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Antibacterial polycaprolactone electrospun fiber mats prepared by soluble eggshell membrane protein-assisted adsorption of silver nanoparticles

Huan-Huan Liu, Qiang Li, Xiao Liang, Xi Xiong, Jian Yu, Zhao-Xia Guo

Key Laboratory of Advanced Materials (MOE), Department of Chemical Engineering, Tsinghua University, Beijing 100084, People's Republic of China

Correspondence to: Z.-X. Guo (E-mail: guozx@mail.tsinghua.edu.cn) and Q. Li (E-mail: liqiang@mail.tsinghua.edu.cn)

ABSTRACT: Antibacterial polycaprolactone (PCL) electrospun fiber mats were prepared by coelectrospinning PCL with soluble eggshell membrane protein (SEP) in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), followed by adsorption of silver nanoparticles (Ag NPs) through hydrogen-bonding interaction between the amide groups of SEP and the carboxylic acid groups capped on the surfaces of Ag NPs. The PCL/SEP fiber mat was characterized by X-ray photoelectron spectroscopy, indicating the presence of some SEP on the fiber surface. The adsorption of Ag NPs was confirmed by transmission electron microscopy and quantitatively characterized by thermogravimetric analysis. The pH value of the silver sol used for adsorption is very important in view of the amount and dispersion state of Ag NPs adsorbed on the fibers. The Ag NP–decorated PCL/SEP fiber mats prepared at pH 3–5 exhibit strong antibacterial activity against both gram-negative *Escherichia coli* and gram-positive *Bacillus subtilis*. Antibacterial PCL fiber mats were also obtained similarly with the assistance of collagen (another protein) instead of SEP, showing that protein-assisted adsorption of Ag NPs is a versatile method to prepare antibacterial electrospun fiber mats. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43850.

KEYWORDS: biomaterials; electrospinning; properties and characterization

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INTRODUCTION

Electrospinning is an efficient nanotechnology for producing ultrafine fibers. The electrospinning products are in the form of fibrous mats as a result of random deposition of the fibers. Polymer electrospun mats have shown a variety of promising applications in various fields, such as filtration, catalysis, protective textiles, tissue engineering, drug delivery, wound dressing, and medical implants, thanks to their unique structural characteristics, such as high specific surface area and high porosity.[1–5] For some applications such as antibacterial filtration and wound dressing, antibacterial activity is a must. However, most polymer electrospun fiber mats do not possess intrinsic antibacterial properties, and antibacterial polymer electrospun fiber mats are usually prepared by incorporation of antibacterial agents, including antibiotics, biocides, metal and metal oxide nanoparticles, and chitosan.[6–12]

Silver has long been used as an antibacterial agent owing to its broad-spectrum antibacterial activity, low toxicity to human cells, and environmentally friendly features.[13–16] It is widely used in medical and food-packaging materials. With the rapid development of nanotechnology in recent years, silver nanoparticles (Ag NPs) are receiving increasing attention because they have a much larger specific surface area than bulk silver, which allows for more efficient release of silver ions, and the nanoparticles themselves possess antibacterial activity.[13,14] The antibacterial action of Ag NPs is more efficient than many other metallic antibacterial nanoparticles, such as copper and zinc.[14]

Polymer electrospun fiber mats containing Ag NPs have been extensively investigated.[12,17–22] There are mainly two preparation methods depending on when Ag NPs are introduced. The first one is coelectrospinning a mixture of polymer/precursor such as $AgNO_3$ or polymer/Ag NPs generated in or blended into the electrospinning solution.[17,18] The second involves attachment of a precursor or Ag NPs on the fiber surfaces by adsorption or magnetron sputtering after electrospinning.[19–22] When a precursor is used, posttreatment of the fibers is needed to generate Ag NPs by means of heating, UV radiation, $NaBH_4$ reduction, plasma treatment, and so on.[20,21] In the first method, a large proportion of Ag NPs is embedded inside the fibers, limiting their antibacterial performance. The second method is advantageous since all the Ag NPs are on the fiber surfaces, facilitating the use of their bactericidal function. However, magnetron sputtering needs special equipment, and adsorption

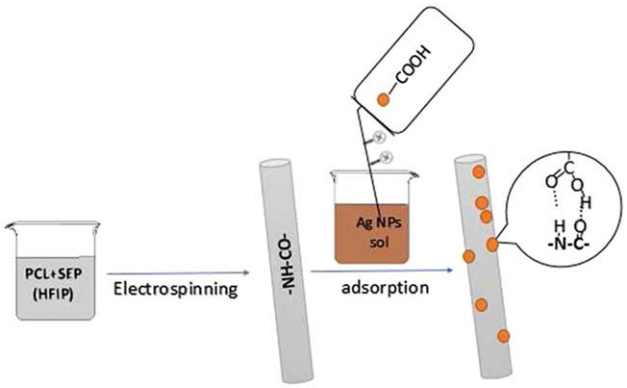
requires that the fibers contain suitable functional groups that have an affinity to silver.

It has been reported that Ag NPs can be adsorbed on nylon 6 fibers through hydrogen-bonding interaction between the amide groups of nylon 6 and the carboxylic acid groups capped on the surfaces of Ag NPs.[23] We would wonder whether this type of interaction could be generally applicable to assist the adsorption of silver nanoparticles onto polymer electrospun fibers by incorporating a protein, which is rich in amide groups similar to nylon 6, via coelectrospinning. This would facilitate the preparation of biocompatible fibers having antibacterial properties. Polycaprolactone (PCL) is a biocompatible and biodegradable polymer and is frequently used in the biomedical field.[24] Soluble eggshell membrane protein (SEP)[25,26] is a protein-rich product isolated from natural eggshell membrane, an abundant proteinous source consisting mainly of proteins such as collagen type I, V, and X.[25] Its processability, biocompatibility, and degradability have been extensively investigated.[26,27] In this work, PCL/SEP blend fiber mat was used as a model system to demonstrate the feasibility of the idea of using a protein to assist adsorption of Ag NPs for the purpose of introducing antibacterial function. The PCL/SEP fiber mat was obtained by coelectrospinning a mixture of PCL and SEP in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and characterized by field emission scanning electron microscopy and X-ray photoelectron spectroscopy. The adsorption of Ag NPs on the surface of the PCL/SEP fiber mat was confirmed by transmission electron microscopy (TEM) and thermogravimetric analysis (TGA). The antibacterial performance of the Ag NP–decorated PCL/SEP fiber mats were evaluated using *Escherichia coli* and *Bacillus subtilis*. Furthermore, SEP was replaced by a type I collagen to confirm the versatility of the method. Compared to the existing methods for the preparation of Ag NP–containing PCL electrospun mats,[28–31] incorporation of a protein such as SEP can considerably improve the biocompatibility and hydrophilicity of the mats[32] and therefore increase their applicability in the biomedical field, as wound dressing for example.

EXPERIMENTAL

Materials

Silver nitrate ($AgNO_3$, AR) and sodium chloride (NaCl, AR) were purchased from Beijing Chemical Works, Beijing, China. Trisodium citrate ($Na_3C_6H_5O_7{\cdot}2H_2O$, AR) was obtained from Sinopharm Chemical Reagent Co, Beijing, China. Sodium borohydride ($NaBH_4$, 98.0–100%) was purchased from Fuchen Chemical Reagent Works in Tianjin, China. Polycaprolactone (800c, $M_w = 80{,}000$, $M_w/M_n \leq 1.8$) was obtained from Shenzhen Esun New Material Co., Shenzhen, China. Collagen (Type 1, Sigma C9789, Shanghai, China) was obtained from bovine Achilles tendon. Hydrochloric acid (HCl, 36–38%) was obtained from Beijing Modern Oriental Fine Chemicals Co., Beijing, China. Agar was purchased from Biotopped, Beijing, China. Tryptone and yeast extract were obtained from Oxoid Ltd., Basingstoke, England. Soluble eggshell membrane protein was prepared according to a published procedure.[33] All the solutions and sols were prepared with deionized water unless otherwise specified, and all chemicals were used without further purification.



Scheme 1. Illustration for the preparation of Ag NP–decorated PCL/SEP fibers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Electrospinning

The electrospinning solutions were prepared by dissolving PCL, PCL/SEP (9:1, mass ratio), and PCL/collagen (9:1, mass ratio) in HFIP at room temperature. All the solution concentrations are 7 wt %, and the solution was placed into a 10 mL syringe with a 12# blunt-end needle. Electrospinning was conducted at ambient conditions with an applied voltage of 12 kV, receiving distance of 12 cm, and feeding rate of 2 mL/h for 2.5 h. The nanofibers were collected on a grounded metal drum wrapped with a layer of aluminum foil of size 12.5 cm × 12.5 cm and then dried at 40 °C under vacuum for 24 h.[34]

Preparation and Adsorption of Ag NPs

The aqueous silver sol containing Ag NPs of a mean size of 11 nm was prepared by a known chemical reduction method.[23,35,36] The preparation procedure used $NaBH_4$ as a reducing agent and $Na_3C_6H_5O_7{\cdot}2H_2O$ as stabilizer. The molar ratio of $AgNO_3/NaBH_4/Na_3C_6H_5O_7{\cdot}2H_2O$ is 1:5:1. Briefly, to a vigorously stirred solution of $AgNO_3$ (11.3 mg) and trisodium citrate (19.6 mg) in water (60 mL) was rapidly added 5 mL of aqueous solution of $NaBH_4$ (12.7 mg). After stirring for 1 h at room temperature, a deep-brown silver sol was formed.

For assembly of Ag NPs onto electrospun mats,[23,34,37] the pH value of the silver sol was adjusted from pH 9.7 (original) to 3.0, 4.0, and 5.0 by adding dropwise 1 *M* HCl just before use. The electrospun mat supported by a filter paper was placed in a vacuum funnel with a diameter of 3 cm. A 10 mL portion of silver sol was filtered through the mat. Then the mat was thoroughly washed with deionized water and dried.

Characterization

Field emission scanning electron microscopy (FESEM) was carried out on a JSM 7401, Tokyo, Japan operated at an accelerating voltage of 3 kV to observe the morphology and diameter of the nanofibers. The specimens were processed with Au spraying before imaging. The average fiber diameters were obtained by measuring and averaging the diameters of 50 fibers using JEOL software (SMILEVIEW, Tokyo, Japan). TEM was performed on a ZEM 2010, Tokyo, Japan operated at an accelerating voltage of 120 kV. For TEM observation, the fibers were directly electrospun onto copper grids covered with an ultrathin carbon layer

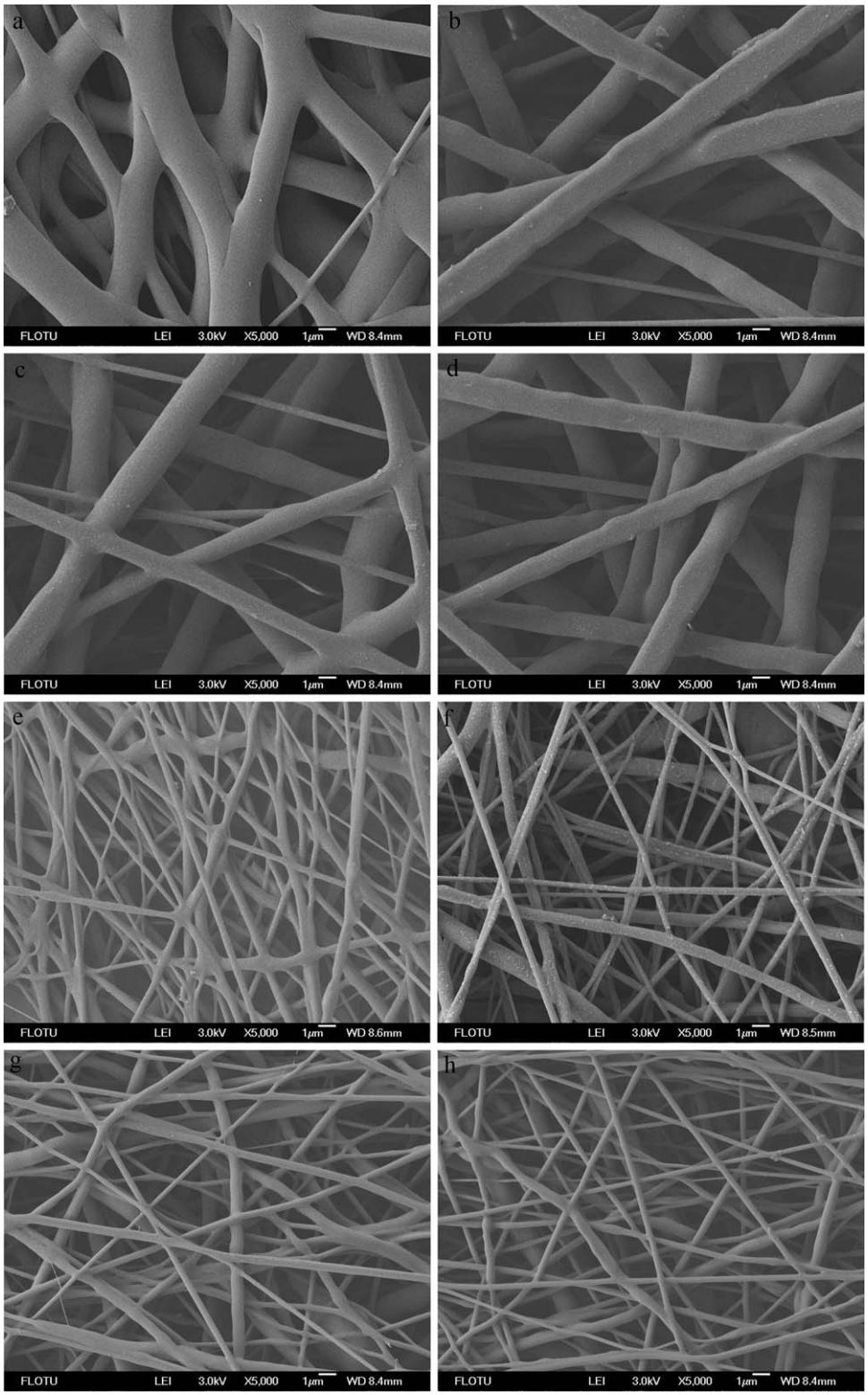


Figure 1. FESEM images of (a) PCL, (e) PCL/SEP, and Ag NP–decorated PCL fibers prepared at different pH values: (b) 3.0, (c) 4.0, (d) 5.0; and Ag NP–decorated PCL/SEP fibers prepared at different pH values: (f) 3.0, (g) 4.0, (h) 5.0.

by putting the copper grids next to the aluminum foil–covered grounded metal drum. After collecting fibers, a droplet of pH-adjusted silver sol was dropped on each fiber-containing grid and allowed to stand for 20 min, followed by washing 10 times with deionized water. X-ray photoelectron spectroscopy (XPS) data were obtained with a Thermo Scientific, ESCALab250Xi, Shanghai, China multifunctional photoelectron spectroscopy analyzer with 200 W Al Kα X-ray as the excitation source. The

Table I. Surface Atom Contents (%) of PCL and PCL/SEP Fibers

	C	O	N	S
PCL	75.73	23.78	0	0
PCL/SEP	74.24	23.71	1.8	0.26

basic vacuum is 3×10^{-10} mbar, and the take-off angle of the XPS was 45°. Water contact angles of the electrospun mats were measured with a sessile drop method using a Dataphysics, OCA-20 contact angle analyzer, Stuttgart, Germany. The droplet volume was 4 μL, and five points were tested on each sample. TGA, was performed on a (DTG-60, Kyoto, Japan) with a heating rate of 20 °C/min under N_2. The test temperature range was from room temperature to 550 °C. The data obtained from TGA were used to determine the silver content of the fiber mats using the principle that inorganic Ag NPs do not lose weight upon heating.The silver content was calculated after deducting the residue of the corresponding electrospun mats used for adsorption of Ag NPs.

Table II. Amounts of Ag NPs (%) Adsorbed on PCL and PCL/SEP Fiber Mats Obtained by TGA

Sample	Neat sample	pH = 3	pH = 4	pH = 5
PCL	0	0.13	0.06	0.02
PCL/SEP	0	3.60	3.09	1.19

Antibacterial Tests

The antibacterial activities of the electrospun mats were examined against *E. coli* and *B. subtilis* according to the AATCC 100 test method with slight modification. The electrospun mats without Ag NPs were used as the control. The detailed procedure is as follows.

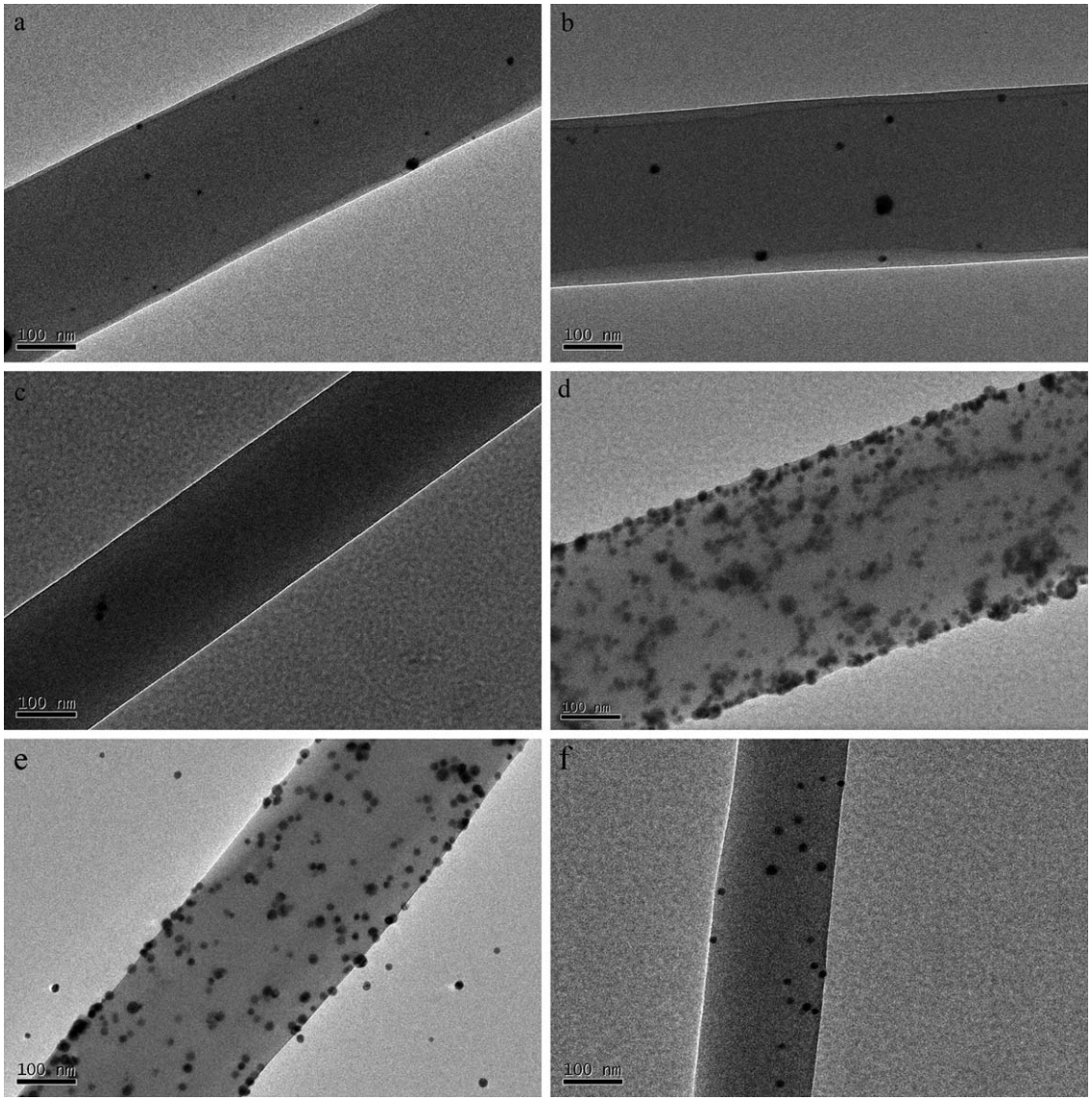


Figure 2. TEM images of Ag NP–decorated PCL fibers prepared at different pH values: (a) 3.0, (b) 4.0, (c) 5.0; and Ag NP–decorated PCL/SEP fibers prepared at different pH values: (d) 3.0, (e) 4.0, (f) 5.0.

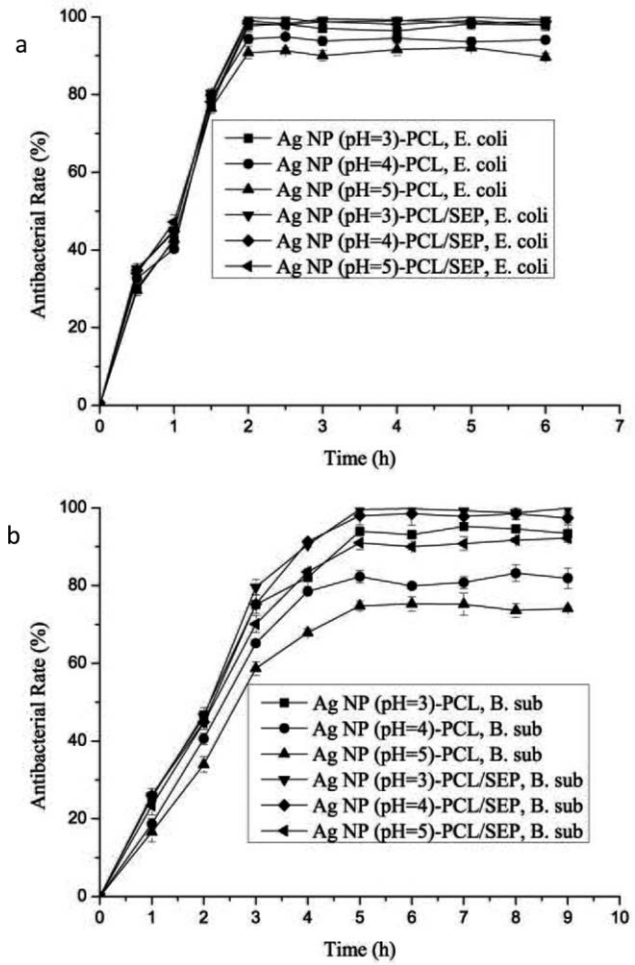


Figure 3. Time course of antibacterial activity of Ag NP–decorated PCL and Ag NP–decorated PCL/SEP fiber mats against (a) *E. coli* and (b) *B. subtilis*.

The electrospun mats in pieces of 1.5 cm × 1.5 cm were immersed into 75% ethanol for 30 min for sterilization and dried in a superclean bench. Then, the mats were put on the Luria-Bertani (LB) medium (containing NaCl 1%, tryptone 1%, yeast extract 0.5%, and agar 2%, and the solvent is deionized water). Care is taken to make sure that the mats and the plates fit fully. A 10 μL portion of an aqueous suspension of bacteria was dropped on each sample. After incubation at 37 °C for a predetermined time, the mats were removed from the LB plates and put in tubes containing 1 mL of deionized water, which were then shaken for 5 min to ensure that the bacteria on the mats were completely washed off. Five batches of bacterial suspension (100 μL each) were taken out and diluted to 10^1, 10^2, 10^3, 10^4, and 10^5 times in sequence. Then 100 μL each of the diluted solutions was placed onto an LB plate and incubated at 37 °C for 18 h. The plate count method[38,39] was used to measure the number of bacteria on each sample. Bacteria concentrations on the test samples were recorded as A_1 (cfu/mL) and that on the reference sample recorded as A_0 (cfu/mL). Every A_1 and A_0 came from the mean values of three tests, and the formula for calculating the antibacterial rate (P) is

$$P = \frac{A_0 - A_1}{A_0} \times 100\%$$

Statistical Analysis

All the data on the antibacterial rates are expressed as the mean plus or minus the standard deviation. One-way analysis of variance was applied to assess the significance difference, where $p < 0.05$ is considered significant.

RESULTS AND DISCUSSION

Preparation of Ag NP–Decorated PCL and PCL/SEP Fiber Mats

The preparation procedure for Ag NP–decorated PCL/SEP fiber mat is illustrated in Scheme 1. First, an electrospinning solution was prepared by dissolving PCL and SEP (10 wt % relative to PCL) in HFIP at room temperature. Second, electrospinning was conducted at ambient conditions, and the PCL/SEP fiber mat was obtained straightforwardly. Third, a silver sol was filtered through the PCL /SEP fiber mat to decorate the mat with

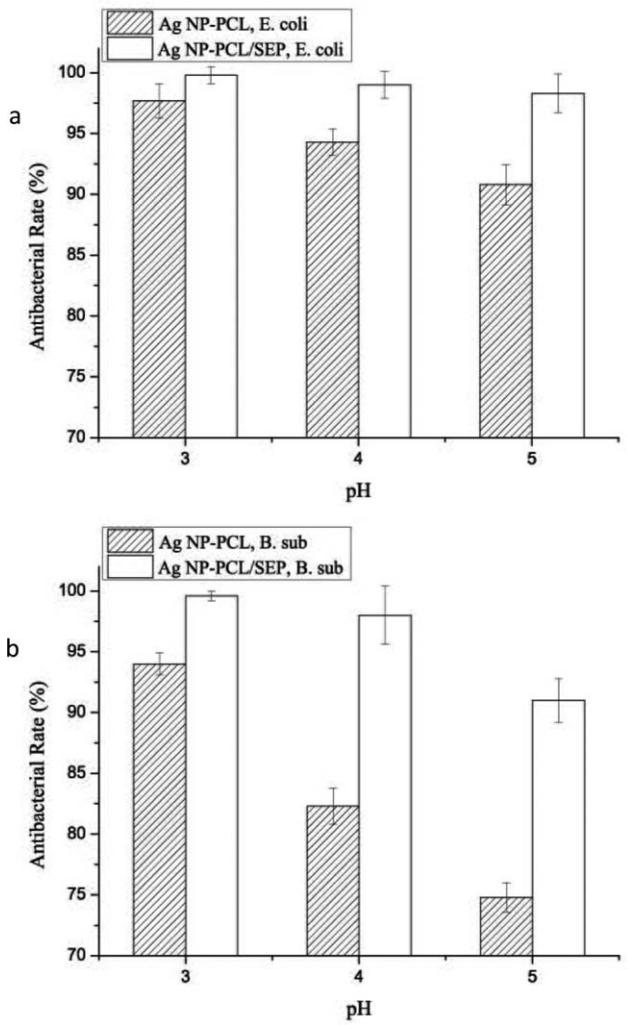


Figure 4. Antibacterial rates of Ag NP–decorated PCL and Ag NP–decorated PCL/SEP fiber mats against (a) *E. coli* and (b) *B. subtilis*. The difference between Ag NP–decorated PCL and Ag NP–decorated PCL/SEP fiber mats at the same pH value is statistically significant ($p < 0.05$).

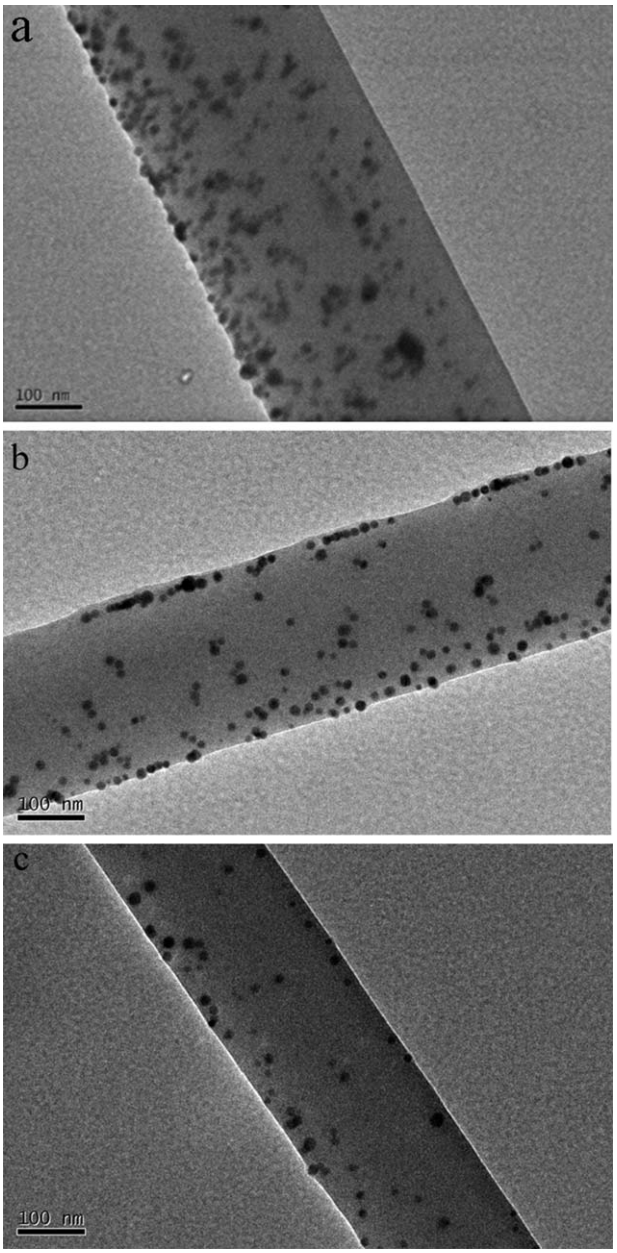


Figure 5. TEM images of Ag NP–decorated PCL/col fibers prepared at different pH values: (a) 3.0, (b) 4.0, (c) 5.0.

Ag NPs. The pH values of the silver sol were 3, 4, and 5 and should be adjusted from the original silver sol (pH = 9.7) freshly just before use to avoid sedimentation of the Ag NPs, which could be severe upon storage, especially at pH 3. The Ag NP–decorated PCL fiber mat is prepared in the same way.

The morphology and the surface elemental compositions of the PCL and PCL/SEP fiber mats were investigated by FESEM and XPS, respectively. As shown in Figure 1, smooth and bead-free fibers were observed with an average diameter of 0.34 ± 0.03 μm for PCL/SEP fibers, which is much smaller than that of neat PCL fibers (1.66 ± 0.03 μm) electrospun from the same solution concentration, similar to the case of PLA/SEP fibers.[32] This is attributed to the increase in solution conductivity and the decrease in solution viscosity, since the molecular weight of SEP is low and it contains polar groups such as amino, amide, and carboxyl groups. The XPS results are listed in Table I. Significant atomic concentrations of N and S were detected on the surface of the PCL/SEP fiber mat, revealing the presence of some SEP on the fiber surface, since neat PCL does not contains N and S elements, but SEP does. Neat PCL fiber mat is hydrophobic with a water contact angle of 136°. In contrast, when water was dropped onto the surface of the PCL/SEP fiber mat during contact angle measurements, it penetrated into the mat immediately, indicating superhydrophilicity. The remarkable improvement in hydrophilicity further confirms the presence of SEP on the fiber surfaces.

The morphology of Ag NP–decorated PCL and PCL/SEP mats prepared at three different pH values was investigated by FESEM. As shown in Figure 1, after adsorption of Ag NPs, the fiber diameter has no obvious change and the porosity of the mats is retained. Because it is difficult to see Ag NPs in FESEM images because of their small sizes even at high magnifications, TEM was envisaged to confirm adsorption of Ag NPs on the fiber surfaces. The sample preparation process for TEM can mimic to a great degree the corresponding mat-preparation process, although the fiber-collection process on a copper grid and the subsequent Ag NP–adsorption process are not exactly the same as those for the fibrous mats. The TEM micrographs are shown in Figure 2. Ag NPs were observed in either monodisperse or slightly agglomerated states. Only a few Ag NPs were found on neat PCL fibers no matter which pH value was used, whereas many more Ag NPs were found on PCL/SEP fiber mats, with the highest amount and highest degree of agglomeration observed at pH 3. The TGA results listed in Table II are basically in agreement with the TEM observation, where less than 0.2% was found for the PCL fiber mats and up to 3.60% for the PCL/SEP fiber mat prepared at pH 3.

These results show clearly that the incorporation of SEP can significantly improve the amount of Ag NPs adsorbed on the fibers. This could be attributed to the protein-rich feature of SEP. SEP contains many amide groups that can have hydrogen-bonding interactions with the carboxyl groups capped on Ag

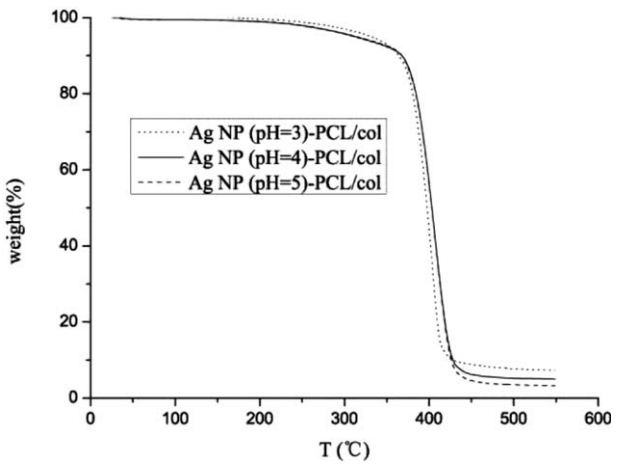


Figure 6. TGA curves of Ag NP–decorated PCL/col fiber mats.

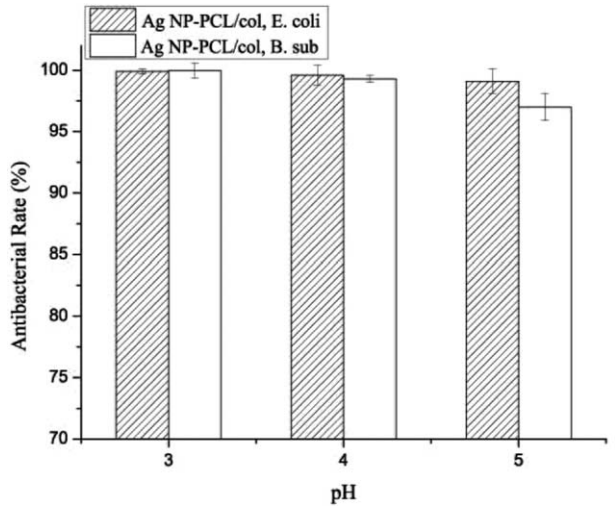


Figure 7. Antibacterial rates of Ag NP–decorated PCL/col fiber mats against *E. coli* and *B. subtilis*.

NPs. PCL contains ester groups in its molecular structure, which can also have hydrogen-bonding interactions with carboxyl acid groups, but the amount and the strength of the bonds formed between ester and carboxylic acid groups are considerably less and weaker than those between amide and carboxyl acid groups.

Antibacterial Properties of Ag NP–Decorated PCL and Ag NP–Decorated PCL/SEP Fiber Mats

The PCL and PCL/SEP fiber mats have no antibacterial activity against both *E. coli* and *B. subtilis* since their antibacterial rates are nearly zero using neat nutrient agar plate as the control. In contrast, effective antibacterial activity against both *E. coli* and *B. subtilis* was found for Ag NP–decorated PCL and Ag NP–decorated PCL/SEP fiber mats. As shown in Figure 3, the antibacterial rates of all the Ag NP–decorated fiber mats increase to a steady level after 2 h and 5 h of incubation for *E. coli* and *B. subtilis*, respectively.

The values of antibacterial rates for all the fiber mats containing Ag NPs at the contact time of 2 h for *E. coli* and 5 h for *B. subtilis* are summarized in Figure 4. Obviously, all the fiber mats containing SEP show significantly better antibacterial performance than those without SEP prepared at the same pH values ($p < 0.05$), especially in the case of *B. subtilis*, showing the usefulness of SEP in improving the antibacterial rates of PCL fiber mats. For example, the antibacterial rate of the Ag NP–decorated PCL/SEP fiber mat prepared at pH 4 is 97.9% against *B. subtilis*, while that of Ag NP–decorated PCL fiber mat prepared at the same pH value is only 82.3%. This difference can be attributed to the difference in the amount of Ag NPs adsorbed on different fiber mats. The fiber mats containing SEP have more Ag NPs on the surface and thus have better antibacterial performance than those without SEP. It is noted that the antibacterial rates of the Ag NP–decorated PCL and PCL/SEP fiber mats increase when the pH of the silver sol used for adsorption decreases from 5 to 3, being in agreement with the increasing tendency of the amount of adsorbed Ag NPs. The increasing tendency from pH 5 to 3 is statistically significant ($p < 0.05$), except for Ag NP–decorated PCL/SEP fiber mats against *E. coli*, where all of the samples have excellent antibacterial rates (over 97%).

The antibacterial performance of all the fiber mats containing Ag NPs against *E. coli* is somewhat better than that against *B. subtilis*, especially for the mats without SEP. For example, the antibacterial rate of Ag NP–decorated PCL fiber mat prepared at pH 4 is 94.3% against *E. coli* after 2 h of incubation, but only 82.3% against *B. subtilis* after 5 h of incubation. This is most likely due to the difference in the structure of the two types of cell walls.[40–42] The cell wall of gram-negative bacteria (2–3 nm) is thinner than that of gram-positive bacteria (20–80 nm),[40] so it is much easier for gram-negative bacteria to be affected by Ag NPs. Also, the cell wall of gram-negative bacteria contains 3–20 times more peptidoglycan than that of gram-positive bacteria.[42] Because peptidoglycans are negatively charged, gram-negative bacteria can permit more Ag^+ to reach the cytomembrane than gram-positive bacteria, and therefore the activity of gram-negative bacteria is more easily decreased by Ag NPs.

Considering the difference in Ag NP contents among the different samples, the effect of Ag NP content on the antibacterial activity is rather small, which is because Ag NPs are a type of highly efficient antibacterial agent. With a small Ag NP content, such as in the case of the PCL/Ag NP samples, the antibacterial activity is already high, over 90% against *E. coli* for all three PCL/Ag NP samples prepared at different pH values. The results for the PCL/SEP/Ag NP samples with higher Ag NP contents than the PCL/Ag NP samples do reveal an improvement in antibacterial activity over the PCL/Ag NP samples, which is due to the effect of Ag NP content, especially in the case of *B. subtilis*.

Universality of the Method

To confirm the universality of the idea of blending a protein to assist adsorption of Ag NPs and increase the antibacterial activity, SEP was replaced by collagen, and a PCL/collagen (PCL/col) 9:1 fiber mat was prepared by electrospinning from HFIP. Ag NPs were easily adsorbed on the fiber mat, as revealed by TEM (Figure 5). The Ag NP content in Ag NP–decorated PCL/col fiber mat was quantitatively measured by TGA (Figure 6). The dispersion state and the amount of Ag NPs on the fiber mats are similar to that of the PCL/SEP fiber mats.

As shown in Figure 7, all of the Ag NP–decorated PCL/col fiber mats prepared at pH 3, 4, and 5 exhibit excellent antibacterial activity against both *E. coli* and *B. subtilis*, demonstrating that collagen, another protein from a different resource than SEP, can also effectively assist in adsorption of Ag NPs and consequently increase antibacterial activity.

CONCLUSIONS

SEP-assisted adsorption of Ag NPs is a viable approach to improve adsorption of Ag NPs and thus the antibacterial activity of PCL electrospun fiber mat. Up to 3.60% of Ag NPs can be adsorbed on the PCL/SEP fiber surface through hydrogen-bonding interaction between the amide groups of SEP and the carboxylic acid groups capped on the surfaces of Ag NPs under acidic conditions (pH 3–5). The antibacterial rates of Ag NP–decorated PCL/SEP fiber mats can be over 99% against both

gram-negative *E. coli* and gram-positive *B. subtilis*, which is an important requirement for applications in the biomedical field. Collagen can also assist adsorption of Ag NPs on a PCL fiber mat, demonstrating the usefulness of a protein in the preparation of antibacterial electospun mats.

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REFERENCES

1. Ahmed, F. E.; Lalia, B. S.; Hashaikeh, R. *Desalination* **2015**, *356*, 15.
2. Hu, X. L.; Liu, S.; Zhou, G. Y.; Huang, Y. B.; Xie, Z. G.; Jing, X. B. *J. Control. Release* **2014**, *185*, 12.
3. Agarwal, S.; Greiner, A.; Wendorff, J. H. *Prog. Polym. Sci.* **2013**, *38*, 963.
4. Choi, J. S.; Kim, H. S.; Yoo, H. S. *Drug Delivery Transl. Res.* **2015**, *5*, 137.
5. Shi, X. M.; Zhou, W. P.; Ma, D. L.; Ma, Q.; Bridges, D.; Ma, Y.; Hu, A. M. *J. Nanomaterials* **2015**, 2015, DOI: 10:1155/app.140716.
6. Panthi, G.; Park, M.; Kim, H. Y.; Park, S. J. *J. Ind. Eng. Chem.* **2015**, *24*, 1.
7. Ignatova, M.; Manolova, N.; Rashkov, I. *Macromol. Biosci.* **2013**, *13*, 860.
8. Liu, Z. Y.; Yan, J. J.; Miao, Y. E.; Huang, Y. P.; Liu, T. X. *Composites, Part B* **2015**, *79*, 217.
9. Zhang, C. L.; Li, C. P.; Bai, J.; Wang, J. Z.; Li, H. Q. *Colloid Polym. Sci.* **2015**, *293*, 2525.
10. Toncheva, A.; Paneva, D.; Maximova, V.; Manolova, N.; Rashkov, I. *Eur. J. Pharm. Sci.* **2012**, *47*, 642.
11. Gao, Y.; Truong, Y. B.; Zhu, Y. G.; Kyratzis, I. L. *J. Appl. Polym. Sci.* **2014**, *131*, DOI: 10.1002/app.40797.
12. Jayakumar, R.; Prabaharan, M.; Shalumon, K. T.; Chennazhi, K. P.; Nair, S. V. *Adv. Polym. Sci.* **2012**, *246*, 263.
13. de Azeredo, H. M. C. *Trends Food Sci. Tech.* **2013**, *30*, 56.
14. Rai, M. K.; Deshmukh, S. D.; Ingle, A. P.; Gade, A. K. *J. Appl. Microbiol.* **2012**, *112*, 841.
15. Rai, M.; Yadav, A.; Gade, A. *Biotechnol. Adv.* **2009**, *27*, 76.
16. Guzman, M.; Dille, J.; Godet, S. *Nanomed. Nanotechnol.* **2012**, *8*, 37.
17. Liu, S.; Liu, S. G.; Liu, X. D.; Zhao, J. W.; Cui, W. G.; Fan, C. Y. *J. Appl. Polym. Sci.* **2013**, *129*, 3459.
18. Xu, X. Y.; Yang, Q. B.; Wang, Y. Z.; Yu, H. J.; Chen, X. S.; Jing, X. B. *Eur. Polym. J.* **2006**, *42*, 2081.
19. Lee, Y. J.; Lyoo, W. S. *J. Polym. Sci., Part B: Polym. Phys.* **2009**, *47*, 1916.
20. Jin, W. J.; Lee, H. K.; Jeong, E. H.; Park, W. H.; Youk, J. H. *Macromol. Rapid Commun.* **2005**, *26*, 1903.
21. Lee, H. K.; Jeong, E. H.; Baek, C. K.; Youk, J. H. *Mater. Lett.* **2005**, *59*, 2977.
22. Klionsky, D. J.; Abdalla, F. C.; Abeliovich, H. *Autophagy*, **2012**, *8*, 445.
23. Dong, H.; Wang, D.; Sun, G.; Hinestroza, J. P. *Chem. Mater.* **2008**, *20*, 6627.
24. Soo, P. L.; Luo, L.; Maysinger, D.; Eisenberg, A. *Langmuir* **2002**, *18*, 9996.
25. Nys, Y.; Gautron, J.; McKee, M. D.; Garcia-Ruiz, J. M.; Hincke, M. T. *World Poultry Sci. J.* **2001**, *57*, 401.
26. Yi, F.; Guo, Z. X.; Zhang, X. C.; Yu, J.; Li, Q. *Biomaterials* **2004**, *25*, 4591.
27. Yi, F.; Lu, J. W.; Guo, Z. X.; Yu, J. *J. Biomater. Sci., Polym. Ed.* **2006**, *17*, 1015.
28. Thomas, R.; Soumya, K. R.; Mathew, J.; Radhakrishnan, E. K. *Appl. Biochem. Biotech.* **2015**, *176*, 2213.
29. Duan, Y. Y.; Wang, S. H.; Jia, J.; Yan, W.; Jin, L.; Wang, Z. Y. *J. Appl. Polym. Sci.* **2007**, *106*, 1208.
30. Paneva, D.; Manolova, N.; Argirova, M.; Rashkov, I. *Int. J. Pharm.* **2011**, *416*, 346.
31. Jia, Y.; Huang, G.; Dong, F.; Liu, Q.; Nie, W. *Polym Compos.* **2015**, DOI: 10.1002/pc.23481.
32. Xiong, X.; Li, Q.; Li, J. W.; Guo, Z. X.; Sun, Z. H.; Yu, J. *J. Biomater. Sci., Polym. Ed.* **2012**, *23*, 1217.
33. Yi, F.; Yu, J.; Guo, Z. X.; Zhang, L. X.; Li, Q. *Macromol. Biosci.* **2003**, *3*, 234.
34. Zhang, X. C.; Xiong, X.; Yu, J.; Guo, Z. X. *Polymer* **2012**, *53*, 5190.
35. Lok, C. N.; Lok, C. M.; Chen, R.; He, Q. Y.; Yu, W. Y.; Sun, H. Z.; Tam, P. K. H.; Chiu, J. F.; Che, C. M. *J. Biol. Inorg. Chem.* **2007**, *12*, 527.
36. Lok, C. N.; Ho, C. M.; Chen, R.; Chen, Q. Y.; Yu, W. Y.; Sun, H. Z.; Sun, P. K. H.; Chiu, J. F.; Che, C. M. *J. Proteome Res.* **2006**, *5*, 916.
37. Han, B. G.; Guo, B.; Zhang, L. W.; Yang, B. S. *Adv. Mater.* **2006**, *18*, 1709.
38. Miles, A. A.; Misra, S. S.; Irwin, J. O. *J. Hygiene* **1938**, *38*, 732.
39. Pfeltz, R. F.; Schmidt, J. L.; Wilkinson, B. *Microb. Drug Resist.* **2001**, *7*, 289.
40. Kawahara, K.; Tsuruda, K.; Morishita, M.; Uchida, M. *Dent. Mater.* **2000**, *16*, 452.
41. Panta, B.; Panta, H. R.; Panta, D. R.; Panthi, G.; Nam, K. T.; Hong, S. T.; Kim, C. S.; Kim, H. Y. *Colloids Surf. A* **2012**, *395*, 94.
42. Wicken, A. J. In Bacterial Adhesion; Savage, D. C., Fletcher, M., Eds.; Plenum Press: New York, London, **1985**, pp 45–70.