Highly Sensitive Surface-Enhanced Raman Spectroscopy Substrates of Ag@PAN Electrospinning Nanofibrous Membranes for Direct Detection of Bacteria

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ABSTRACT: Surface-enhanced Raman spectroscopy (SERS) can be applied for biological detection because of its high sensitivity and noninvasiveness for analytes. Herein, we engineer plasmonic free-standing substrates composed of Ag nanoparticles (NPs) supported on polyacrylonitrile (PAN) electrospinning nanofibrous felts as sensors for bacterial detection. Ag NPs are evenly distributed on PAN nanofibers after preimpregnation and impregnation of PAN nanofibers in Tollens’ reagent. The size and loading density of Ag NPs are tunable by adjusting the reaction time of glucose and Tollens’ reagent, thereby allowing the tuning of the surface plasmon resonance. Using 4-mercaptoophenol (4-MPh) and 4-mercaptobenzoic acid (4-MBA) as probe molecules, SERS effects of Ag@PAN composite nanofibers are investigated, and the substrates allow the detection of 4-MPh and 4-MBA at a low concentration of $10^{-9}$ mol/L. Importantly, the substrates exhibit a high sensitivity of SERS performance for bacterial identification without a specific bacteria–aptamer conjugation. The SERS substrates also show good uniformity of SERS response for bacterial organelles. Furthermore, the antimicrobial property was evaluated, and the results indicate that the sample of Ag@PAN nanofiber mats possesses excellent antibacterial properties against Escherichia coli and Staphylococcus aureus.

INTRODUCTION

Bacteria are liable to cause infectious diseases to animals and human beings because of their various species, widespread routes, and variability for medical resistance. Thus, it is a particularly important task to detect and effectively inhibit pathogenic bacteria. Surface-enhanced Raman scattering (SERS) has been intensively applied in fields of environmental monitoring, bio-diagnostics, bioinstrumentation, and chemical analysis because of its high sensitivity, nondestruction, high accuracy, repeatability, and rapid signal export. Interestingly, SERS signals can express abundant spectral information on lipids, proteins, and bacteria. Generally, the common SERS substrates are restricted to noble metals, especially Au and Ag, owing to their high enhancement. Meanwhile, a Ag nanomaterial is a class of typical inorganic antibacterial agents, exhibiting wide-spectra, and durable and efficient antibacterial properties against bacteria and fungi.

However, to obtain ultrasensitive detection, the substrates should possess SERS “hot spots” to dramatically enhance signals and large loading rates to adsorb target molecules. Thus, various SERS substrates have been present, including nanospheres, polymer nanofibers, inorganic nanofibers, and nano-arrays. Among them, three-dimensional (3D) hierarchical nanostructures consisting of nanofibers can offer not only large specific surface area but also high density of “hot spots.” Similarly, to overcome the agglomeration tendency of Ag nanoparticles (NPs), a common and effective method is employed by using substrate materials to support NPs. Coincidentally, one-dimensional (1D) [nanowires (NWs) and nanofibers] and 3D hierarchical nanostructures are well suited as supporting materials for antimicrobials because of their high porosity, breathability, and high-loading density. Electrospinning technology, as a convenient and versatile strategy, can be qualified for fabrication of polymers and inorganic (TiO$_2$, SiO$_2$, etc.) and composite nanofibers.

In our previous work, we reported polymer nanofibers incorporated with Ag NPs or Ag NWs and verified the antibacterial and SERS activities for target molecules of 4-mercaptophenol (4-MPh). Subsequently, in order to achieve multifunctional applications of the nanofibrous membrane, we fabricated TiO$_2$ electrospinning substrates loaded with Ag NPs and provided not only a possibility for SERS detection of bacteria but also excellent antibacterial activities. However, after hydrolyzation and calcination of the precursor tetrabutyl titanate, the TiO$_2$ nanofiber membranes show obvious brittleness, which greatly reduced the advantages (free-standing and flexibility) of the nanofibrous membranes.
fabricated via electrospinning. Herein, we successfully obtained polymer/metal composite nanofibrous mats of Ag NPs directly decorated on the polycrylonitrile (PAN) electrospinning nanofiber surface, with a tunable size and loading density, by the preimpregnation and impregnation methods. First, PAN nanofibrous mats were fabricated via an electrospinning method. Afterward, PAN nanofelts were preimpregnated in AgNO₃ solution to adsorb silver ions on the PAN surface. Then, Ag NPs were grown on the nano fiber surface through the reaction of glucose with Tollens’ reagent, and the loading density was tuned by controlling the immersion time. The morphology and structures of Ag@PAN electrospun nanofibrous felt were investigated by field emission scanning electron microscopy, transmission electron microscopy (TEM), X-ray powder diffraction (XRD), and X-ray photoelectron spectroscopy (XPS). SERS activities were evaluated by the SERS signal of target molecules using 4-MPh and 4-mercaptobenzoic acid (4-MBA). SERS detection of bacteria was performed on Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) by using the dropping method and soaking method. The antibacterial capability was tested by the turbidimetric method, inhibition zone, and observation of the bacteria morphology. Based on the above characterization and detection, we demonstrate that the as-prepared Ag@PAN electrospinning nanofibrous substrates not only show excellent antibacterial properties but also possess high SERS sensitivity, as well as realize direct SERS identification for bacteria without prior aptamer binding.

RESULTS AND DISCUSSION

Characterization of Ag@PAN Nanofibrous Mats. To functionalize PAN nanofibers with Ag NPs, a two-step procedure was carried out. First, PAN nanomembranes were saturated and adsorbed with Ag⁺ ions in AgNO₃ solution for 12 h so that the absorbed Ag⁺ ions on PAN nanofibrous were employed as seeds for Ag crystalline growth. Then, Ag NPs were obtained by the reduction of Tollens’ reagent with glucose. The diameter variations and alternative surface of PAN nanofibers can be clearly seen from SEM images. Figure 1 illustrates the SEM image of neat PAN (a) and Ag@PAN nanofibers with different deposition time: (b) 5, (c) 15, and (d) 30 min. Smooth and bead-free neat PAN fibers can be observed in Figure 1a. It is found that the diameter distribution of PAN nanofibers is narrow, and the mean diameter is 299 ± 25 nm. After functionalization for electrospinning nanofibers, the surface of PAN nanofibers is no longer smooth but decorated with spherical particles, indicating that Ag NPs are loaded onto the nanofiber surface. Furthermore, it is observed that Ag NPs are distributed along PAN nanofibers without obvious splitting away off the fiber surface, which implies that Ag NPs are closely adsorbed on PAN nanofibers through the above-mentioned seed-growth two-step process. By comparison, Ag NPs can hardly be deposited on PAN nanofibers without the preimpregnation procedure (the TEM data are not shown in this paper). It is worth noting that PAN nanofibers are slowly wrapped by Ag NP-coating layers with the deposition time. After 5 min deposition, Ag NPs sporadically appear along the PAN fibrous substrates, as shown in Figure 1b. With further increasing deposition time to 15 and 30 min, it shows that many Ag NPs are attached on PAN nanofibers. It is obviously seen that when the deposited time reaches 15 min, the adsorbed Ag NPs are in a large loading amount and uniform size. Furthermore, the distance between Ag NPs is even and in nanometer scale, which would help to generate SERS “hot spots,” thus would favor for SERS detection. However, when the deposition time reaches 30 min, the particle size of Ag NPs becomes so large that Ag NPs aggregate and the diameter of fibers increases significantly. To reveal the change in the size of Ag NPs with deposition time, statistical analysis was applied by using software of Scion Image (the result is shown in Figure S1). By comparison, deposition time has a significant effect on the loading amount and the size of Ag NPs. The average diameter of Ag NPs becomes larger with the increasing deposition time, resulting in the whole diameter of Ag@PAN composite nanofibers reaching 546 nm, as shown in Figure 1d.

Besides SEM observation, TEM and high-resolution TEM (HRTEM) characterization were also applied to reveal the morphology of the as-prepared nanofibers in a more microscopic scale. Figure 2 shows TEM images of neat PAN (a) and Ag@PAN nanofibers with different deposition time: (b) 5, (c) 15, and (d) 30 min; the inset in Figure 2c is a...
HRTEM image of adsorbed Ag NPs. It is seen from Figure 2a that neat PAN nanofibers are homogeneous solid, smooth-faced, and semitransparent in appearance. What’s more, though PAN felts are preimpregnated in AgNO3 solution for 12 h, it seems that the morphology of PAN nanofibers is not being affected. However, as shown in Figure 2b, when the deposition time is 5 min, scattered NPs are emerging on the PAN nanofiber surface, which shows a significant difference in the transparency because of diverse electron transmittance. It is seen from Figure 2c, when deposition time is increased to 15 min, the particle size is slightly enlarged, and the loading amount is increased dramatically. Whereas, Ag NPs absorbed on PAN nanofibers are homogeneous without obvious aggregation. Compared with the SEM image of the same sample, it seems that the density of Ag NPs shown in TEM is not as high as that in SEM, supposedly owing to the difference in magnification times and the image-forming principle of two characterization methods. From the representative HRTEM image of the attached particles (the inset shown in Figure 2c), the distinct lattice fringes can be distinguished. The lattice spacing is measured to be 2.36 Å, which can be indexed to the (111) planes of Ag crystalline. As shown in Figure 2d, when the deposition time is further increased to 30 min, there are more Ag NPs adsorbed on the surface of PAN nanofibers, compared with the sample of Ag@PAN-15. However, the particle size is dramatically increased, and the distance between Ag NPs is further reduced, some aggregates are even emerged.

Combined with the above SEM and TEM observation for Ag NPs and Ag@PAN nanofibers, it is found that both the loading amount and the Ag NP size are increased with the prolongation of deposition time. However, it is supposed that the sample of Ag@PAN-15 would exhibit more pronounced activities in SERS detection and antibacterial properties due to the relatively appropriate particle size, uniform particle size distribution, and suitable gap scale between Ag NPs.

The crystal structural characterization for Ag@PAN nanofibrous mats was investigated by means of XRD analysis. Figure 3a illustrates XRD patterns of PAN and Ag@PAN nanofibers with different deposition time (5, 15, and 30 min) as SERS substrates, and SERS spectra of probe molecules [4-MPh (a) and 4-MBA (c)] with different concentrations from $10^{-3}$ to $10^{-9}$ mol/L recorded on the surface of Ag@PAN-15 nanofibrous felts.

Figure 4. SERS spectra of probe molecules [4-MPh (a) and 4-MBA (c)] with the concentration at $10^{-1}$ mol/L using Ag@PAN nanofibrous felts with different deposition time (5, 15, and 30 min) as SERS substrates, and SERS spectra of probe molecules [4-MPh (b) and 4-MBA (d)] with different concentrations from $10^{-3}$ to $10^{-9}$ mol/L recorded on the surface of Ag@PAN-15 nanofibrous felts.
and (200) lattice planes. It may be due to that the particle size distribution, loading density, and crystallinity of Ag NPs are optimal for the composite nanofibers with deposition time of 15 min.

To understand the chemical states and surface chemical compositions of the as-prepared Ag@PAN composite nanofibrous mats, the full and Ag 3d XPS spectra were measured, as shown in Figure 3b,c. It is observed that the peaks of C, N, and Ag are detected in the full XPS spectrum, which is consistent with the chemical composition of the composite nanofibers. For Ag@PAN composite nanofibers, the peak at 284.6 eV related to C (1s) and the peak at 398.4 eV related to N (1s) are observed, indicating the existence of PAN. Moreover, Figure 3c shows that the binding energy of Ag 3d_{5/2} and Ag 3d_{3/2} is located at 368.2 and 374.2 eV, respectively. Furthermore, the splitting energy of the Ag 3d doublet, as shown in Figure 3c, is 6.0 eV, which verifies that composite nanofibers contain metallic Ag (Ag^0) on the PAN surface but do not contain Ag^+.

**SERS Performance for Probe Molecules.** To evaluate the SERS signal enhancement by free-standing Ag@PAN nanofibrous felts, 4-MBA and 4-MPh were used as probe molecules. Figure 4a shows SERS spectra of 4-MPh (10^{-1} mol/L) using Ag@PAN nanofibrous felts with different deposition time (5, 15, and 30 min) as SERS substrates. As shown in Figure 4a, no Raman signals are detected for 4-MPh deposition time (5, 15, and 30 min) as SERS substrates. As without SERS substrates and with neat PAN nanofibers. In a similar manner, it can also be seen that the prominent peaks of 4-MPh are centered at 523, 983, 1079, 1108, 1170, 1492, and 1598 cm^{-1} as an example, the SERS signal intensity of Ag@PAN-15 is about 6.5 and 1.3 times, compared with that of Ag@PAN-30 and Ag@PAN-5, respectively. This result is related with the variation in morphologies and structures of different samples, as the above-mentioned analysis. Thus, Ag@PAN-15 nanofibrous felts were applied as SERS substrates for further study on the sensitivity. Figure 4b shows SERS spectra of 4-MPh (10^{-3} to 10^{-9} mol/L) recorded on the Ag@PAN-15 nanofibers mats. It can be seen that overall SERS peaks are acquired for all selected concentrations of 4-MPh. The intensity of SERS peaks is decreased with the decreasing concentration of probe molecules. However, explicit SERS signals still can be detected even when the concentrations reduced to 10^{-9} mol/L, suggesting superb SERS sensitivity of Ag@PAN nanofibrous. The sample of Ag@PAN-15 shows explicit SERS signals at concentrations of 4-MPh up to 10^{-9} mol/L. This detection limit is comparative with other composite electrospinning nanofibers, including our previous work of Ag@TiO_2 nanofibrous membranes. Furthermore, the matrix electrospinning nanofibers of PNA do not show background noise in the SERS spectra, which is superior to that of TiO_2.
respectively. Other SERS signal assignments are shown vibrational modes, including 733 cm$^{-1}$ of E. coli bacteria by using the as-prepared Ag@PAN nanofibers. It demonstrated the reliability of qualitative analysis for study are highly consistent with the references reporting. Thus, the distribution of Ag NPs on the PAN nanofibers is more intensive than that of Ag@PAN-5 and Ag@PAN-30, which implies that this sample is the most appropriate for bacterial SERS detection in the sense of sensitivity. It is because of the relative high-loading density and appropriate for bacterial SERS detection in the sense of performance, two approaches for immobilization of bacterial cells on SERS substrates (i.e., dropping and soaking) were both applied in this study. Figure S5 shows SERS spectra of E. coli and S. aureus treated with immersing Ag@PAN nanofelts in the bacteria suspension for 2 h and leached with distilled water. Compared with Figure 5a, b, no obvious changes in SERS peak positions for both E. coli and S. aureus are found, but the peak intensity ratios are enlarged. In other words, compared with the most intensive peaks at 658 and 733 cm$^{-1}$ of E. coli and S. aureus, the bands of other vibration of molecule groups are enlarged. The reason may be due to the sufficient interaction between bacteria and Ag@PAN nanofelts through the immersion method. Furthermore, compared with our previous work of Ag@TiO$_2$ nanofibrous felts as SERS substrates, the present study exhibits higher sensitivity, in addition to a clean signal background and flexible matrix materials, which shows that it has more advantage in the application for bacteria detection.

For a qualified SERS substrate, except for sensitivity, the homogeneity is another important demanding characteristic. To evaluate the homogeneity of Ag@PAN nanofibrous felts as SERS substrates for bacteria detection, 16 different sites on Ag@PAN-15 membranes were randomly selected, and the corresponding SERS spectra were recorded, as shown in Figure 6. It is observed from Figure 6a that for various SERS spectra, the peak positions and relative intensity are basically consistent, which suggests a high uniformity of the as-fabricated SERS substrates. A similar finding for bacteria of S. aureus is obtained, as shown in Figure 6b. Furthermore, statistical analysis was performed based on the most intensive SERS peak at 658 cm$^{-1}$ for E. coli and at 733 cm$^{-1}$ for S. aureus. The average intensity (integral area found by software of Origin) and relative standard deviation (RSD) are shown in Figure S2. Figure S2 exhibits that RSD of SERS peak intensity is 7.1% for E. coli and 8.5% for S. aureus. The same result is observed as compared with the other work. Thus, it can be concluded that the as-fabricated Ag@PAN electrospinning nanofibrous SERS substrates possess high homogeneity. One non-negligible reason is that whole felts substrates are composed of numerous random laid nanofibers, resulting in a very uniform distribution of Ag NPs. It is an additional significant advantage of electrospinning nanofibrous felts, except for free-standing and flexibility.

**Antibacterial Activities of Ag@PAN Nanofibrous Felts.** The antimicrobial properties of neat PAN electro-
spinning nanofibers and Ag@PAN nanofibrous felts were evaluated by the turbidity method, inhibition zone method, and bacteria morphology SEM observation. Figure 7a shows the UV−vis absorption spectrum of bacteria treated with PAN and Ag@PAN nanofibers by the turbidity method against E. coli and S. aureus. For the turbidity method, absorbance of the band centered at 600 nm was collected, which reflects the turbidity of the bacteria suspension. The weaker the absorbance, the lower concentration of surviving bacteria, indicating an obvious antimicrobial effect. As shown in Figure 7a, after addition of Ag@PAN nanofibrous felts into the bacteria suspension, a significant decrease of absorbance is observed for both E. coli and S. aureus, compared with the suspension treated with PAN nanofibers. It indicates that Ag NPs in composite nanofibers exhibit their intrinsic antibacterial activities. Figure 7a also illustrates that Ag@PAN-15 nanofibrous felts manifested the highest antimicrobial effect in the three samples, which is due to the relatively small particle size and abundant deposition density of adsorbed Ag NPs.

Figure 7b,c shows photographs of the inhibition zone against E. coli and S. aureus, where the circles of 1-4 are corresponding to neat PAN, Ag@PAN-5, Ag@PAN-15, and Ag@PAN-30 nanofibrous felts. Herein, the neat PAN nanofibrous mats are tested as the control. Whereas, pellucid areas around Ag@PAN nanofibers mats are clearly seen in agar plates (marked with red circles), which indicates that the bacteria growth are inhibited due to the antibacterial effects from the nanofibers. This area is defined as the inhibition zone, and its diameter is determined by the bacteriostasis of samples. As shown in Figure 7b,c, no obvious inhibition zone is observed for PAN nanofibrous mats. On the other hand, clear inhibition zones are detected around Ag@PAN nanofibrous felts, which indicates the antibacterial effects visually. Overall, the larger inhibition zones of Ag@PAN-15 are observed than that of Ag@PAN-5 and Ag@PAN-30 against E. coli and S. aureus, which is consistent with the result of the turbidity test. Furthermore, the detailed difference values (D-value) of the outer ring and inner ring for inhibition zones are shown in Figure S3. It is clear that Ag@PAN-15 exhibits more significant bacteriostasis compared with the other two samples. Meanwhile, the strain of S. aureus is more susceptible to the inhibiting effect of Ag@PAN nanofibrous mats than E. coli.

To further explore the antibacterial mechanism of Ag@PAN nanofibers against bacteria, microstructure observation was applied to reflect the change in the bacteria morphology. In this method, glutaraldehyde (GA) was used as a capturing agent for both Gram-positive and Gram-negative bacteria because of its excellent cross-linking effects on the surface of bacteria cell walls. Figure 8 shows SEM images of bacteria (E. coli and S. aureus) without treatment (a,c) and treated with Ag@PAN-15 nanofibrous felts (b,d). Moreover, the inserted red arrows in the figure point to cells which are severely damaged. Figure 8a shows that the normal E. coli cells exhibit the plump and uniform short rod morphology. However, it is observed from Figure 8b that wizened, shaggy, and fragmentized cells are emerged in shape, implying that the cells have been killed to death absolutely. Similarly, comparing Figure 8c with 8d, the normal S. aureus shows a smooth spherical or spheroidal morphology, whereas the bacteria cells are significantly deformed after treatment with Ag@PAN-15 nanofibrous felts. In addition, some particles with different contrasts are found to be adhered on the surface of cells, indicating the occurrence of interactions between the released Ag NPs and bacteria cells.

Based on the result of antibacterial tests and the observation of microstructures of bacteria, a possible antibacterial mechanism of Ag@PAN nanofibrous felts can be proposed. First, the contact action mechanism is easily inferred and has been testified by our previous work. In this process, bacteria would be adsorbed on the nanofiber surface and filled into nanofibrous felts gaps. Simultaneously, parts of Ag NPs are inclined to be stripped away from nanofibers, adsorbed,
and inserted into bacteria cells to destroy their normal physical activity. In addition, the possible antibacterial mechanism of Ag@PAN also involves superabundant reactive oxygen species and silver ion releasing. As far as Ag@PAN nanofibers in this study are concerned, whatever the mechanism is working, the high specific surface area and adsorptive property of electrospinning nanofibers provide a perfect carrier for Ag NPs sustained release, which are in favor of their durable antibacterial property.

### CONCLUSIONS

In the present study, we have developed a convenient and cost-effective electrospinning technique to fabricate Ag@PAN nanofibrous felts for bacteria detection. The morphology and structure of the as-prepared nanofibers were intensively characterized by techniques of SEM, TEM, HRTFM, XRD, and XPS. It is found that the even distributed Ag NPs along the matrix PAN fibers were obtained when the deposition time in Tollens’ reagent is 15 min. Probe molecules of 4-MPH and 4-MBA were selected to evaluate the SERS performance of Ag@PAN nanofibrous felts, and the substrate showed high sensitivity with a detection limit of $10^{-9}$ mol/L. More importantly, the free-standing Ag@PAN nanofibrous felts as SERS substrates can detect biomacromolecules such as bacteria of *E. coli* and *S. aureus* directly without previous bacteria–aptamer conjugation. Meanwhile, it exhibits remarkable homogeneity for SERS detection because of its homogeneous structure. The antibacterial activities of Ag@PAN nanofibrous felts were tested by the method of turbidity, inhibition zone, and morphology observation. The results show that the as-prepared Ag@PAN nanofibers possess excellent antibacterial properties, especially for the sample of Ag@PAN-15. Thus, combined with SERS detection and antimicrobial properties, the as-prepared Ag@PAN nanofibrous mats have brilliant potential applications in fields such as water ultrapurification and trace detection for microorganism.

### EXPERIMENTAL SECTION

**Materials.** PAN (average $M_w = 150,000$) was purchased from Macklin Biochemical Co., Ltd (Shanghai, China). Potassium hydroxide (KOH), N,N-dimethylformamide (DMF), $\alpha$-glucose (99%), and ammonia solution (NH$_3$, H$_2$O) were supplied by Tianjin Kermel Chemical Reagent Co., Ltd. (Tianjin, China). Silver nitrate (AgNO$_3$, 99.8%) was obtained from Beijing Sinopharm Chemical Reagent Co., Ltd (Beijing, China). 4-MPH and 4-MBA were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). All chemicals were of analytical grade (AR) and used as received without further treatment. In addition, both bacteria strains (*E. coli* (ATCC 25922) and *S. aureus* (ATCC 6538)) were acquired from Guangdong HuanKai Microbiology Science & Technology Co., Ltd. (Guangzhou, China). Agar medium and nutrient broth were chosen as the bacteria biology Science & Technology Co., Ltd. (Guangzhou, China).

**Preparation of PAN Electrospinning Nanofibrous Felts.** The electrospinning solution (14 wt % PAN in DMF) was moderately stirred at the ambient temperature for 24 h to obtain pale yellow transparent solutions. Before the spinning, the solution was extracted into a 10 mL disposable plastic syringe with an 18-gauge stainless-steel needle. The high-voltage electric field between the collector and metal needle tip was 15 kV. The feeding rate of the polymer solution was 9 μL/min, and the distance of tip-to-collector was kept at 9 cm. Afterward, the electrospinning nanofibers were collected on a piece of grounded collector of aluminum foil. Finally, the as-prepared nanofibers were stripped from aluminum foil and dried in a vacuum drying oven for 48 h before the next treatment.

**Fabrication of Ag@PAN Electrospun Nanofibrous Felts.** In a typical procedure, first, the as-prepared PAN electrospinning nanofibrous felts were immersed in AgNO$_3$ (12 wt %) solution and shaken for 12 h to make Ag$^+$ being adsorbed on the PAN nanofibrous felts, which were served as silver formation seeds in the following reduction reaction. Then, the nanofibrous felts were washed with distilled water. Second, the Ag@PAN composite electrospinning nanofibrous felts were fabricated through Tollens’ reagent reduction reaction which was described in our previous work. The specific experimental process is briefly described as follows. First, the ammonia solution (15 mol/L) was added into AgNO$_3$ (0.05 mol/L) solution dropwise with vigorous stirring until the brown sedimentation appears. Subsequently, 5 mL KOH (0.80 mol/L) solution was dropped into the above system until the solution turns into clarification. Then, ammonia solution was added to the above solution until being colorless. To obtain PAN embedded with Ag NP composite nanofibers, PAN nanofibrous was first pretreated with AgNO$_3$ solution and then immersed in Tollens’ reagent for 2 min. Glucose (0.12 mol/L) was poured in the mixed solution. After several minutes, the membranes were taken out, washed with distilled water three times, and vacuum dried. The as-prepared Ag@PAN membranes were denoted as Ag@PAN−x, where x was the deposition time (min).

**SERS Measurement for Target Analytes.** Before the detection of bacteria, two targeted analytes with small molecule cation. Then, 5 mL KOH (0.80 mol/L) solution was dropped into the above system until the solution turns into clarification. Then, ammonia solution was added to the above solution until being colorless. To obtain PAN embedded with Ag NP composite nanofibers, PAN nanofibrous was first pretreated with AgNO$_3$ solution and then immersed in Tollens’ reagent for 2 min. Glucose (0.12 mol/L) was poured in the mixed solution. After several minutes, the membranes were taken out, washed with distilled water three times, and vacuum dried. The as-prepared Ag@PAN membranes were denoted as Ag@PAN−x, where x was the deposition time (min).

**SERS Detection of Bacteria.** Herein, two sample preparation methods were applied to detect bacteria: dropping and soaking. The process of dropping is described as follows. A
SERS band assignments for bacteria (PDF)

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**Notes**

The authors declare no competing financial interest.

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