fibroin nanofibers by plasma treatment and acrylic acid grafting. In another study, a colloidal solution of ZnO NPs was applied to a nanofibrous mat that had been prepared earlier and left to dry [49]. In none of the procedures was the sample washed to remove non-adsorbed nanoparticles. However, a study focused on the release of Zn^+ ions showed that Zn^+ ions were gradually released over 5 days with no initial burst release.

Some researchers also incorporated secondary additives, such as essential oils (EO) [16], antimicrobial polymers [10][43], antibiotics [46][47], and enzymes [18], into the nanofibrous mats they used for the preparation of antimicrobial dressings. Nanofibers with a combination of ZnO NPs and cinnamon EO displayed enhanced antibacterial effectiveness against *S. aureus* and cytocompatibility with human dermal fibroblasts compared to nanofibers loaded only with ZnO NPs. Most importantly, nanofibers loaded with a combination of additives proved to be more efficient than other nanofibrous solutions in healing full-thickness incision wounds infected with *S. aureus* in rats in terms of bacterial inhibition following a single application, healing speed, and the quality of skin structure recovery, which was verified by morphological, microbiological, and histopathological studies [16]. Ahmed et al. (2018) [10] and Ranjbar-Mohammadi et al. (2021) [43] chose two different methods of integration of CHIT, which is known for its antibacterial properties. The first collective of researchers prepared nanofibers from CHIT and PVA, while the second collective incorporated a nanofibrillated CHIT/ZnO NPs composite into keratin/polylactic acid (PLA)/PVA nanofibers as an additive. In both cases, the combination of CHIT and ZnO NPs produced an excellent antibacterial effect.

Most of the reported studies focused on preparing materials for wound healing, though some were intended for more specific applications, such as the treatment of burns or diabetic wounds. For diabetic wound healing, nanofibrous mats made from a combination of PVA/CHIT or PVA/CHIT/ZnO NPs were tested on incision wounds in diabetic rabbits and compared with untreated controls. Both types of nanofibrous mats accelerated wound closure, but wounds treated with the PVA/CHIT/ZnO NPs mats showed greater wound contraction and better quality of new tissue [10]. When used for the treatment of second-degree burns induced in mice and rats, silk fibroin/ZnO wound dressing materials decreased the inflammatory response, enhanced skin regeneration, and stimulated the growth of hair follicles, the formation of sebaceous glands, and the deposition of collagen to a greater degree than nanofibrous mats made solely from silk fibroin and untreated controls [18][44]. The positive effect of ZnO NPs on wound closure in vivo was also evident when the NPs were incorporated in CHIT/PVA nanofibers. Wound healing was accelerated, with almost complete wound healing

achieved in 12 days. The wound space was matured, filled with granular tissue, and relatively thick squamous epithelium formed on the wound surface. Chitosan, which is degraded to N-acetylglucosamine during the wound healing period, supported fibroblast proliferation, collagen formation, and hyaluronic acid deposition at the wound site [45].

Table 2. Nanofibrous materials containing ZnO NPs.

Polymers	Additives	Incorporation	Antimicrobial Activity	Application	References
Silk fibroin, HA	ZnO NPs	Blend	*E. coli*, *S. aureus*	Burn wound healing	Hadisi et al. (2020) [44]
Chitosan, PVA	ZnO NPs	Blend	*E. coli*, *S. aureus*	Wound dressing, tissue engineering	Rezaei et al. (2023) [45]
HA, PVA, PEO	ZnO NPs, cinnamon EO	Blend	*S. aureus*	Wound dressing	El-Aassar et al. (2021) [16]
Chitosan, PVA	ZnO NPs, chitosan	Blend	*E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis*	Diabetic wound healing	Ahmed et al. (2018) [10]
PLA, PVA, keratin	ZnO NPs, chitosan	Blend	*E. coli*, *S. aureus*	Tissue engineering applications	Ranjbar Mohammadi et al. (2021) [43]
PCL, gelatin	ZnO NPs, amoxicilin	Blend	*S. aureus*	Wound dressing	Jafari et al. (2020) [47]
Sodium alginate, PVA	ZnO NPs, ciprofloxacin	Blend	*E. coli*, *S. aureus*	Wound dressing	Sadeghi et al. (2023) [46]
Soy protein isolate, Eudragit	ZnO-halloysite nanotubes, allantoin	Blend	*E. coli*, *S. aureus*	Skin tissue-engineered constructs	Jaberifard et al. (2023) [50]
Gelatin	ZnO NPs	Side-by-side (spraying/spinning)	*E. coli*, *S. aureus*	Wound dressing	Chen et al. (2019) [48]
Silk fibroin	ZnO NPs (polydopamine coated), bromelain	Post-process immobilization	*E. coli*, *S. aureus*	Burn wound healing	Hasannasab et al. (2021) [18]
Gum arabic, PVA, PCL	ZnO NPs	Post-process immobilization	*E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans*	Wound dressing, antimicrobial and antibiofilm coating	Harandi et al. (2021) [49]

2.3. Other Nanoparticles

There are several other types of metal-based nanoparticles but their incorporation into natural polymer-based nanofibrous mats is not commonly reported, such as in the case of Ag NPs and ZnO NPs. Thus, this section summarizes the literature describing the use of other metal-based nanoparticles, namely Fe_2O_3 NPs, TiO_2 NPs, and Cu-based NPs, for antimicrobial applications in the medical field (**Table 3**).

Similar to other presented types of NPs, the antimicrobial action of Cu-based NPs is attributed to the small size and high specific surface area. The mechanism involves release of copper ions and their interaction with bacterial cell walls. The ions are also able to adhere on and penetrate the cell wall, alter its permeability, or destroy it and cause leakage of cytoplasm. Moreover, the production of ROS, damaging DNA, denaturation of proteins, and altering the enzyme function, is involved [51][52][53]. Cu-based NPs can find applications in medicine (reducing hospital infections, treatment of wounds, and preventing colonization of medical devices) or for water purification. The advantage in comparison with widely used Ag and Au NPs is their lower price [51][52][53]. There are some examples of nanofibers/Cu-based NPs proposed for medicinal use found in the literature. Haider et al. (2021) [20] prepared cellulose nanofibers, and the CuO NPs were synthetized on their surface by reduction of the precursor. Nanofibrous mats showed good antibacterial efficacy against both G− and G+ bacteria, cytocompatibility, antioxidant activity, and the moisture vapor transmission rate, thus being a good candidate for wound healing applications. Lu et al. (2022) [19] developed chitosan nanofibers with CuS NPs and fucoidan immobilized on their surface. The antibacterial action is unique to other reported studies—the bacteria is killed by photothermal and photocatalytic bactericidal effects. CuS can convert near-infrared light into heat, and thus it produces local hyperthermia for thermal ablation of bacteria. The release of fucoidan and Cu ions promoted alkaline phosphatase

activity of osteoblast cells and capillary tube formation of endothelial cells, which is suitable for treating wound or bone infections and for tissue regeneration, after further in vivo study. Elevated concentrations of Cu ions increase the risk of toxicity because they can interfere with the homeostasis of other metals, damage DNA, and generate reactive oxygen species. To avoid these possible toxic side effects of Cu ions caused by their high concentration, Zirak Hassan Kiadeh et al. (2021) [54] incorporated Cu-based MOF with gradual release of Cu ions into pectin/PEO nanofibers. In addition, folic acid for stabilizing the Cu-MOF and enhancing angiogenesis, fibroblast migration, and proliferation was incorporated. The prepared nanofibers exhibited antibacterial activity while being biocompatible, indicating that the Cu ions' release was in the safe range.

The use for medicine (wound healing) applications was also described for Fe_2O_3 NPs. The nanoparticles were prepared by green synthesis using bacteria *Lactobacillus acidophilus* and *Lactobacillus plantarum* and then coated onto the gum Arabic/PVA/PCL nanofibers. The Fe_2O_3 NPs can penetrate the cell wall and cause cell membrane injury and cell death due to the small size and high specific surface area. Probiotic *Lactobacillus* bacteria can, on top of that, produce inhibitory compounds and reactive oxygen species; thus, these two agents act synergistically. The nanofibrous mats were able to inhibit bacteria, fungi, as well as biofilm by adsorbing the microorganisms onto the nanofibers while maintaining excellent cell viability [55].

Titanium dioxide nanoparticles (TiO_2 NPs) are among the most used nanoparticles in different industrial and consumer products due to their catalytic and antimicrobial properties. TiO_2 NPs are also used in many areas—building engineering (windows, tiles, wall paint, and coatings), agriculture (fertilizers and pesticides), environmental protection (water and sewage treatment), pharmaceuticals, food products, and the cosmetics industry [56][57]. The antimicrobial activity is related to several mechanisms—interaction between NP and bacterial surface, release of ions, and production of ROS under UV light [58]. Blantocas et al. (2018) [9] prepared chitosan nanofibers consequently coated by TiO_2 NPs via the plasma-enhanced chemical vapor process for wastewater remediation. The original idea was to blend photo-catalytic metal oxide and chitosan with antimicrobial properties. However, pure chitosan nanofibers showed no inhibitory effect. On the other hand, composite material showed clear inhibition zones in disc diffusion assay, with their size depending on the plasma deposition time.

In a study by Shahverdi et al. (2023) [59], the use of cerium aluminate NPs ($CeAlO_3$ NPs) in potential nanofibrous wound dressing was reported for the first time. NPs were dispersed in a solution of chitosan and PVA, together with cephalexin, an antibiotic model. Composite nanofiber mats with PCL were prepared by side-by-side electrospinning. The incorporation of NPs caused an increase in tensile strength and modulus. $CeAlO_3$-incorporated nanofibers did not affect nanofiber biocompatibility, and fibroblast cells could better grow, differentiate, and cover the scaffold surface compared to the neat fibers. The prepared nanofibers could be a promising wound dressing material prepared by electrospinning directly onto a fabric dressing.

Table 3. Summary of various inorganic NPs incorporated in natural polymer-based nanofibrous mats.

Polymers	Additives	Incorporation	Antimicrobial Actitivy	Application	References
Pectin, PEO	Cu MOF, folic acid	Blend	*E. coli*, *S. aureus*	Drug delivery system	Zirak Hassan Kiadeh et al. (2019) [54]
Cellulose	CuO NPs	Post-process in situ synthesis	*E. coli*, *S. aureus*	Wound dressing	Haider et al. (2021) [20]
Chitosan	CuS NPs, fucoidan	Post-process immobilization	*E. coli*, *S. aureus*	Treating bone and wound infections, tissue regeneration	LuLu et al. (2022) [19]
Gum arabic, PVA; PCL coated	Fe_2O_3 NPs	Post-process adsorption	*E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans*	Wound healing applications	Harandi et al. (2022) [55]
Chitosan	TiO_2 NPs	Post-process	*S. aureus*	Wastewater remediation	Blantocas et al. (2018) [9]
Chitosan, PVA, PCL	$CeAlO_3$ NPs, cephalexin	Blend	*E. coli*, *S. aureus*	Wound dressing	Shahverdi et al. (2022) [59]

3. Non-Metallic Inorganic Antimicrobial Agents

This section presents other inorganic nanomaterials, including graphene derivatives and clay minerals, which have been used in recent years as fillers in nanofibrous mats based on natural polymers intended for antimicrobial applications (summarized in **Table 4**). Graphene derivatives should possess inherent antimicrobial properties; in the case of clay minerals, these only serve as carriers for other antimicrobial substances.

Graphene and its derivatives (graphene-based materials—GM) have great mechanical and conducting properties, and they are usually used for preparation of nanofibrous nanocomposites with potential in tissue engineering or sensor technologies [60][61]. The reported antimicrobial activity of GM is attributed to the physicochemical interaction between GM and bacteria and physical damage of the bacterial membranes, leading to the inactivation of bacteria due do leakage of the intracellular matrix. The antimicrobial effect further includes various mechanisms, such as inducing oxidative and membrane stress, deterioration of cellular components (protein, lipids, and nucleic acids), or interaction with RNA/DNA hydrogen groups [62][63]. In recent years, graphene oxide or reduced graphene oxide was incorporated into silk fibroin nanofibers by blending the additive in the spinning solution. This method should suppress possible toxic effects of the nanofillers. The prepared nanofibrous mats were found effective against both Gram-positive and Gram-negative bacteria and biocompatible (in vitro) [13][64][65].

Inorganic nanoparticles based on clay minerals, such as halloysite nanotubes (halloysite NTs), montmorillonite, or kaolinite, are often used as carriers for drugs or nanoparticles. Clay minerals are generally chemically inert with good biocompatibility and low toxicity. Drug loading is influenced by many factors, including the surface area, cation exchange capacity, charge density, and swelling degree of clay [66]. Fatahi et al. (2021) [67] loaded levofloxacin, an antibiotic drug, into halloysite NTs, which were incorporated into sodium alginate/PEO by blend electrospinning. Resulting nanofibrous mats possessed biocompatible and good antimicrobial efficiency, which was slightly lowered in comparison to sodium alginate/PEO/levofloxacin nanofibers. Most importantly, in vitro release analysis indicated that nanocomposite fibrous mats have great potential for the sustained release of the antibiotic. Zou et al. (2020) [68] prepared chitosan/PCL nanofibers blended with chlorogenic acid/halloysite NTs. Chlorogenic acid is a naturally occurring plant-derived bioactive compound with antimicrobial and antioxidant properties. The composite nanofibrous mats showed improved hydrophilicity and long-term sustained release of chlorogenic acid driven by Fickian diffusion. However, the material was meant for its usage in the food industry, so no in vivo tests were performed.

Hydroxyapatite (HAp) is a bioceramic material with similar mineral components and crystal structure as mammalian bones. HAp is biocompatible, bioactive, osteoconductive, and stable (both chemically and thermally), but it lacks other certain important properties, such as mechanical strength or antimicrobial activity. However, HAp can be modified by incorporation of a wide range of ions into the crystal lattice while the crystal remains stable, introducing additional properties. The doping by naturally occurring elements (Mg, Zn, Mn, Cu, Sr, Ag, Ce, and Cu) with intrinsic antimicrobial activity could be used to turn HAp into a drug carrier, preventing bacterial infections and promoting other biological processes in tissue engineering [14][69]. Antibacterial activity against *S. aureus* and *E. coli* grew simultaneously with higher doping by Cu ions. At the same time, human fibroblast cells were cultivated on the scaffolds in vitro and were adhered to, spread, and proliferated both on fibers' surface and inside the scaffold [53]. Similar results were reported by Kandasamy et al. (2020) [14], when the zone of microorganism inhibition increased with the increase in concentration of Zn and Mn co-dopants. Carboxymethyl cellulose/PVP/HAp were also found hemocompatible, non-toxic (supported the attachment and proliferation of HOS cells), and performed biomineralization activity in vitro. The material was proposed for bone tissue engineering applications after further in vivo studies.

Ramírez-Agudelo et al. (2018) [70] incorporated HAp nanoparticles and doxycycline into gelatin/PCL nanofibrous mats for cancer therapy. Doxycycline is a well-known antibiotic with activity against cancer cells, and it was reported that even HAp nanoparticles themselves inhibit the proliferation of several kinds of cancer cells while supporting the proliferation of normal bone cells. The synergic dual-effect was confirmed by a higher cytotoxic effect on selected cancer cells and higher bacteria inhibition performed by combinational nanofibrous mats compared to single-agent nanofibers. Moreover, HAp nanoparticles might be used as drug carriers, similarly to nano-sized clay minerals. Wang et al. (2021) [71] adsorbed tetracycline hydrochloride (TCH) on the surface of HAp nanoparticles and the composite was then blended with the spinning solution and electrospun. The release of TCH from TCH/HAp/gelatin/chitosan nanofibers was significantly prolonged in comparison to the release of TCH from TCH/gelatin/chitosan nanofibers, from 4 to 14 days. The antibacterial activity was tested by the disc-diffusion method also on "pre-released" samples, meaning the samples were left in a release medium for certain time and then the antibacterial tests were conducted. TCH/gelatin/chitosan nanofibers showed prominent inhibitory zones after 1 and 4 days, and a small inhibition zone after 6 days of pre-release. In contrast,

TCH/HAp/gelatin/chitosan nanofibers showed a distinctive inhibition zone even after 14 days of pre-release. The authors propose the prepared material as an attractive candidate for applications in wound dressings and drug release systems.

Table 4. Summary of other inorganic materials incorporated in natural polymer-based nanofibrous mats.

Polymers	Additives	Incorporation	Antimicrobial Activity	Application	References
Silk fibroin	Graphene oxide	Blend	*E. coli*, *S. aureus*	Wound dressing	Wang et al. (2018) [72]
Silk fibroin	Reduced graphene oxide	Blend	*E. coli*, *S. aureus*	Tissue engineering	Zhang et al. (2021) [13]
Silk fibroin	Reduced graphene oxide/TiO_2	Blend	*S. aureus*	Tissue engineering	Zhang et al. (2021) [64]
Sodium alginate, PEO	Halloysite NT, levofloxacin	Blend	*E. coli*, *S. aureus*	Drug carrier, wound dressing	Fatahi et al. (2021) [67]
Cellulose acetate	HAp + Cu ions	Blend	*E. coli*, *S. aureus*	Regeneration of skin tissues	Elsayed et al. (2020) [69]
Carboxymethyl cellulose, PVP	HAp, doped by Zn and Mn	Blend	*E. coli*, *S. aureus*, *C. albicans*	Bone tissue engineering	Kandasamy et al. (2020) [14]
Gelatin, PCL	HAp, doxycyclin	Blend	*S. aureus*, *P. gingivalis*	Drug delivery	Ramírez-Agudelo et al. (2018) [70]
Gelatin, chitosan	HAp, tetracycline hydrochloride	Blend	*E. coli*, *S. aureus*	Antimicrobial wound dressing	Wang et al. (2021) [71]

References

1. Heunis, T.D.J.; Dicks, L.M.T. Nanofibers Offer Alternative Ways to the Treatment of Skin Infections. J. Biomed. Biotechnol. 2010, 2010, 510682.
2. Liu, M.; Duan, X.P.; Li, Y.M.; Yang, D.P.; Long, Y.Z. Electrospun nanofibers for wound healing. Mater. Sci. Eng. C 2017, 76, 1413–1423.
3. Sylvester, M.A.; Amini, F.; Tan, C.K. Electrospun nanofibers in wound healing. Mater. Today Proc. 2020, 29, 1–6.
4. Bombin, A.D.J.; Dunne, N.J.; McCarthy, H.O. Electrospinning of natural polymers for the production of nanofibres for wound healing applications. Mater. Sci. Eng. C Mater. Biol. Appl. 2020, 114, 110994.
5. Hamdan, N.; Yamin, A.; Hamid, S.A.; Khodir, W.K.W.A.; Guarino, V. Functionalized Antimicrobial Nanofibers: Design Criteria and Recent Advances. J. Funct. Biomater. 2021, 12, 59.
6. Bombin, A.D.J.; Dunne, N.; McCarthy, H. A microRNA ‘cocktail’ by electrospun PVA/alginate/ciprofloxacin nanofibres: A genetic nanomedicine for impaired wound healing. In Proceedings of the 31st Conference of the European Society for Biomaterials, Porto, Portugal, 5–9 September 2021.
7. Hajipour, M.J.; Fromm, K.M.; Ashkarran, A.A.; de Aberasturi, D.J.; de Larramendi, I.R.; Rojo, T.; Serpooshan, V.; Parak, W.J.; Mahmoudi, M. Antibacterial properties of nanoparticles. Trends Biotechnol. 2012, 30, 499–511.
8. Kalwar, K.; Hu, L.; Li, D.; Shan, D. AgNPs incorporated on deacetylated electrospun cellulose nanofibers and their effect on the antimicrobial activity. Polym. Adv. Technol. 2017, 29, 394–400.
9. Blantocas, G.Q.; Alaboodi, A.S.; Mekky, A.-B.H. Synthesis of Chitosan—TiO2 Antimicrobial Composites via a 2-Step Process of Electrospinning and Plasma Sputtering. Arab. J. Sci. Eng. 2017, 43, 389–398.
10. Ahmed, R.; Tariq, M.; Ali, I.; Asghar, R.; Khanam, P.N.; Augustine, R.; Hasan, A. Novel electrospun chitosan/polyvinyl alcohol/zinc oxide nanofibrous mats with antibacterial and antioxidant properties for diabetic wound healing. Int. J. Biol. Macromol. 2018, 120, 385–393.
11. Maharjan, B.; Joshi, M.K.; Tiwari, A.P.; Park, C.H.; Kim, C.S. In-situ synthesis of AgNPs in the natural/synthetic hybrid nanofibrous scaffolds: Fabrication, characterization and antimicrobial activities. J. Mech. Behav. Biomed. Mater. 2017, 65, 66–76.
12. Nudelman, R.; Alhmoud, H.; Delalat, B.; Fleicher, S.; Fine, E.; Guliakhmedova, T.; Elnathan, R.; Nyska, A.; Voelcker, N.H.; Gozin, M.; et al. Jellyfish-Based Smart Wound Dressing Devices Containing In Situ Synthesized Antibacterial

Nanoparticles. Adv. Funct. Mater. 2019, 29, 1902783.

13. Zhang, C.; Wang, X.; Fan, S.; Lan, P.; Cao, C.; Zhang, Y. Silk fibroin/reduced graphene oxide composite mats with enhanced mechanical properties and conductivity for tissue engineering. Colloids Surf. B Biointerfaces 2021, 197, 111444.
14. Kandasamy, S.; Narayanan, V.; Sumathi, S. Zinc and manganese substituted hydroxyapatite/CMC/PVP electrospun composite for bone repair applications. Int. J. Biol. Macromol. 2020, 145, 1018–1030.
15. El-Aassar, M.R.; Ibrahim, O.M.; Fouda, M.M.G.; El-Beheri, N.G.; Agwa, M.M. Wound healing of nanofiber comprising Polygalacturonic/Hyaluronic acid embedded silver nanoparticles: In-vitro and in-vivo studies. Carbohydr. Polym. 2020, 238, 116175.
16. El-Aassar, M.R.; El-Beheri, N.G.; Agwa, M.M.; Eltaher, H.M.; Alseqely, M.; Sadik, W.S.; El-Khordagui, L. Antibiotic-free combinational hyaluronic acid blend nanofibers for wound healing enhancement. Int. J. Biol. Macromol. 2021, 167, 1552–1563.
17. Eghbalifam, N.; Shojaosadati, S.A.; Hashemi-Najafabadi, S.; Khorasani, A.C. Synthesis and characterization of antimicrobial wound dressing material based on silver nanoparticles loaded gum Arabic nanofibers. Int. J. Biol. Macromol. 2020, 155, 119–130.
18. Hasannasab, M.; Nourmohammadi, J.; Dehghan, M.M.; Ghaee, A. Immobilization of bromelain and ZnO nanoparticles on silk fibroin nanofibers as an antibacterial and anti-inflammatory burn dressing. Int. J. Pharm. 2021, 610, 121227.
19. Lu, H.-T.; Huang, G.-Y.; Chang, W.-J.; Lu, T.-W.; Huang, T.-W.; Ho, M.-H.; Mi, F.-L. Modification of chitosan nanofibers with CuS and fucoidan for antibacterial and bone tissue engineering applications. Carbohydr. Polym. 2022, 281, 119035.
20. Haider, K.; Ullah, A.; Sarwar, M.N.; Saito, Y.; Sun, L.; Park, S.; Kim, I.S. Lignin-mediated in-situ synthesis of CuO nanoparticles on cellulose nanofibers: A potential wound dressing material. Int. J. Biol. Macromol. 2021, 173, 315–326.
21. Shi, Q.; Vitchuli, N.; Nowak, J.; Caldwell, J.M.; Breidt, F.; Bourham, M.; Zhang, X.; McCord, M. Durable antibacterial Ag/polyacrylonitrile (Ag/PAN) hybrid nanofibers prepared by atmospheric plasma treatment and electrospinning. Eur. Polym. J. 2011, 47, 1402–1409.
22. Rodríguez-Tobías, H.; Morales, G.; Grande, D. Comprehensive review on electrospinning techniques as versatile approaches toward antimicrobial biopolymeric composite fibers. Mater. Sci. Eng. C 2019, 101, 306–322.
23. Rather, A.H.; Khan, R.S.; Wani, T.U.; Rafiq, M.; Jadhav, A.H.; Srinivasappa, P.M.; Abdal-Hay, A.; Sultan, P.; Rather, S.-U.; Macossay, J.; et al. Polyurethane and cellulose acetate micro-nanofibers containing rosemary essential oil, and decorated with silver nanoparticles for wound healing application. Int. J. Biol. Macromol. 2023, 226, 690–705.
24. Alexander, J.W. History of the Medical Use of Silver. Surg. Infect. 2009, 10, 289–292.
25. Sim, W.; Barnard, R.T.; Blaskovich, M.A.T.; Ziora, Z.M. Antimicrobial silver in medicinal and consumer applications: A patent review of the past decade (2007–2017). Antibiotics 2018, 7, 93.
26. Bruna, T.; Maldonado-Bravo, F.; Jara, P.; Caro, N. Silver Nanoparticles and Their Antibacterial Applications. Int. J. Mol. Sci. 2021, 22, 7202.
27. Yin, I.X.; Zhang, J.; Zhao, I.S.; Mei, M.L.; Li, Q.; Chu, C.H. The Antibacterial Mechanism of Silver Nanoparticles and Its Application in Dentistry. Int. J. Nanomed. 2020, ume 15, 2555–2562.
28. Azizi-Lalabadi, M.; Garavand, F.; Jafari, S.M. Incorporation of silver nanoparticles into active antimicrobial nanocomposites: Release behavior, analyzing techniques, applications and safety issues. Adv. Colloid Interface Sci. 2021, 293, 102440.
29. Jane Cypriyana, P.J.; Saigeetha, S.; Lavanya Agnes Angalene, J.; Samrot, A.V.; Suresh Kumar, S.; Ponniah, P.; Chakravarthi, S. Overview on toxicity of nanoparticles, it's mechanism, models used in toxicity studies and disposal methods—A review. Biocatal. Agric. Biotechnol. 2021, 36, 102117.
30. Ferdous, Z.; Nemmar, A. Health Impact of Silver Nanoparticles: A Review of the Biodistribution and Toxicity Following Various Routes of Exposure. Int. J. Mol. Sci. 2020, 21, 2375.
31. Jatoi, A.W.; Kim, I.S.; Ni, Q.Q. A comparative study on synthesis of AgNPs on cellulose nanofibers by thermal treatment and DMF for antibacterial activities. Mater. Sci. Eng. C 2019, 98, 1179–1195.
32. Moon, J.Y.; Lee, J.; Hwang, T.I.; Park, C.H.; Kim, C.S. A multifunctional, one-step gas foaming strategy for antimicrobial silver nanoparticle-decorated 3D cellulose nanofiber scaffolds. Carbohydr. Polym. 2021, 273, 118603.
33. Sofi, H.S.; Akram, T.; Shabir, N.; Vasita, R.; Jadhav, A.H.; Sheikh, F.A. Regenerated cellulose nanofibers from cellulose acetate: Incorporating hydroxyapatite (HAp) and silver (Ag) nanoparticles (NPs), as a scaffold for tissue engineering applications. Mater. Sci. Eng. C 2020, 118, 111547.

34. Chen, J.; Huang, Z.; Zhang, H.; Zhang, Z.; Wang, D.; Xia, D.; Yang, C.; Dong, M. Three-dimensional layered nanofiber sponge with in situ grown silver- metal organic framework for enhancing wound healing. Chem. Eng. J. 2022, 443, 136234.

35. Fereydouni, N.; Zangouei, M.; Darroudi, M.; Hosseinpour, M.; Gholoobi, A. Antibacterial activity of chitosan-polyethylene oxide nanofibers containing silver nanoparticles against aerobic and anaerobic bacteria. J. Mol. Struct. 2023, 1274, 134304.

36. Jatoi, A.W.; Kim, I.S.; Ni, Q.-Q. Cellulose acetate nanofibers embedded with AgNPs anchored TiO2 nanoparticles for long term excellent antibacterial applications. Carbohydr. Polym. 2019, 207, 640–649.

37. Li, C.; Liu, Z.; Liu, S.; Tiwari, S.K.; Thummavichai, K.; Ola, O.; Ma, Z.; Zhang, S.; Wang, N.; Zhu, Y. Antibacterial properties and drug release study of cellulose acetate nanofibers containing ear-like Ag-NPs and Dimethyloxallyl Glycine/beta-cyclodextrin. Appl. Surf. Sci. 2022, 590, 153132.

38. Srivastava, C.M.; Purwar, R.; Gupta, A.P. Enhanced potential of biomimetic, silver nanoparticles functionalized Antheraea mylitta (tasar) silk fibroin nanofibrous mats for skin tissue engineering. Int. J. Biol. Macromol. 2019, 130, 437–453.

39. Yang, J.; Wang, K.; Yu, D.-G.; Yang, Y.; Bligh, S.W.A.; Williams, G.R. Electrospun Janus nanofibers loaded with a drug and inorganic nanoparticles as an effective antibacterial wound dressing. Mater. Sci. Eng. C 2020, 111, 110805.

40. Cotton, G.C.; Lagesse, N.R.; Parke, L.S.; Meledandri, C.J. Antibacterial Nanoparticles. In Comprehensive Nanoscience and Nanotechnology; Academic Press: Cambridge, MA, USA, 2019; pp. 65–82. ISBN 9780128122952.

41. Jiang, J.; Pi, J.; Cai, J. The Advancing of Zinc Oxide Nanoparticles for Biomedical Applications. Bioinorg. Chem. Appl. 2018, 2018, 1062562.

42. Siddiqi, K.S.; Rahman, A.U.; Tajuddin, N.; Husen, A. Properties of Zinc Oxide Nanoparticles and Their Activity Against Microbes. Nanoscale Res. Lett. 2018, 13, 141.

43. Ranjbar-Mohammadi, M.; Shakoori, P.; Arab-Bafrani, Z. Design and characterization of keratin/PVA-PLA nanofibers containing hybrids of nanofibrillated chitosan/ZnO nanoparticles. Int. J. Biol. Macromol. 2021, 187, 554–565.

44. Hadisi, Z.; Farokhi, M.; Bakhsheshi-Rad, H.R.; Jahanshahi, M.; Hasanpour, S.; Pagan, E.; Dolatshahi-Pirouz, A.; Zhang, Y.S.; Kundu, S.C.; Akbari, M. Hyaluronic Acid (HA)-Based Silk Fibroin/Zinc Oxide Core–Shell Electrospun Dressing for Burn Wound Management. Macromol. Biosci. 2020, 20, e1900328.

45. Rezaei, A.; Katoueizadeh, E.; Zebarjad, S.M. Investigating of the influence of zinc oxide nanoparticles morphology on the properties of electrospun polyvinyl alcohol/chitosan (PVA/CS) nanofibers. J. Drug Deliv. Sci. Technol. 2023, 86, 104712.

46. Sadeghi, M.; Rahimnejad, M.; Adeli, H.; Feizi, F. Matrix–Drug Interactions for the Development of pH-Sensitive Alginate-Based Nanofibers as an Advanced Wound Dressing. J. Polym. Environ. 2023, 31, 1242–1256.

47. Jafari, A.; Amirsadeghi, A.; Hassanajili, S.; Azarpira, N. Bioactive antibacterial bilayer PCL/gelatin nanofibrous scaffold promotes full-thickness wound healing. Int. J. Pharm. 2020, 583, 119413.

48. Chen, Y.; Lu, W.; Guo, Y.; Zhu, Y.; Song, Y. Electrospun Gelatin Fibers Surface Loaded ZnO Particles as a Potential Biodegradable Antibacterial Wound Dressing. Nanomaterials 2019, 9, 525.

49. Harandi, F.N.; Khorasani, A.C.; Shojaosadati, S.A.; Hashemi-Najafabadi, S. Living Lactobacillus–ZnO nanoparticles hybrids as antimicrobial and antibiofilm coatings for wound dressing application. Mater. Sci. Eng. C 2021, 130, 112457.

50. Jaberifard, F.; Ramezani, S.; Ghorbani, M.; Arsalani, N.; Moghadam, F.M. Investigation of wound healing efficiency of multifunctional eudragit/soy protein isolate electrospun nanofiber incorporated with ZnO loaded halloysite nanotubes and allantoin. Int. J. Pharm. 2023, 630, 122434.

51. Makvandi, P.; Wang, C.Y.; Zare, E.N.; Borzacchiello, A.; Niu, L.N.; Tay, F.R. Metal-Based Nanomaterials in Biomedical Applications: Antimicrobial Activity and Cytotoxicity Aspects. Adv. Funct. Mater. 2020, 30, 1910021.

52. Raffi, M.; Mehrwan, S.; Bhatti, T.M.; Akhter, J.I.; Hameed, A.; Yawar, W.; ul Hasan, M.M. Investigations into the antibacterial behavior of copper nanoparticles against Escherichia coli. Ann. Microbiol. 2010, 60, 75–80.

53. Usman, M.S.; El Zowalaty, M.E.; Shameli, K.; Zainuddin, N.; Salama, M.; Ibrahim, N.A. Synthesis, characterization, and antimicrobial properties of copper nanoparticles. Int. J. Nanomed. 2013, 8, 4467–4479.

54. Kiadeh, S.Z.H.; Ghaee, A.; Farokhi, M.; Nourmohammadi, J.; Bahi, A.; Ko, F.K. Electrospun pectin/modified copper-based metal–organic framework (MOF) nanofibers as a drug delivery system. Int. J. Biol. Macromol. 2021, 173, 351–365.

55. Harandi, F.N.; Khorasani, A.C.; Shojaosadati, S.A.; Hashemi-Najafabadi, S. Surface modification of electrospun wound dressing material by Fe2O3 nanoparticles incorporating Lactobacillus strains for enhanced antimicrobial and antibiofilm

activity. Surf. Interfaces 2022, 28, 101592.

56. Baranowska-Wójcik, E.; Szwajgier, D.; Oleszczuk, P.; Winiarska-Mieczan, A. Effects of Titanium Dioxide Nanoparticles Exposure on Human Health—A Review. Biol. Trace Elem. Res. 2020, 193, 118–129.
57. Waghmode, M.S.; Gunjal, A.B.; Mulla, J.A.; Patil, N.N.; Nawani, N.N. Studies on the titanium dioxide nanoparticles: Biosynthesis, applications and remediation. SN Appl. Sci. 2019, 1, 310.
58. Amjadi, S.; Almasi, H.; Ghorbani, M.; Ramazani, S. Preparation and characterization of TiO2NPs and betanin loaded zein/sodium alginate nanofibers. Food Packag. Shelf Life 2020, 24, 100504.
59. Shahverdi, F.; Barati, A.; Salehi, E.; Arjomandzadegan, M. Biaxial electrospun nanofibers based on chitosan-poly (vinyl alcohol) and poly (ε-caprolactone) modified with CeAlO3 nanoparticles as potential wound dressing materials. Int. J. Biol. Macromol. 2022, 221, 736–750.
60. Al-Dhahebi, A.M.; Gopinath, S.C.B.; Saheed, M.S.M. Graphene impregnated electrospun nanofiber sensing materials: A comprehensive overview on bridging laboratory set-up to industry. Nano Converg. 2020, 7, 27.
61. Shang, L.; Qi, Y.; Lu, H.; Pei, H.; Li, Y.; Qu, L.; Wu, Z.; Zhang, W. Graphene and Graphene Oxide for Tissue Engineering and Regeneration. In Theranostic Bionanomaterials; Cui, W., Zhao, X., Eds.; Elsevier: Amsterdam, The Netherlands, 2019; pp. 165–185. ISBN 978-0-12-815341-3.
62. Kumar, P.; Huo, P.; Zhang, R.; Liu, B. Antibacterial Properties of Graphene-Based Nanomaterials. Nanomaterials 2019, 9, 737.
63. Mohammed, H.; Kumar, A.; Bekyarova, E.; Al-Hadeethi, Y.; Zhang, X.; Chen, M.; Ansari, M.S.; Cochis, A.; Rimondini, L. Antimicrobial mechanisms and effectiveness of graphene and graphene-functionalized biomaterials. A scope review. Front. Bioeng. Biotechnol. 2020, 8, 465–486.
64. Zhang, C.; Wang, X.; Liu, A.; Pan, C.; Ding, H.; Ye, W. Reduced graphene oxide/titanium dioxide hybrid nanofiller-reinforced electrospun silk fibroin scaffolds for tissue engineering. Mater. Lett. 2021, 291, 129563.
65. Wang, S.-D.; Ma, Q.; Wang, K.; Chen, H.-W. Improving Antibacterial Activity and Biocompatibility of Bioinspired Electrospinning Silk Fibroin Nanofibers Modified by Graphene Oxide. ACS Omega 2018, 3, 406–413.
66. Dong, J.; Cheng, Z.; Tan, S.; Zhu, Q. Clay nanoparticles as pharmaceutical carriers in drug delivery systems. Expert Opin. Drug Deliv. 2021, 18, 695–714.
67. Fatahi, Y.; Sanjabi, M.; Rakhshani, A.; Motasadizadeh, H.; Darbasizadeh, B.; Bahadorikhalili, S.; Farhadnejad, H. Levofloxacin-halloysite nanohybrid-loaded fibers based on poly (ethylene oxide) and sodium alginate: Fabrication, characterization, and antibacterial property. J. Drug Deliv. Sci. Technol. 2021, 64, 102598.
68. Zou, Y.; Zhang, C.; Wang, P.; Zhang, Y.; Zhang, H. Electrospun chitosan/polycaprolactone nanofibers containing chlorogenic acid-loaded halloysite nanotube for active food packaging. Carbohydr. Polym. 2020, 247, 116711.
69. Elsayed, M.T.; Hassan, A.A.; Abdelaal, S.A.; Taher, M.M.; Ahmed, M.K.; Shoueir, K.R. Morphological, antibacterial, and cell attachment of cellulose acetate nanofibers containing modified hydroxyapatite for wound healing utilizations. J. Mater. Res. Technol. 2020, 9, 13927–13936.
70. Ramírez-Agudelo, R.; Scheuermann, K.; Gala-García, A.; Monteiro, A.P.F.; Pinzón-García, A.D.; Cortés, M.E.; Sinisterra, R.D. Hybrid nanofibers based on poly-caprolactone/gelatin/hydroxyapatite nanoparticles-loaded Doxycycline: Effective anti-tumoral and antibacterial activity. Mater. Sci. Eng. C 2018, 83, 25–34.
71. Wang, J.; Cai, N.; Chan, V.; Zeng, H.; Shi, H.; Xue, Y.; Yu, F. Antimicrobial hydroxyapatite reinforced-polyelectrolyte complex nanofibers with long-term controlled release activity for potential wound dressing application. Colloids Surf. A Physicochem. Eng. Asp. 2021, 624, 126722.
72. Wang, J.; Planz, V.; Vukosavljevic, B.; Windbergs, M. Multifunctional electrospun nanofibers for wound application—Novel insights into the control of drug release and antimicrobial activity. Eur. J. Pharm. Biopharm. 2018, 129, 175–183.