Hindawi
Journal of Nanomaterials
Volume 2021, Article ID 9920755, 15 pages
https://doi.org/10.1155/2021/9920755

Review Article

Antimicrobial Effect of Electrospun Nanofibers Loaded with Silver Nanoparticles: Influence of Ag Incorporation Method

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Received 3 March 2021; Accepted 29 July 2021; Published 17 August 2021

Academic Editor: Antonios Kelarakis

The antimicrobial bioactivity of silver nanoparticles is well known, and they can be used widely in many applications, becoming especially important in the biomedical industry. On the other hand, the electrospun nanofibers possess properties that can enhance silver nanoparticle applicability. However, silver nanoparticle bioactivity differs depending on the loading of silver ions into electrospun nanofibers. This review is aimed at comparing different silver incorporation methods into electrospun nanofibers and their antimicrobial activity, discussing each procedure's limitations, and presenting the most promising one. This review showed that the preferred techniques for incorporating silver nanoparticles were *direct blending* and *ultraviolet irradiation* methods due to their simplicity and efficient results. Besides, polyacrylonitrile nanofibers (PAN) have been the most reported system loaded with silver nanoparticles. Finally, independently of the technique used, silver nanoparticle-loaded nanofibers show high antimicrobial activity in all cases.

1. Introduction

In the last decades, the interest of the biomedical industry in nanomaterials has increased due to its promising applications against different diseases. In this sense, AgNPs are among the most studied nanomaterials principally due to their highly efficient antimicrobial properties [1–6]. It is known that AgNP efficiency increases using a carrier [4]. Electrospun nanofibers are ideal carriers for AgNPs since their small dimensions permit homogeneous distribution and avoid mass aggregation. The blend of technologies between nanofibers and nanoparticles maximizes both structure properties, making them an ideal amalgam for many applications.

Electrospun nanofibers are synthesized by the electrospinning technique, which allows the generation of ultrafine

fibers using natural or synthetic biomaterials [7–9]. These three-dimensional scaffolds are produced in a 10–1000 nm diameter's range [10–18]. Nanofiber's properties such as nanometric thickness, controllable porosity, and high surface contact area [7, 8] allow the potential applications in tissue engineering [19, 20], drug delivery systems [21–24], biosensor fabrication [25, 26], energy storage [27], solar cells [28], water filtration [29, 30], catalysis [31, 32], and sensing [33, 34], among other uses. Specifically, the loading of electrospun nanofibers with AgNPs has become attractive [35–37] for many applications such as food packaging [38], filters [39]. Moreover, there are investigations of electrospun nanofibers loaded with AgNPs for their use in healthcare and other biomedical applications [37, 40, 41], including wound dressing [9] and implants [40].

Thus, this amalgam of nanofiber/AgNP system properties has been proved and attributed to the metallic nanoparticle proportion, size, and spatial distribution of AgNPs in the fibers [42] (*entry 3*).

The AgNP-loaded electrospun systems can control the silver ions released through the immobilization of the AgNPs [43]. Strategies for the fabrication of AgNP-loaded nanofibers include incorporation of silver nitrate ($AgNO_3$) by *direct blending* into the polymeric solution followed by photoreduction with ultraviolet (UV) *irradiation* [44–46], *thermal reduction* [47] (*entry 14*), or *the silver mirror reaction* [48] (*entry 12*), among other methods.

A comparative study of AgNP loading methods on PVA was performed by electrospinning $AgNO_3$-polymeric solutions followed by a reduction posttreatment. In that study, the AgNPs were mixed in the polymer solution before the electrospinning process, and the resulting polymeric scaffolds were immersed in a silver solution followed by a reduction process (comparing UV and thermal treatments). The authors reported that UV reduction was the more efficient method to incorporate AgNPs on nanofibers' surfaces [49].

In this work, we compare the results published regarding the incorporation of AgNPs in nanofibers, including all the above methods. Nevertheless, AgNP-loaded nanofibers differ in their activity depending on the silver loading method into the nanofibers. Hence, this review compares different techniques of AgNP incorporation into electrospun nanofibers and their antimicrobial effectivity to identify the most promising method and discuss the current limitation of each of them.

2. Methods of AgNP Immobilization for Loading of Electrospun Nanofibers

In literature, several strategies merge the electrospun nanofibers and AgNPs, which coat the nanofibers' surface or are embedded into the bulk [50].

The parameters considered in this work to determine the best among the discussed methods include the AgNP distribution in/on the electrospun fibers. Bortolassi et al. discussed that the bioactivity of the AgNPs depends on their capacity to attach to the microbial cell membrane's surface, altering the permeability and cellular homeostatic, thereby AgNP distribution and availability over the surface of the fibers become crucial for the bioactivity. Hence, the combination of the high specific surface area of the electrospun fibers and an AgNP high loading with homogeneous distribution over the surface of the fibers become a desirable design for the high final antimicrobial activity [51, 52]. For instance, the loaded AgNP nanofibers fabricated by the direct addition of AgNPs into the polymeric solution decreased the antimicrobial efficiency of AgNPs due to their aggregation. When the AgNPs are incrusted in the fibers, they are not exposed for direct contact with the cellular membrane [53].

Also, it is remarkable that the presence of AgNPs into/on the polymeric nanofibers affects its intrinsic properties. The most important characteristic that is modified is its bioactivity, which is improved [46, 51–53]. Among other properties that are affected by the presence of the AgNPs on/in the fibers are the mechanical properties such as reduction in surface tension [54], increase in average fiber diameter [36], and changes in thermal properties including the glass transition temperature, degradation temperature, and temperature-dependent mass loss. In general, AgNP-loaded nanofibers are more resistant to heat. All property changes mentioned above are caused by the structural changes of the polymeric backbone [36, 46, 47].

Below, we present and compare several methods to create AgNP-loaded electrospun nanofiber scaffolds.

2.1. Direct Blending Method. A facile method to produce AgNP-loaded nanofibers is the *direct blending* of premade AgNPs in the polymer solution before electrospinning [35], being preferred the AgNP colloidal solutions for easy incorporation into the nanofibers [55]. The encapsulation of AgNPs within the poly(ε-caprolactone) (PCL) microfibers without Ag at the surface of microfibers allows controlling release of AgNPs from the hybrid constructs in combination with high antibacterial activity [56].

Several polymeric systems have been reported to be electrospun and loaded with AgNPs, such as the case of poly(vinylidene fluoride) (PVDF) [20] (*entry 4*), PVA/poly(urethane) (PU) [57] (*entry 2*), nylon [42, 58], and poly(vinyl pyrrolidone) (PVP) [59] *(entry 1)*, among others. This versatility of modified nanofibers is because AgNPs can be prepared with various solvents such as formic acid (FA), dimethylacetamide (DMAc), water (H_2O), and hexafluoro propanol (HFIP). Also, it has been recognized by several studies that the loaded amount of AgNPs can greatly differ from 0.1 to 30% wt., of the total polymeric mass. On the other hand, it has been observed that the higher the content of AgNPs added to the polymeric solution, the higher the conductivity, promoting smaller fiber's diameters [35, 55]. These resultant fiber morphologies can be rough due to the coating and immersion of AgNPs mentioned before [55].

2.1.1. Experimental Conditions of the Direct Blending Method. For a reported direct blending technique, the first step was the dissolution at 50°C of nylon 6 (15% wt.) in formic acid, a reduction agent for $AgNO_3$. In this study, the $AgNO_3$ (0.5 and 1.25% wt.) was slowly added to the polymer solution, kept in darkness with constant stirring at room temperature for 24 h, enough time for the reduction of

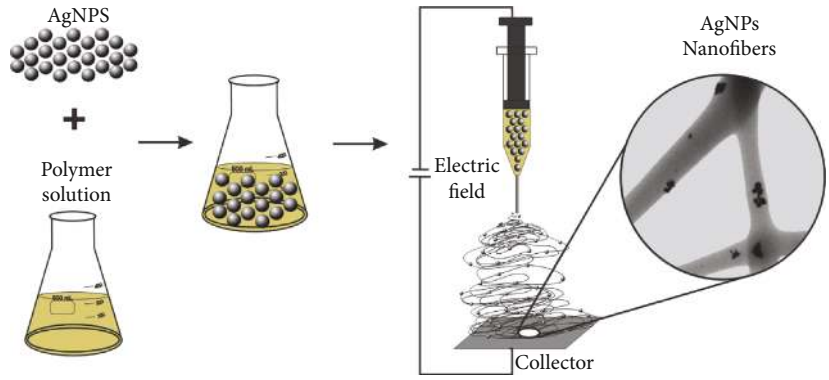

Figure 1: *Direct blending method* for incorporation of AgNPs on nanofibers. Based on [41, 55, 59].

$AgNO_3$. After that, the final dispersion was electrospun [42] (*entry 3*).

In another study, tannic acid prepared in an ammonia solution ($NH_3{\cdot}H_2O$) was added to the polymer solution as a reducing agent of $AgNO_3$. After one hour, the powder obtained by solution concentration was resuspended in acetone and filtered. Synthesized AgNPs were extracted using vacuum drying at room temperature and then added to a PVP solution before electrospinning (Figure 1).

2.2. UV-Irradiation Method. The amount of AgNPs loaded on nanofibers is more significant when a *UV-irradiation method* is used to produce the initial silver burst release, either in the form of AgNPs or in the residual Ag^+ ions. This method promotes incorporating the AgNPs into nanofibers' surface in a random distribution [44–46]. The method induces the silver ion migration from the core to the surface during the formation of the AgNPs [45]. Also, it has been described that the UV-irradiation method achieves smaller AgNP diameters with narrower distributions than the other methods. These results are relevant for antimicrobial activity because the high surface area of AgNPs promotes a faster Ag^+ ion release [60] (entries 8 and 16).

Moreover, the biological activity of electrospun scaffolds is related to the $AgNO_3$ content. It was reported that AgNP-loaded poly(ether amide) (PEBA) fibers were fabricated using 0.15% of $AgNO_3$ in the polymer solution for electrospinning inhibits >99.99% of *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) cultures. However, when the $AgNO_3$ concentration decreased (0.05% $AgNO_3$), the bacterial inhibition decreases (20% less activity in both bacterial strains) [61] (*entry 7*). A similar activity was observed on loaded AgNPs in gelatin nanofibers evaluated against *S. aureus* and *E. coli*, with selective activity for the later strain. Authors suggest that the different biocidal effect is due to the generous peptidoglycan layer next to the cell membrane of Gram-positive bacteria such as *S. aureus*, which serves as a protective structure for external threats reducing the AgNP introduction to the bacterial cell [60].

Phan et al. [46] (*entry 5*) synthesized silver/polyacrylonitrile (Ag/PAN) nanocomposite membranes testing their antibacterial activity. In this study, electrospun AgNP/PAN nanofibers were prepared from the solution of PAN and $AgNO_3$ using the *UV-irradiation method* to reduce the Ag^+ ions into AgNPs. The nanofiber antibacterial activity was tested against *E. coli* and *Bacillus subtilis* (*B. subtilis*), finding long-term bactericidal effects. The authors claim that these nanofiber systems are useful for water purification, bacterial filtration, and biomedical devices [46] (*entry 5*).

2.2.1. Experimental Conditions of the UV-Irradiation Method. In the case of the UV-irradiation method, most of the studies prepared the Ag-loaded nanofibers by mixing the polymeric and $AgNO_3$ solutions previous to the electrospinning process [44, 45, 60–62] except for Phan et al. [46] (*entry 5*) that electrospun the fibers before the $AgNO_3$ incorporation. An advantage of this method is that, as reported, no extra time or additional solvent is required [44–46, 60–63]. Besides, no further treatments are needed after the irradiation step, and the exposition time to UV light can be changed depending on the desired results. Interestingly, it has been reported that after four hours of irradiation, the number and size of the AgNPs continuously increased [44]. Figure 2 explains the UV-irradiation method general procedure, where the incrusted AgNPs can be appreciated over the nanofiber surface.

Additionally, the electrospinning conditions in both methods (*direct blending* and *UV irradiation*) are selected depending on the polymer system used [62]. After the electrospinning process, the UV irradiation step is developed. Here, the UV light's specific wavelengths are 254 and 365 nm [46] (*entry 5*), but not a specific irradiation time is presented. Several conditions are reported as follows: UV light (254 nm) irradiation for 24 h [46] (*entry 5*), UV light (254 nm) for 6 h [61] (*entry 7*), UV light (254 and 365 nm) irradiation from 10 min to 8 h [44] (*entry 10*), UV light (254 nm) irradiation for 10 min [45] (*entry 9*), UV light (365 nm) irradiation from 3 h [62] (*entries 8 and 16*), and UV light (not defined wavelength) irradiation for 4 h [63] (*entry 6*).

2.3. Silver Mirror Reaction Method (SMR). The *SMR method* is a versatile method that uses different substances as reducing agents for $AgNO_3$ solution to produce the AgNPs, which coat the surface of an object submerged in the reaction solution. This reduction can generate a visual phenomenon that

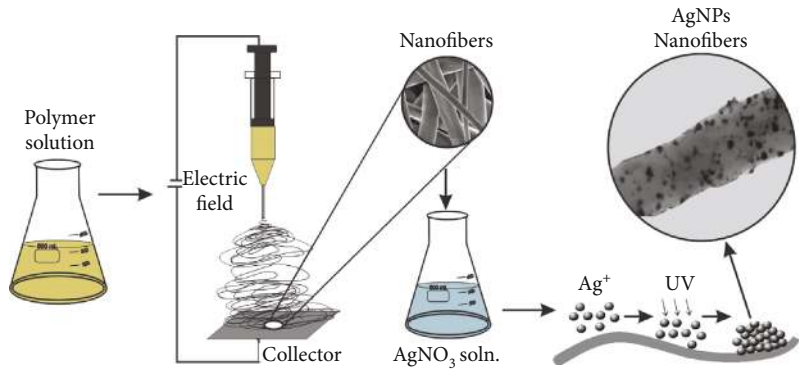

Figure 2: UV-irradiation method for incorporation of AgNPs on nanofibers. Based on [44–47, 60–63].

resembles a shiny mirror coat over the upper surface of the solution or over an object submerged.

It is used to create a controllable smooth coating over different surfaces: for large surfaces (e.g., telescope glasses) and extremely small surfaces (nanofibers) [64] (*entry 13*). The reaction occurs at ambient conditions, which is very appropriate for nanotechnology applications [65] (*entry 11*). Even though using this method is impossible to achieve shape control of AgNPs, the technique can produce quasispheres, wires, rods, right bipyramids, beams, spheres, cubes, or octahedrons [65, 66].

Several applications have been reported for SMR synthesized nanofibers. Excellent antimicrobial activities against bacteria and fungi were observed with PAN/AgNP nanofibers with AgNPs evenly dispersed on the nanofiber's external surface. Those were prepared with PAN nanofibers pretreated in $AgNO_3$ aqueous solution followed by the SMR process [48]. Also, noble metal nanoparticles loaded on electrospun nanofibers have been synthesized due to their potential use as sensors [67]. Another application is for silver nanowire membranes, in this case, Ag^+ ions coated poly(acrylonitrile–co–phenylethylene) (P(AN-S)) nanofibers used as a template for the reduction of a silver solution on the nanofibers' surface. The SMR process allows silver deposition on the nanofiber's surface. Thus, nanowires were developed and characterized by scanning electron microscopy (SEM), energy dispersive spectrometry (EDS), and X-ray diffraction (XRD). These nanowires have different resistance and transmittance on PET and glass: 15 Ω/sq and 80%, and 37 Ω/sq and 81%, respectively [65].

2.3.1. Experimental Conditions of the SMR Method. The reported methodologies differ in time and posttreatment steps [53, 54], but an advantage over other methods is that the reaction can be made at room temperature. In two manuscripts, the polymeric solution needs a 12 h reaction at room temperature before the electrospinning process [48, 65]. In another study, just 30 min were used, and a sol-gel process was done before the electrospinning step [64]. Hence, the polymer solution preparation time is not standardized and differs depending on the methodology used to achieve complete polymer dissolution. Wang et al. prepared PAN nanofibers stirring the polymeric solution just for 1 hour at room temperature, using the same polymer as the compared studies. Hence, these polymeric solution reaction times can vary depending on the polymer chosen and the AgNP incorporation method [11]. Within the reported procedures, we can find nanofibers exposed to the $AgNO_3$ solution for 18 and 24 h in a dark room at room temperature [48], and $AgNO_3$ solution mixed with the polymer solution before the electrospinning process [64, 65]. Hence, the $AgNO_3$ solution can be added before or after the formation of fibers.

Regarding electrospinning, parameters depend more on the polymeric solution than on the AgNP immobilization method or the presence of $AgNO_3$. The electrospinning parameters determine the morphology, diameter, porosity, and distribution of the fibers [20]. After fiber preparation, several posttreatments are needed. Obtained fibrous mats are submerged into $AgNO_3$ solution at the desire concentration, usually in a 1 : 2 $AgNO_3$/polymer solution ratio, but other relative proportions $AgNO_3$/polymer were also tested [64, 65]. Ag^+ ions are deposited over the fiber's surface when the $AgNO_3$ solution is added to the fibers earlier prepared. In contrast, Ag^+ ions get encapsulated into the fibers if $AgNO_3$ solution is added before fiber preparation [68]. Other procedures include fibrous scaffolds freeze-drying before the SMR [48] or a two-step filtration with an organic-free filter followed by a 0.2 μm filter to removed particles [64].

Other differences were observed for the reported SMR step. In some cases, the authors dropped ammonium hydroxide (NH_4OH) into $AgNO_3$ solution to prepare a diamine silver (I) ($Ag(NH_3)_2^+$) solution [65] (*entry 11*). Others used a hydrazinium hydroxide (N_2H_5OH) solution as a reducing agent [64] (*entry 13*). On the other hand, Shi et al. [48] (*entry 12*) submerged the fibers into the $AgNO_3$ solution, then added concentrated ammonia (NH_3) (2.5% wt.) into the beakers just until the brown precipitate dissolved, and then added formaldehyde (HCHO) (1% wt.) as the final step. Additional steps after SMR as a threefold washing procedure with distilled water or 40°C vacuum oven drying are reported [64, 65].

A simplified schematization of the silver mirror reaction method showing the AgNP distribution on the nanofiber's surface is represented in Figure 3.

2.4. Thermal Reduction Method. This method is promising due to the easy steps to perform. Some advantages of this method are as follows: uniform AgNP distribution on the

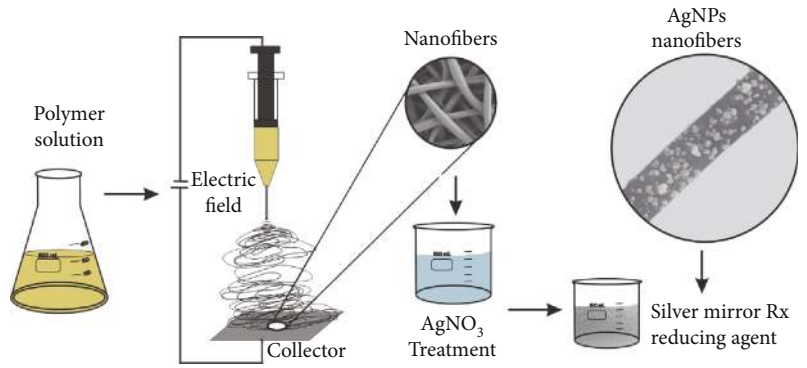

Figure 3: Silver mirror reaction method for incorporation of AgNPs on nanofibers. Based on [48, 61, 65, 68].

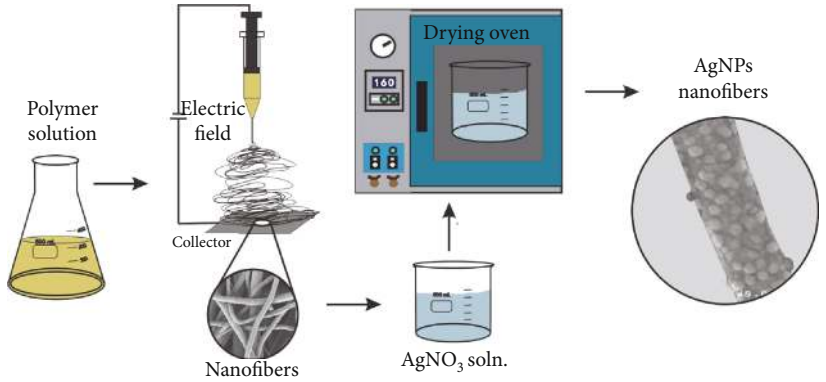

Figure 4: Thermal treatment method for incorporation of AgNP on nanofibers. Based on [47, 62, 70].

nanofiber's cross-section [69], decomposition temperature decreases when Ag content increases on PVA-AgNPs [49, 62], and more desired stability (less probability of AgNP aggregation). However, AgNP leaching could inactivate the membrane once the first Ag^+ release from the nanofiber surface has occurred [62].

Silver conducting nets were synthesized using the thermal reduction method over a plane scaffold prepared with electrospun nanofibers of poly(methyl methacrylate) (PMMA), and silver trifluoroacetate (STA) deposited on transparent substrates. Those nets had high transmittance and low resistance [70]. STA is reduced to AgNPs with a 100°C posttherm al treatment that decomposes the organic polymer to achieve the one-dimensional net configuration. The nets' sheet resistance was as low as 15 Ω/sq correlated with the morphology and the STA/PMMA ratio. Properties as surface plasmon resonances (SPRs), fiber morphologies, and electrical and optical properties (diffusive optical transparency of ≈54%) were determined.

2.4.1. Experimental Conditions of the Thermal Reduction Method. For this method, several strategies are reported to incorporate the Ag^+ ions into the nanofibers. Jatoi et al. [47] (*entry 14*) prepared acetate cellulose (CA) solution under constant stirring for 24 h and then electrospun the solution. On the other hand, Chen et al. [70] (*entry 15*) replaced the $AgNO_3$ solution with an STA solution and mixed it with the PMMA solution using a reaction time of 24 h at room temperature. Similarly, Lin et al. [62] (*entries 8 and 16*) mixed the previously prepared (~2 h) PVA solution with the $AgNO_3$ solution stirring vigorously for an additional 30 min, taking just 2.5 h before the electrospinning step.

For the electrospinning technique, no special modifications were added. The optimization of the fiber formation and the electrospinning parameters were chosen depending on the properties (viscosity, concentration, conductivity, and surface tension) of the polymer solution [20, 47, 62, 70].

CA fibrous mats were synthesized with a simple alkaline treatment (NaOH) by submerging the fibers in the solution for 48 h, creating cellulose fibers (CEF). The CEAg samples were obtained immersing the CEF for 24 h at 23°C in an $AgNO_3$ solution then dried for 2 h. Finally, the reduction process of CEAg was done in a drying oven at 160°C for 1 h, 1.5 h, and 2 h [47]. Silver nets also could be obtained by thermal decomposition at 500°C of PMMA for 3 h under air or nitrogen atmosphere. In this case, AgNPs were obtained by reduction of silver precursors for 12 h at 100°C [70]. Contrary to the previous two discussed methods, before the electrospinning process, Lin et al. [62] (*entries 8 and 16*) pretreated the polymeric/$AgNO_3$ solution at 105°C for 1 h, no further treatments after the obtention of the fibers.

Figure 4 represents the general procedure of the *thermal reduction method.*

AgNP particle size and average nanofiber diameter obtained with four AgNP incorporation methods are presented in Table 1.

Table 1: Physicochemical and antimicrobial properties of AgNP-nanofiber systems prepared by different methods.

Entry	AgNP electrospun system	Method used	AgNP size	Nanofibers/fiber size	[$AgNO_3$]/[polymer]	[loaded AgNPs]/[polymer] % wt.	[$AgNO_3$]/[loaded AgNPs]	Characterization	Method (medium used)	Antimicrobial activity (% effectivity)	Applications	Ref.
1	AgNPs/FK/PVP/PEO	*Direct blending method*	13.67 ± 2.95 nm	~140-300 nm	120 mmol/L $AgNO_3$/12% FK	0-3% AgNPs/12% FK	120 mmol/L $AgNO_3$/0-3% AgNPs	SEM-EDX, TEM, XRD, TGA, tensile stress, antibacterial activity	Inhibition zone method (LB agar)	8.24 mm inhibition zone, (*E. coli*) 2.08 mm inhibition zone, (*S. aureus*)	Biomedical applications	[58]
2	AgNPs/WPU/PVA	*Direct blending method*	5.1 ± 0.6 nm	290 ± 35 nm	1% AgNPs/15% WPU/PVA	Not tested/15% WPU/PVA	1% AgNPs/not tested	TEM/SEM, TGA, XPS, antibacterial assay, cytotoxicity assay	Inhibition zone assay (nutrient agar)	2.4-fold inhibition (*S. aureus*) 1.6-fold (*E. coli*)	Antimicrobial agents, wound dressings, and water or air purification techniques. A	[56]
3	AgNPs/nylon 6	*Direct blending method*	2–4 nm	~50–150 nm	0.5-1.25% $AgNO_3$/15% nylon 6	Not tested/15% nylon 6	0.5-1.25% $AgNO_3$/not tested	Viscosity, conductivity, SEM, TEM, Ag release profile, antibacterial activity	Viable cell-counting method (LB broth and TSA broth)	4 log reduction 0.5% $AgNO_3$; 5.8 log reduction 1.25% $AgNO_3$ (*E. coli*) 3.4 log reduction 0.5% $AgNO_3$; 3.4 log reduction 1.25% $AgNO_3$ 99.99% (*B. cereus*)	Energy storage, biomedical materials, catalysis, sensors	[41]
4	AgNPs/PVDF	*Direct blending method*	5.1 nm	600 ± 176 nm	280-676 ppm AgNPs/10-25% PVDF	310-730 ppm AgNPs/10-25% PVDF	280-676 ppm AgNPs/310-730 ppm AgNPs	SEM, TEM, XPS, ICP, viscosity, antibacterial activity	Growth inhibition rate (nutrient broth)	43-77% growth inhibition (*S. aureus*) *57-77%* growth inhibition (K. pneumonia)	Water filters, wound dressings, or antiadhesion membranes	[7]
5	AgNPs/PAN	*UV-irradiation method*	2.0 ± 0.6 nm	~400-500 nm	0.1, 0.3, and 0.5 M $AgNO_3$/8% PAN [1 fold:5 fold]	Not tested/8% PAN	0.1, 0.3, and 0.5 M $AgNO_3$/not tested	SEM, XRD, Ag release profile, FTIR, antibacterial activity	Kirby–Bauer Method; disk diffusion test (LB broth)	89.67 ± 1.7% (*E. coli*) 87.67 ± 4.03% (*B. subtilis*)	Water purification, bacterial filtration, biomedical devices	[46]
6	AgNPs/PAN	*UV-irradiation method*	2-50 nm	~600-900 nm	0.05-1% $AgNO_3$/8% PAN [1 fold:8 fold]	Not tested/8% PAN	0.05-1% $AgNO_3$/not tested	SEM, EDAX, UV-Vis, XRD, AFM, air filtration efficiency test, antibacterial activity	Inhibition zone method (nutrient broth)	17 mm inhibition zone (*E. coli*) 18 mm inhibition zone (*S. aureus*) (nutritive broth)	Antimicrobial filters	[64]
7	AgNPs/PEBA	*UV-irradiation method*	13.5-16.5 nm	~100-300 nm	0.05-0.25% $AgNO_3$/5%-2.5% PEBA [1 fold:100 fold]	No tested/5%-2.5% PEBA	0.05-0.25% $AgNO_3$/no tested	TEM-EDS, SEM, DSC, TGA, XPS, antibacterial activity	Pour-plate culture Method (nutrient broth)	99.99% *E. coli*, *S. aureus*	Biomedical materials, sports apparatus, and laminating films	[59]

Table 1: Continued.

Entry	AgNP electrospun system	Method used	AgNP size	Nanofibers/fiber size	[$AgNO_3$]/[polymer]	[loaded AgNPs]/[polymer] % wt.	[$AgNO_3$]/[loaded AgNPs]	Characterization	Method (medium used)	Antimicrobial activity (% effectivity)	Applications	Ref.
8	AgNPs/PVA	*UV-irradiation method*	~29.7 nm	200-300 nm	1% $AgNO_3$/15% PVA [1 fold:15 fold]	2.6% wt., AgNPs/15% PVA	1% $AgNO_3$/2.6% wt., AgNPs	XRD, SEM, TEM, EDS, DLS, XPS, antibacterial activity	Inhibition zones assay	2-fold log reduction (*S. aureus*), 1.4-fold log reduction (*E. coli*)	Biological sensors, conductive interconnects, optoelectronic devices, effective bioactive materials	[61]
9	AgNPs/PAN	*UV-irradiation method*	4-7.8 nm	185-629 nm	0.5, 1.5, and 2.5% $AgNO_3$/10% PAN [1 fold:10 fold]	2.93-15.30 mg/g AgNPs/10% PAN	0.5, 1.5 and 2.5% $AgNO_3$/2.93-15.30 mg/g AgNPs	SEM, TEM, EDX, mechanical properties, Ag release rate, antimicrobial activity	Disk diffusion method Mueller Hinton agar in	99.99% *E. coli*, *S. aureus*	Antibacterial applications	[45]
10	AgNPs/CA	*UV-irradiation method*	~21 nm	~610 nm	0.5% $AgNO_3$/10% CA	Not tested/10% CA	0.5% $AgNO_3$/Not tested	TEM, UV-Vis, antibacterial activity	Nonwoven fabric attachment method (not defined)	99.99% *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*	Separation filters, wound dressing materials	[44]
11	AgNPs/(P(AN-S))	*The silver mirror reaction method*	Not tested	~500–600 nm	10% wt., $AgNO_3$/20% wt., 25% wt., and 30% wt., (P(AN-S)) [1 fold/2, 2.5, 3-fold]	30.1% wt., AgNPs/20% wt., 25% wt., and 30% wt., (P(AN-S))	30.1% wt., AgNPs/10% wt., $AgNO_3$	SEM, EDS, XRD, conductive test,	Not tested	Not tested	Flexible electronics materials, optoelectronics	[64]
12	AgNPs/PAN	*The silver mirror reaction method*	20–30 nm	~200-500 nm	2% wt., 5% wt., $AgNO_3$/7%, 10%, 14%, 17%, 22% PAN [1 fold/3 fold]	90-92% AgNPs/7%, 10%, 14%, 17%, 22% PAN	2-10% $AgNO_3$/90-92% AgNPs	SEM, TEM, SAED, XRD, TGA, antibacterial activity	Viable cell-counting method (nutritive agar and broth	*100%* *E. coli*, *S. aureus*, *Monilia albicans*	Biomedical applications	[48]
13	AgNPs/PAN	*The silver mirror reaction method*	<5.8 nm	~390 nm	[1 fold]/[2 fold] [%/8% w/v] PAN $AgNO_3$/PAN molar ratio of 0.05 and 0.005	No tested	No tested	XRD, TEM, UV-Vis, viscosity, antibacterial activity	Inhibition zone method (LB agar plates)	0.05 (2.5 mm); 0.005 (1 mm) (*E. coli*) 0.05 (2.5 mm); 0.005 (1 mm) (*S. aureus*)	Catalytic, surface-enhanced Raman scattering activity, electrically conductive, antimicrobial materials	[63]
14	AgNPs/CE	*Thermal reduction method*	2.1-7.4 nm	~250-320 nm	200 mM $AgNO_3$/17% CE	30-40% AgNPs/17% CE	200 mM $AgNO_3$/ 30-40% AgNPs	FTIR, XRD, EDX, XPS, TEM, DTA, swelling, mechanical properties, antibacterial activity	Kirby–Bauer Method; disk diffusion test (LB agar and broth)	*100%* *E. coli*, *S. aureus*	Cosmetic, biomedical, and pharmaceutical application; wound dressings and impermanent skin covering materials	[47]
15	AgNPs/PMMA	*Thermal reduction method*	2 – 10 nm	~640-3000 nm	15.3-24.5% STA/21% PMMA	AgNPs/21% PMMA 0/1, 0.5/1, 0.6/1, 0.7/1, 0.8/1, 0.9/1	15.3-24.5% STA	SEM, TEM, UV-Vis, TGA, XRD, viscosity, conductivity	Not tested	Not tested	Microelectromechanical systems (MEMS) and optoelectronic applications	[69]
16	AgNPs/PVA		~29.7 nm	200-300 nm								[61]

Table 1: Continued.

Entry	AgNP electrospun system	Method used	AgNP size	Nanofibers/fiber size	[$AgNO_3$]/[polymer]	[loaded AgNPs]/[polymer] % wt.	[$AgNO_3$]/[loaded AgNPs]	Characterization	Method (medium used)	Antimicrobial activity (% effectivity)	Applications	Ref.
		Thermal reduction method			1% $AgNO_3$/15% PVA [1 fold:15 fold]	2.6% wt., AgNPs/15% PVA	1% $AgNO_3$/2.6% wt., AgNPs	XRD, SEM, TEM, EDS, DLS, XPS, antibacterial activity	Inhibition zones assay	2-fold log reduction (*S. aureus*), 1.4-fold log reduction (*E. coli*)	Biological sensors, conductive interconnects, optoelectronic devices, effective bioactive materials	

Materials: AgNPs: silver nanoparticles; CA: cellulose acetate; CE: cellulose; PAN: poly(acrylonitrile); FK: feather keratin; PMMA: poly(methyl methacrylate); PVA: poly(vinyl alcohol); PEO: poly(ethylene oxide); PEBA: poly(ether block amide); P(AN-S): poly(acrylonitrile–co–phenylethylene); STA: silver trifluoroacetate. Assays: AFM: atomic force microscopy; DTA: thermal stability; EDAX: energy dispersive X-ray analysis; SEM: scanning electron microscopy; EDX: energy dispersive X-ray spectroscopy; ICP: inductively coupled plasma; TEM: transmission electron microscopy; XRD: X-ray diffraction; XPS: X-ray photoelectron spectroscopy; TGA: thermogravimetric analysis; UV-Vis: ultraviolet-visible light spectroscopy.

3. Antimicrobial Activities of Ag Nanofibers

AgNPs are well known to have antimicrobial bioactivity. This bioactivity occurs through cell membrane damage, free radical generation, and DNA interaction, among others (Figure 5) [71, 72]. One of the proposed mechanisms of AgNP action is the effect on the lipid bilayer induced by the AgNP accumulation in the bacterial cell wall [59]. Thus, membrane permeability increased, causing cell damage and death. Moreover, the effect increases while the AgNP size decreases [73]. Another mechanism suggested for cell death induced by AgNPs is the reaction with thiol groups (–SH) of cysteine and phosphorus compounds on the cell wall, affecting respiration and replication processes [59, 74]. Another explanation for the antimicrobial activity is that metal depletion may cause the formation of irregularly shaped pits in the outer membrane and change membrane permeability, which is caused by a progressive release of lipopolysaccharide molecules and membrane proteins [63] (entry 6).

AgNPs or the released silver ions (Ag^+) can also enter the bacterial cells and interact with compounds containing sulfur and phosphorus, preventing DNA replication and inactivating proteins. Besides, they can inhibit the activity of endocellular ATP levels, thereby preventing the cell's respiratory function. Furthermore, AgNPs have been reported to induce the release of reactive oxygen species (ROS), forming free radicals with strong bactericidal effects (Figure 5) [75]. AgNPs have a broad antibacterial spectrum covering aerobic, anaerobic, and Gram amphoteric bacteria [76], low incidence of resistance [77], and sustained antibacterial activity [78], thus have been widely used in antibacterial wound dressings [75].

The antimicrobial activity of AgNPs depends on the surface area of the nanomaterial [72]. The highest concentrations of released Ag^+ ions have been observed from AgNPs with the highest surface area. On the contrary, Ag^+ ion's low release has been found for AgNPs with low surface area, resulting in weak antimicrobial properties [79]. Most electrospun nanofibers do not affect microbial cell reproduction by themselves, but only with the presence of AgNPs. Such is the case of PAN nanofibers which were endowed with excellent antibacterial properties due to the introduction of AgNPs. The authors claim that AgNPs have strong antibacterial properties since they attach to the cell walls and disturb cell-wall permeability and cellular respiration [48] (entry 12).

Poly(vinyl alcohol-co-vinyl acetate)/octadecyl amine-montmorillonite) (P(VA-co-VAc)/ODA-MMT) nanofibers loaded with AgNPs showed high antimicrobial activity against fungus (*Candida albicans*, *tropicalis*, *glabrata*, *keyfr*, and *krusei*) and bacteria (*S. aureus* and *E. coli*) [80]. It has been demonstrated that Ag^+ release confers the AgNP microbicidal effect [81–83]. Moreover, the internalization of AgNPs into the fibers permits a longer bioavailability of the silver on the application site because the release of the A^+ ions depends on the time of fiber degradation [84]. This could explain that 10 nm AgNPs were more toxic for *E. coli* than 20-80 nm due to a more efficient cell-particle contact. Thus, AgNP toxicity correlates with size and content because of the Ag^+ initial release rate, affecting the fibrous scaffold cytotoxicity. However, the AgNP amount also regulates the long-term Ag^+ release rate and, therefore, the microbicidal activity [72, 75].

AgNP-loaded electrospun membranes with antibacterial properties have been tested in food packaging material to delay food spoilage or bacteria contamination [63, 78]. Chaudhary et al. [63] (*entry 6*) used an electrospun AgNP/-PAN composite filter media to cover a nutrient media in room conditions and pass ambient air through the filter media. The nutrient media protected by the nanofibrous filter remained free of bacteria growth after two months, while the unprotected nutrient media show microorganism growth. Taking advantage of the excellent dispersion of AgNPs in electrospun nanofibers, Castro-Mayorga et al. [85] reported electrohydrodynamic processing, which combines the electrospraying and electrospinning techniques to produce a multilayer system comprising a poly(hydroxy alkanoate) (PHA) substrate and an electrospun PHA coating containing AgNPs. The materials reduced the *Salmonella enterica* population below the detection limits at a very low silver loading of 0.002% wt.

Table 1 shows the physicochemical and antimicrobial properties of AgNP-nanofiber systems prepared by the discussed methods. It can be observed that each of the four methods affects the resulted size of the incorporated AgNPs. Moreover, small particle sizes (<10 nm) can be achieved in all methods.

It should be noted that the data in Table 1 include the following: (1) inhibition zone diameter, which may not be comparable, or (2) results of distinct AgNP concentrations leading to approximately 100% inhibition of microorganism growth, which is also not representative. In the future, the minimal inhibitory concentration (MIC) and the inhibitory concentration (IC_{50}) should be presented as data; moreover, for this purpose, the microdilution test to evaluated antimicrobial activity should be used [37].

Also, it can be appreciated that information about the resulting AgNP size is not reported in some articles. The antimicrobial results are reported in different ways, making it difficult to compare studies.

4. Comparisons among Methods of Incorporation of AgNPs

Metal nanoparticles tend to aggregate in the polymer matrix during nanofiber formation, highlighting the need for new and better methods that allow better metal nanoparticle dispersion on the polymer nanofiber matrix [42] (*entry 3*). Another requirement is to reduce the number of reaction steps and reduce the use of toxic chemical agents to form AgNP/composite polymeric scaffolds. The search for a facile and ecofriendly method is an important task [86]. There is not a noticeable difference in AgNP size and antimicrobial activity for the studied methods; they all reported similar results (Tables 1 and 2). Hence, in our further discussion, we can focus on finding the method possessing the most advantages other than appropriate AgNP size.

Among the compared methods, the *direct blending method* is the easiest, more accessible, and most commonly

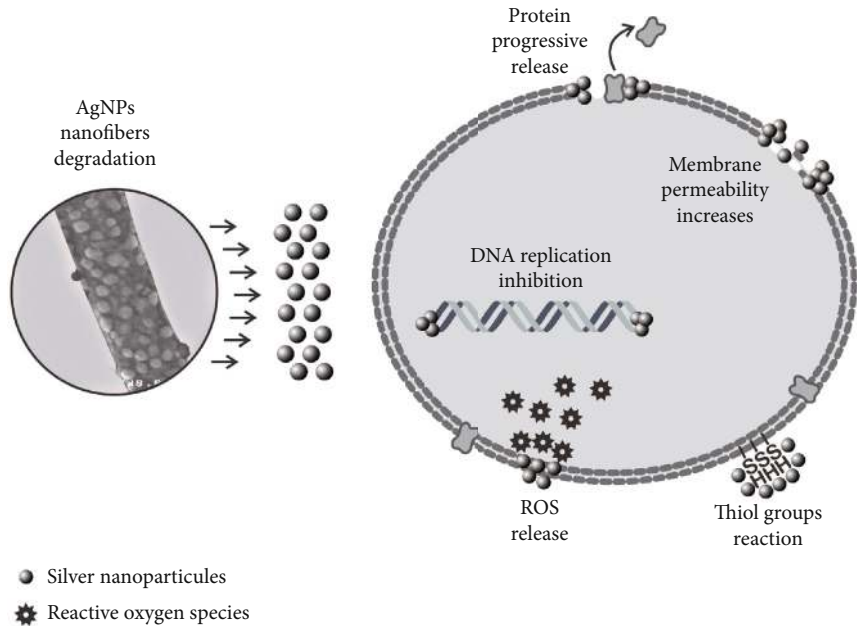

Figure 5: AgNP release from nanofibers and mechanisms of action of AgNPs on microbial cells.

used technique to incorporated AgNPs into nanofiber mats [35, 55]. It has been reported that the electrospinning conditions do not affect AgNP bioactivity on microbial cells; however, fiber-matrix encapsulation could reduce it [87]. Despite that, the lack of chemical treatment, irradiation, and thermal treatment made the direct blending method preferable compare to others that consume more time and energy [42] (*entry 3*). However, it has been described that the mechanical mixing of the AgNPs with the polymeric solution by the *direct blending method* affects the homogeneity of particle dispersion, facilitating their aggregation, increase viscosity of the polymeric solution, which increase the surface tension, complicating nanofiber formation [66] (*entry 13*).

Otherwise, comparing the *UV-irradiation method* with the *direct blending*, an initial burst release of the AgNPs incorporated into the nanofibers is observed only in the UV method. As mentioned above, this burst release was attributed to the silver ion's migration from the core to the nanofiber surface [45]. Moreover, in the *UV-reduction method,* the AgNP diameters are smaller with narrower distribution than other methods. In its turn, the size of AgNPs is an essential parameter of antimicrobial activity [62] (*entries 8 and 16*). AgNP/PVA nanofibers prepared by the UV irradiation method showed more effectivity against *S. aureus* than those prepared by the *thermal reduction method.* In the first case, after 30 minutes of incubation, no bacterial colonies were detected. In comparison, microbial colonies were detected in both treated and untreated AgNP/PVA scaffolds. The authors discussed the last result is ascribed to a higher dispersion diameter resulted from the $AgNO_3$ reduction from the thermal treatment in the PVA samples [62] (*entries 8 and 16*).

In the thermal reduction method, the size and spatial distribution of AgNPs can be tuned by varying the $AgNO_3$ solution concentration. The strategy to immobilizing and manipulating the size of the AgNPs on polymer nanofibers may be extended to other particle systems for various applications such as catalysis, energy, sensing, photonic, and biomedical applications [88]. Unfortunately, this method has been observed that leaching of AgNPs in the initial stage can occur [62] (*entries 8 and 16*). However, UV irradiation prepared AgNPs are preferred over those loaded on electrospun poly(vinyl alcohol) (PVA) by heat treatment of $AgNO_3$ for its cytotoxic and microbicidal properties.

The *silver mirror reaction method* produces different morphologies of Ag nanostructures (quasispheres, wires, rod, right bipyramid, beam, spheres, cube, and octahedron) which cannot be predefined [66], limiting its use for the synthesis of a particular Ag nanostructure. However, to our knowledge, none of the methods compared in this review allow us to design the nanostructure. On the other hand, the *silver mirror reaction method* is easily controlled, and it takes place at ambient conditions but requires further processing (filtration, purification, and washing) [64] (*entry 13*). Nanofiber morphology has no significant differences if prepared by SMR or the *thermal reduction method* [47].

Hence, from the above analysis, we can conclude that all four considered methods presented difficulties in loading AgNPs into the nanofibers, but this does not impact the antimicrobial effectivity of the nanofiber/nanoparticle systems (Table 2). It would be interesting to compare the concentration of $AgNO_3$ is used and how much Ag is loaded. Still, at the moment, several studies do not evaluate the Ag loaded on the nanofibers, which does not allow to do these correlations.

5. Future Perspectives and Limitations

The properties as porosity, a high surface area/volume ratio, and porous interconnection that can be reached with electrospun polymeric nanofibers make them attractive in different

Table 2: Comparison of advantages and disadvantages of the methods for incorporation AgNPs in electrospun nanofibers.

Method	Advantage	Disadvantage	Ref.
Direct blending method	(i) Single-step process (ii) Polymeric solution is the reducing agent (iii) Tiny particle sizes obtained <10 nm (iv) Faster and simpler than other compared methods	(i) Stabilizing and protection agents are used to avoiding NP aggregation (ii) Posttreatment needed (purification, extractions, etc.) (iii) Lacking size homogeneity in dense matrices	[42, 64]
UV-irradiation method	(i) Not necessarily extra time in polymeric solution preparation (ii) No additional solvents are required (iii) Tiny particle sizes obtained <10 nm	(i) Limited to the use of UV-sensitive polymers (ii) Not recommendable the use irradiation greater than 380 nm (polymer degradation) (iii) Extra UV irradiation treatment (~3-24 h) (iv) When the irradiation time is prolonged, even though the formation of nanoparticles increases, the size also increases	[44–46, 60–62]
Silver mirror reaction method	(i) The reaction is at room temperature (ii) Facilitate the surface coating of big devices (iii) Coating of micro- and nanostructures such as nanofibers (iv) Formation of smooth coatings (v) Easy control (vi) The reducing agent can vary (vii) Tiny particle sizes obtained <10 nm	(i) The high volume of the reaction (ii) Posttreatments needed (purification, filtration; washing, vacuum drying) (iii) 2.5 h posttreatment after electrospinning (iv) Use of surfactants for stabilizing nanostructures (v) 12 h for solution preparation	[64, 66]
Thermal reduction method	(i) Easy to perform (ii) Uniform distribution of loaded AgNPs on the fibers (iii) Size and distribution of the AgNPs are controllable with the time and temperature applied (iv) Very small particle sizes obtained <10 nm	(i) Extra thermal irradiation treatment (~1-12 h) (ii) Limited to stable polymers at temperatures above 100°C (polyesters, natural polymers) (iii) Leaching of AgNPs	[47, 62, 70]

fields [89]. Thus, in the last years, applications for filtering, tissue engineering, wound dressing, drug delivery, and biosensors have been reported using electrospun nanofibers with inorganic particles [90]. However, several variables affect the electrospinning process. Such variables are not yet fully described [48].

The electrospinning method is also limited for parameters that have to be optimized. For example, the large-scale production may present polymer blockage, and electrospinning flux may alter the electric field. It has been reported that this can be fixed using a multirow component in the injection system [91]. Also, it is desired to control nanofiber thickness with collecting devices with different characteristics [9], such as centrifugal electrospinning, or surface-free systems [92]. Other improvement points are precision and reproducibility; for those, the control of temperature and humidity is essential [9]. Finally, stability and mechanical properties can be upgraded with new electrospinning formulations [92–94].

The microbicidal effect of silver has been related to Ag^+ ion interaction with the cell membrane. It has been described that in AgNPs, Gram-negative bacteria inhibition depends on the concentration associated with cell wall damage [63], but a precise mechanism is still unknown. Still, more studies have to be implemented to investigate the mechanism of the antimicrobial action of the AgNPs. Most importantly, it is necessary to develop the ideal method that does not present any of the troubles mentioned in this review.

The literature data analysis made in the present review indicates that at the present moment, there are numerous publications dedicated to the application of different methods of deposition of AgNPs on nanofibrous scaffolds. Nevertheless, these results are not enough to conclude which method is the most promising for the best control of Ag^+ ion release. Research where all methods are applied in similar experimental conditions (the same silver nanoparticles type, including stabilizer, particle size, hydrodynamic diameter, and Ag concentration) is necessary to carry out to answer this question.

6. Conclusions

The analysis carried out in this work showed that the methods of AgNP incorporation into the electrospun nanofibers could alter the size of AgNPs, their distribution on the electrospun nanofibers, and their antimicrobial activity. The differences among the four analyzed methods also come from

the incorporation time, method difficulty, complexity, and cost of the equipment and solvents used. The latter is not determined by the technique but by the selected nanofiber polymer. Until now, polyacrylonitrile nanofibers are the most frequently chosen system.

We found that the preferred methods are *direct blending* and *UV irradiation*. The other two methods (*silver mirror reaction* and *thermal reduction*) are less used and less studied. It was found that the *UV treatment* and *thermal reduction methods* can manipulate the size and concentration of AgNPs by varying the time of exposition. The *direct blending* and *silver mirror reaction* methods cannot control the AgNPs size, and small-sized AgNPs can be incorporated. The silver mirror reaction method's advantage is that it is a unique technique that can cover large surfaces with AgNPs.

On the other hand, taking into account the presented data, it is proposed that the best method to control the release of Ag^+ ions from the nanofibrous scaffolds is the SMR method because it already reported that the UV treatment and thermal reduction methods presented a leaching phenomenon after the AgNP loading on the fibers. In the case of the direct blending method, agglomerations of the AgNPs occur affecting controlled release. Moreover, the SRM method provides a homogeneous and well-defined distribution where the AgNP release can be controlled by optimizing the polymeric nanofiber's degradation.

Conjugation of well-known antimicrobial activity of AgNPs with electrospun nanofibers could take AgNP antimicrobial properties to their highest efficiency. However, up to now, the antimicrobial efficiency of AgNP-loaded nanofibers has not been explored thoughtfully. The mechanisms of antimicrobial action of AgNPs still need to be addressed. Finally, it is recommended to accompany the future performance of four methods discussed here with a comprehensive determination of physicochemical and antimicrobial characteristics of obtained AgNP-loaded nanofibers. This will allow making a more objective comparison of these methods.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

The team of Research Institute of Clinical and Experimental Lymphology–Branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences thanks to the Ministry of Education and Science of Russia (theme No. 0324-2019-0046). Dr. Alexey Pestryakov thanks Tomsk Polytechnic University Task Program "Science" project FSWW-2020-0011 and Sevastopol State University Task Program "Science" project No. FEFM-2020-0003 and Research grant 42-01-09/169/2021-4.

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