Silver nanoparticles decorated, flexible SiO2 nanofibers with long-term antibacterial effect as reusable wound cover

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\textbf{Abstract}

As wound cover, electrospinning-derived organic polymer nanofibers usually cannot bear calcinations to remove contaminants from wound for regeneration of their functionality, thus are not reusable. In this investigation, we make an exploration of inorganic SiO\textsubscript{2} nanofibers as reusable wound cover. SiO\textsubscript{2} nanofibers here are fabricated with the sol–gel technique and the electrospinning method. Silver nanoparticles (Ag NPs) are grafted on fiber surface through post treatment to endow this material with antibacterial effect. Our results demonstrate the SiO\textsubscript{2} nanofibers are very soft and flexible. They can be conveniently patterned into nonwoven film (the required shape of wound cover). The Ag NPs grafted SiO\textsubscript{2} nanofibers can efficiently inhibit the proliferation of Escherichia coli with a long-term antibacterial effect. More importantly, this inorganic antibacterial wound cover can be renewed through calcinations without lost of its flexibility and antibacterial effect. Consequently, the Ag NPs grafted SiO\textsubscript{2} nanofibers in this investigation are very suitable to be applied as reusable wound cover.

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1. Introduction

Electrospinning (ES) is currently reckoned as a facile approach for preparation of continuously long fibers with their diameter down to nanoscale. Compared with other methods, electrospinning is simpler, more cost-effective, and more environmentally friendly, etc. [1]. Very recently, electrospun biocompatible polymer nanofibers as wound cover has generated a lot of interest [2–7], because the nonwoven films composed of continuously long nanofibers possess huge specific surface area, which is beneficial for regeneration of damaged skin. In addition, the substantially existing interconnected voids in the nonwoven films are beneficial for gaseous exchange, which is also useful for recovery of the wound.

Recent investigations have verified that Ag NPs have efficient and broad-spectrum antibacterial ability with minimal cytotoxicity to human cells [8–12]. The MIC (minimum inhibitory concentration) of Ag NPs is one order lower than the one of silver ions [9]. Nevertheless, in practical applications, Ag NPs are easy to oxidized or aggregate, thus its efficacy could be degraded or even deprived [12]. As wound cover, electrospun polymer nanofibers are usually functionalized with antibacterial effect through addition of silver nanoparticles [13–15]. The polymer matrix can effectively inhibit the oxidation and aggregation of Ag NPs. Now that disposal of used wound cover with incineration or other approaches will inevitably bring about pollutions, development of reusable wound cover will effectively alleviate the environmental degradation. However, due to poor thermal and chemical resistance, electrospun polymer nanofiber wound cover usually cannot be renewed through post treatment to possess reusability.

SiO\textsubscript{2} is a kind of inorganic material extensively applied in many fields [16–19], due to its relative ease of preparation, hydrophilic nature, physical and chemical stability and good biocompatibility, etc. [20]. Recently, investigations on SiO\textsubscript{2} nanofibers (SNFs) in biological applications have been prosperus [21,22]. For example, Yamaguchi et al. [21], applied electrospun SNFs as substrate for culturing cells. They found that Chinese hamster ovarian cells CHO-K1 and human cell line HepG2 cells grew much faster on SNFs than on HAPS (hydroxyapatite-pulp composite fiber sheet). This triggered our imagination of electrospun SNFs nonwoven film as reusable wound cover. And antibacterial effect can be obtained...
with SNFs through incorporation of Ag NPs. However reported investigations on Ag–SNFs nanocomposites mostly focused on doping Ag NPs inside the SNFs [23,24]. This loading style of Ag NPs will inevitably degrade the antibacterial performance, now that the biocidal effect of Ag NPs is mainly through direct contact with microorganisms [9].

Here, we report on preparation of flexible SNFs with Ag NPs grafted on the surface as reusable antibacterial wound cover. In preparation of SNFs using electrospinning, no polymer was used and the precursor SiO2 sol was synthesized with a sol–gel technique, thus the calcination for removal of polymer was circumvented. Consequently, the obtained SNFs are very flexible and soft, making it suitable to be applied as wound cover. Ag NPs are grafted on the fiber surface simply by incubating the SNFs in silver nitrate solution to obtain antibacterial ability. We investigate the morphology, structure, texture, environmental stability and antibacterial ability of the Ag NPs decorated SNFs. We also make a further exploration of the reusability of this material as wound cover.

2. Experimental

2.1. Materials

TEOS (tetraethoxysilane, >98 wt%), ETOH (ethanol, >99.7 wt%) and AgNO3 (silver nitrate, >99.99 wt%) were all purchased from Sinopharm Chemical Reagent Co., Ltd.; HCl (hydrochloric acid, 36–38 wt%) was purchased from Hangzhou Chemical Reagent Co., Ltd. All reagents were used without further purification.

2.2. Sample preparation

In preparation of SiO2 sol, TEOS (30 mL) and ETOH (30 mL) was mixed in a 100 mL glassy vessel, deionized water (4.8 mL) and HCl (0.2 mL) was added drop wise, then the mixed solution was hydrolyzed under 80 °C for 2 h. The parameters of the electrospinning process were optimized as follows: the diameter of the needle was 1.2 mm, collecting distance, applied DC voltage and feeding rate of the sol varied in the ranges of 10–20 cm, 8–16 kV and 0.2–1.6 mL/h, respectively. The whole electrospinning process proceeded at room temperature and room humidity. After 5 h of collection, SNFs (SiO2 nanofibers) were scraped off the aluminum drum and dried at 100 °C for 12 h. For grafting of Ag NPs, 0.1 g SNFs were immersed in water (100 mL) for 12 h, then the SNFs were collected by centrifugation at 10,000 rpm for 10 min, and dried at 60 °C in a vacuum oven. The obtained SNFs were dispersed in 100 mL aqueous solution of AgNO3 in a 100 mL glassy flask (concentration of the silver nitrate solution was set to be 0.05 to 0.1, then to 0.15 M). The flask was placed in an oil bath pot and heated under different temperature for different time, accompanied by magnetic stirring, then the fibers were filtered out, washed with deionized water for three times and dried at room temperature in dark for 12 h. The Ag NPs grafted SiO2 nanofibers were named as SNF@Ag. As the sample prepared with the solution concentration, incubation temperature and time to be 0.1 M, 90 °C and 2 h, respectively, will be repeatedly discussed as a model in the following, so we named this sample as SNF@Ag-m ("m" means model) for convenience.

2.3. Characterization

Digital images of SNFs nonwoven film were recorded with a Canon PowerShot: A640 digital camera. Structure of the SNF@Ag was investigated using a Rigaku D/MAX-RA diffractometer with Cu Kα ray as the incident radiation source; UV–vis spectra of the samples were measured with a U-4100 Spectrophotometer; SEM and TEM images were recorded with a Hitachi S-4800 scanning electron microscope and a CM2000UFT transmission electron microscope; silver content of the sample was estimated using EDX (Energy-dispersive spectroscopy) with an EMAX 350 energy dispersive spectrometer.

2.4. Evaluation of antibacterial ability

Inhibition zones of the SNF@Ag were investigated using Escherichia coli as a model microorganism. Briefly, freshly prepared SNF@Ag (0.1 g) and SNF@Ag-m stored under ambient condition (temperature: 9–24 °C; humidity: 25–80%) for one month (0.1 g) were patterned into small disks with a diameter of 2 cm. 100 μL E. coli strain DH5α culture suspension in logarithmic phase diluted to OD (optical density) 0.500 was added to the surface of 10 cm glass dish with 10 mL congealed agarose LB medium (10 g of tryptone, 5 g of yeast extract, and 5 g of NaCl per liter). The as-patterned disks of SNF@Ag were immediately overlayed on the medium/bacterial surface separately. The dishes were incubated under 37 °C overnight.

Quantitative evaluation of antibacterial effect of the SNF@Ag was investigated by studying the growth dynamic in LB liquid media. 100 μL E. coli strain DH5α culture suspension in logarithmic phase diluted to OD 0.500 was added to 5 mL liquid LB medium in glass tubes. Gradient masses of SNF@Ag-m were added into the glass tube, and the tube was incubated in a 200-rpm shaker at 37 °C. Absorbance at 600 nm wavelength was measured at selected time intervals using an Eppendorf Bio Photometer.

To investigate the antibacterial efficiency of the SNF@Ag, gradient masses of SNF@Ag-m were added to the incubation medium with initial bacteria concentration being 1 × 108 cells/mL. Then the above mixtures were spread uniformly in Petri dishes, and the dishes were sealed and incubated at 37 °C for 2 days. The surface spread-plate method was applied to measure the cell counts of the E. coli with viability.

2.5. Investigation of cytotoxicity of the sample

BMSCs (bone mesenchymal stem cells) were cultured in H-DMEM medium (Invitrogen, USA) containing 10% heat-inactivated FBS (fetal bovine serum) at 37 °C in the humidified atmosphere with 5% CO2. The cells were seeded in 96-well plates at a density of 10 × 104 cells/cm² and grew overnight prior to studies. Then, the cells were incubated with fresh media with gradient doses of SNF@Ag-m (from 20 μg/cm² to 500 μg/cm²). After incubation for 4 days, 20 μL MTT (thiazolyl blue tetrazolium bromide, 10 mg/mL, Sigma–Aldrich, USA) solution was added to each well of the plate, then the plate was incubated at 37 °C for 4 h. Finally, the cells were lysed using DMSO (Sigma, USA); A microplate reader (Bio-Rad 680, USA) was applied to monitor the absorbance of the supernatants at 570 nm. This experiment was repeated for three times.

3. Results and discussion

In an electrospinning process, many variables, typically including the collecting distance, applied voltage and feeding rate of sol, can be operated to adjust the morphology and size of the products. Fig. 1 shows the SEM images of electrospun SiO2 fibers derived from various parameters. Along with the increase of the applied voltage, both homogeneity of the diameter along one single SNF and uniformity of the diameter between individual SNFs are significantly improved, as shown by Fig. 1(a1)–(a3). Consequently, relatively high applied voltage is beneficial to improve the fiber morphology. Feeding rate plays an important role in adjustment of the products size. When keeping collecting distance and applied voltage stable and changing feeding rate from 0.2 mL/h to 0.8 mL/h, then to 1.6 mL/h, average diameter of the SNFs varied from 210 to 260 and
finally to 340 nm. Increase of collecting distance, in essence, possesses the same effect as decrease of applied voltage, because both of them lead to weakening of the electric field strength. As a result, both homogeneity of the diameter along one single SNF and uniformity of the diameter between individual SNFs degenerate with increase of the collecting distance, as shown by Fig. 1(c1)–(c3).

Fig. 2(a) shows the digital image of a SNFs nonwoven film (here, the SNFs were fabricated with the collecting distance, applied voltage and feeding rate to be 15 cm, 12 kV and 1 mL/h, respectively).

As can be seen, the sample can be easily folded up without breakage. Fig. 2(b) shows the same nonwoven mat after being unfolded. Obviously, the folding did not cause any rapture to the sample, indicating that it is very soft and flexible. This soft and flexible texture of the SNFs is dispensable for application as wound cover. In addition, it can also bring the patients with comfort.

Fig. 3(a) and (b) shows the SEM images of SNFs and SNF@Ag-m, respectively. The as-spun SNFs are continuously long fibers with smooth surface and uniform diameter. Length of the SNFs was
observed to be at the centimeter-scale, and its average diameter was calculated to be about 260 nm. As can be seen, a lot of interconnected voids were formed due to the random compile of the SNFs. These substantially existed voids make the nonwoven mat highly fluffy, which is beneficial for gaseous exchange and proliferation of skin cells, when applied as wound cover. After incubation in silver nitrate solution, surface of the SNFs was decorated with a layer of Ag NPs with roughly spherical shape, as shown in Fig. 3(b). Mean diameter of the Ag NPs was calculated to be about 24 nm, and distribution of the Ag NPs on the SNFs was relatively homogenous. Fig. 3(c) and (d) shows the corresponding diameter distributions of SNFs and Ag NPs, respectively.

TEM image of the SNF@Ag-m is shown in Fig. 4. As can be seen, gray SNFs are decorated with relatively dark Ag NPs. The inset in Fig. 4 is a HRTEM (high-resolution TEM) of one single Ag NPs (the white-circled one in Fig. 4). Regular one-directional arranged crystal fringes are observed with the whole Ag NP, indicating a single crystalline structure. The gaps between adjacent lattice fringes are measured to be about 0.238 nm, corresponding to (1 1 1) face of cubic silver crystal.

Fig. 5 shows the XRD patterns of the SNF@Ag-m. The big broadened peak at about 2θ is a typical diffraction peak of amorphous SiO₂, being ascribed to SNFs. Small peaks at about 2θ = 38.18°, 44.3°, 64.32° and 77.69° correspond to diffractions from (1 1 1), (2 0 0), (2 2 0) and (3 1 1) faces of cubic Ag NPs (JCPDS, File No. 01-087-0720) [25]. Inset in Fig. 5 shows the EDX patterns of the SNF@Ag-m. Characteristic peaks of silver can be observed clearly, and the mass ratio of Ag NPs was measured to be about 0.2 wt%.

In this investigation, we also studied the influence from incubation parameters to the size and morphology of the grafted Ag NPs. As shown in Fig. 6, increase of all the three variables, including the concentration of silver nitrate solution, incubation temperature and time, significantly increase the density of Ag NPs on fiber surface. Increase of solution concentration slightly enhanced the size of Ag NPs (mean diameter of Ag NPs in Fig. 6(a1)–(a3) is 21 nm, 24 nm and 28 nm, respectively). Reversely, increase of incubation temperature caused significant decrease of the Ag NPs size, especially when the temperature was as high as 120 °C (mean diameter of Ag NPs in Fig. 6(b1)–(b3) is 25 nm, 22 nm and 14 nm, respectively). Both variation of solution concentration and incubation temperature did not change the shape of Ag NPs (sphere-shaped). Prolonging of incubation time remarkably enhanced the size and meanwhile tremendously changed the shape of Ag NPs. When incubation time increased to 3 h, the grafted Ag NPs

![Fig. 3. (a) and (b): SEM images of SNFs and SNF@Ag-m; (c) and (d): diameter distribution of SNFs and grafted Ag NPs.](image1)

![Fig. 4. TEM image of SNF@Ag-m. The inset shows the HRTEM image of one single Ag NP marked with white circle.](image2)
Fig. 5. XRD pattern of SNF@Ag-m. The inset shows the EDX pattern of SNF@Ag-m.

become irregularly-shaped rather than sphere-shaped, and their size almost increased to be more than two times large as the one of 1-h-incubated particles (mean size of Ag NPs in Fig. 6(c1)–(c3) is 15 nm, 23 nm and 38 nm, respectively).

Previous researches have proved individual Ag NPs (without capping agent or stabilizing matrix) are easy to be oxidized and/or aggregate in air [26–28]. Such oxidation or aggregation can cause degradation or even lost of the antibacterial ability of Ag NPs. Fig. 7(a) shows the UV–vis spectra of Ag NPs aqueous suspension prepared by fierce ultrasonic washing the aqueous suspension of SNF@Ag-m and properly centrifuging away the fibers. Obviously, both the Ag NPs stored in dark and in light have significant spectral change with substantial intensity decrease, indicative of oxidation and/or aggregation of the Ag NPs. Light played an important role in oxidation or aggregation of Ag NPs. As a result, spectral change of the Ag NPs stored in light was more significant. Stabilizing Ag NPs with SNFs can effectively alleviate the oxidation and/or aggregation. Parallel investigation indicates that the SNF@Ag-m prepared here is very environmentally stable, change of its UV–vis spectra is very slight, no matter stored in dark or in light even for one month, as shown in Fig. 7(b). The high stability of the SNF@Ag should be ascribed to the spatial distribution and impregnation of the Ag NPs stabilized on the surface of SNFs, which anchored the Ag NPs and prevented their oxidation or aggregation.

It has been experimentally verified that, elemental nanosilver is a kind of potent and broad-spectrum biocide, whose effectiveness is one order higher than that of silver salts [9,29]. Considering the various advantages of SNFs on biological applications, the SNF@Ag prepared here is suitable to be used as antibacterial wound dressing material. We tested inhibitive effect of SNF@Ag with different loading amount of Ag NPs (samples in Fig. 8(a1), (b1) and (c1) are the same as the samples in Fig. 6(a1), (a2), (a3), respectively) against proliferation of E. coli. Clear side inhibition zones can be observed with all the three samples, and their area increased sequentially, indicating an Ag NPs dose dependent antibacterial effect. Area of inhibition zones of the three samples is measured to be 0.53 cm², 0.66 cm² and 0.75 cm², respectively.

As mentioned above, polymer nanofiber wound covers usually are vulnerable to post treating process, such as incineration, for regeneration of their functionality, thus are not reusable. Quite different from polymer nanofibers, SNF@Ag is thermally and chemically stable. After its use, it can be incinerated to remove the

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Silver concentration increasing (temperature=90 °C, time=2 h)

Temperature increasing (silver concentration=0.1 M, time=2 h)

Time increasing (silver concentration=0.1 M, temperature=90 °C)

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Fig. 6. Influence from incubation condition to the morphology and size variation of Ag NPs. From (a1) to (c1), concentration for silver nitrate solution was 0.05 M, 0.1 M and 0.15 M, respectively; from (a2) to (c2), incubation temperature was 60 °C, 90 °C and 120 °C, respectively; from (a3) to (c3), incubation time was 1 h, 2 h and 3 h, respectively.
remnant from wounds, such as body fluid or adsorbed bacteria, for its decontamination. Consequently, this material, as wound cover, is reusable. We treated the samples in Fig. 8 (after test of the inhibition zone) with calcinations in air at 380 °C for 2 h to remove the contaminant from the incubation medium, and resteted its inhibition zones. As shown in Fig. 8(a2), (b2) and (c2), the sample still exhibited effective antibacterial ability, clear side inhibition zone was formed after 12-h-incubation. The area of the side inhibition zones of the sample in Fig. 8(a2), (b2) and (c2) are measured to be 0.06 cm², 0.17 cm² and 0.28 cm². In addition, we also found that the calcined SNF@Ag-m retained their softness and flexibility very well. These results indicate that the SNF@Ag fabricated here is quite suitable to be applied as reusable wound cover.

Above results have demonstrated the SNF@Ag is environmentally stable, so long-term antibacterial effect is expected to be possessed by this material. As shown by Fig. 9, after being stored in light under ambient condition for one month, the antibacterial effect of SNF@Ag-m was well preserved. Compared with the one of freshly prepared SNF@Ag-m, the inhibition zone area of the SNF@Ag-m stored for one month is only slightly decreased, indicative of a long-lasting antibacterial ability.

Growth dynamic of E. coli incubated with gradient doses of SNF@Ag-m was investigated to give a quantitative evaluation of the sample's antibacterial ability. As shown in Fig. 10, the growth of E. coli exhibited a sensitive dose-dependent antibacterial effect of the sample. The lag phase length of the growth curve increased gradually with the increase of the sample concentration. When the sample concentration reached 500 ppm, the lag phase extended to longer than 10 h. Absolute inhibition of E. coli in the whole incubation process was obtained when the sample concentration reached more than 1000 ppm, also indicating that the SNF@Ag has a long-term antibacterial effect. Fig. 11 shows the antibacterial efficiency of the SNF@Ag-m. 100% antibacterial efficiency was achieved, when the sample concentration reached 1000 ppm. According to the EDX result, then the MIC of the SNF@Ag-m was calculated to be about 2 μg/mL (in terms of silver element).

Fig. 7. (a) UV–vis spectra of freshly prepared Ag NPs (solid line) and Ag NPs stored in dark (dashed line) and in light (dashed and dotted line) for one week; (b) UV–vis spectra of freshly prepared SNF@Ag-m (solid line) and SNF@Ag-m stored in dark (dashed line) and in light (dashed and dotted line) under ambient condition for one month.

Fig. 8. Inhibition zones of SNF@Ag with different loading amount of Ag NPs against proliferation of E. coli. The samples in (a1), (b1) and (c1) were prepared with the same incubation temperature (90 °C) and time (2 h), but the concentration of silver nitrate solution for them was 0.05 M, 0.1 M and 0.15 M, respectively. Samples in (a2), (b2) and (c2) were obtained through calcined the samples in (a1), (b1) and (c1) after the germinal test of their inhibition zones. Experimental parameters for the inhibition zone test of these two groups were identical.
Investigations from other groups have verified that SiO₂ has good biocompatibility. In addition, electrospun SNFs as culture substrate for human cells also has been well studied. Fig. 12 shows the result of cytotoxicity investigation of the SNF@Ag-m. It indicates that the SNF@Ag-m can slightly promote the proliferation and viability of BMSCs, when the sample dose was lower than 200 μg/cm². Antibacterial efficiency experiment has shown that sample concentration of 1000 ppm (equivalent to 13 μg/cm²) already has 100% antibacterial efficiency, thus the SNF@Ag can effectively avoid infection from microorganisms and at the same time promote the regeneration of the damaged skin, when applied as wound cover.

Fig. 9. Inhibition zones of (a) freshly prepared SNF@Ag-m and (b) SNF@Ag-m stored in light under ambient condition for one month.

Fig. 10. Growth curves of E. coli incubated with gradient concentrations of SNF@Ag-m.

Fig. 11. Evolution of antibacterial efficiency of SNF@Ag-m as a function of sample concentration.

Fig. 12. Cytotoxicity investigation of SNF@Ag. The viability of BMSCs incubated with gradient doses of SNF@Ag-m for 4 days.

4. Conclusion

In summary, we propose a simple and productive strategy for preparation of flexible Ag NPs decorated SNFs (SNF@Ag) as antibacterial wound cover. SNFs were prepared with a sol–gel combined electrospinning method first, then Ag NPs were grafted on the fiber surface through incubating the SNFs in aqueous solution of silver nitrate. Morphology and diameter of the SNFs can be conveniently adjusted by variation of electrospinning parameters, while shape and size of the Ag NPs can be easily controlled through changing of incubation condition. The SNF@Ag exhibited efficient inhibitory ability against proliferation of E. coli. With SNFs as a matrix for stabilizing Ag NPs, the SNF@Ag is environmentally stable. Effective antibacterial activity of the SNF@Ag was well preserved for a long time of storage under ambient condition. Cytotoxicity investigation indicates that the SNF@Ag not only has no toxicity to human cells, but also can promote the growth of human cells in a wide concentration range. The used SNF@Ag nonwoven films can be conveniently renewed through calcinations in air to obtain reusability. Considering its soft and flexible texture, efficient and persistent antibacterial effect and reusability, the SNF@Ag could be a kind of promising wound cover.

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References