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Research Article

A Biomedical Surface Enhanced Raman Scattering Substrate: Functionalized Three-Dimensional Porous Membrane Decorated with Silver Nanoparticles

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We fabricated a simple, cheap, and functional surface enhanced Raman scattering substrate for biomedical application. Hot spots between two close silver nanoparticles distributed in the skeleton of a three-dimensional porous membrane, especially in the pores, were formed. The dual poles of micropores in the membrane were discussed. The pores could protect the silver nanoparticles in the pores from being oxidized, which makes the membrane effective for a longer period of time. In addition, *Staphylococcus aureus* cells could be trapped by the micropores and then the Raman signal became stronger, indicating that the functional surface enhanced Raman scattering substrate is reliable.

1. Introduction

Surface enhanced Raman scattering (SERS) is widely used in biomedical sensing, chemical analysis, and environmental monitoring owing to its extremely high sensitivity. It is a powerful analytic tool in the detection of small organic molecule, such as deoxyribonucleic acid [1–3], proteins [4], tumor cells [5], bacteria [6, 7], and enzymes [8]. The electromagnetic resonance properties of a substrate, such as a roughened surface on gold nanoparticles and silver nanoparticles, are used for generating the SERS effect. However, achieving a reliable, stable, and uniform SERS signal substrate is challenging.

To fabricate silver nanoparticle arrays with high SERS activity and better uniformity, several groups have prepared periodic nanostructure arrays via lithography [9–12]. The electric field of incident light is usually enhanced in the interstitial regions or the gaps among the closely spaced nanostructures, which are known as "hot spots." Therefore, the improvement of the intensity and density of hot spots is important for designing and fabricating SERS substrates. The preparation of hot spots on a two- or three-dimensional surface is a good method for increasing the density of hot spots. Wang et al. have fabricated hierarchical silver nanocap-type array using a porous anodic aluminum oxide template, in which hot spots are engineered on a three-dimensional surface [13]. In addition, extremely high SERS activity was observed on the three-dimensional surface with the hot spots. Jung et al. have developed a chitosan-silver nanoparticle hybrid three-dimensional porous structure as a SERS substrate for biomedical applications [1]. However, the function of the pores in the membrane was not analyzed.

Here we attempted to develop a simple and functional surface enhanced Raman scattering substrate with hot spots distributed in the skeleton of a three-dimensional porous membrane, especially in the pores. Its potential use for biomedical application was evaluated.

2. Method

2.1. Preparation of Surface Enhanced Raman Scattering Substrate. Silver nitrate, glucose, and ammonium hydroxide were purchased from China Sinopharm Chemical Reagent Company Limited, and the filter membrane was purchased from Haining Zhenghao Filter Company Limited. The experimental steps are as follows: (1) cleaning the filter membrane: the membrane was soaked in 70°C deionized water for 2

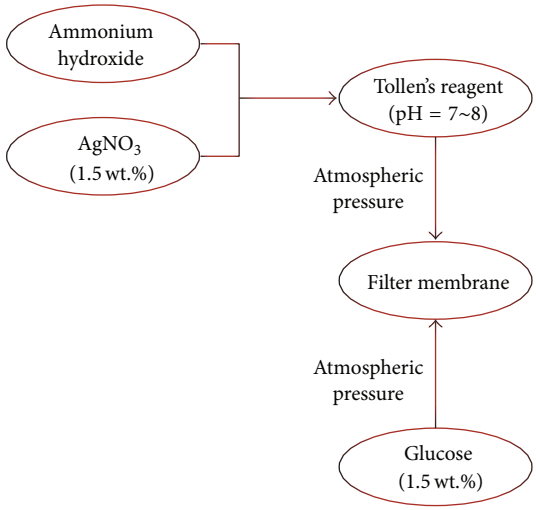


Figure 1: Schematic representation of the experiment.

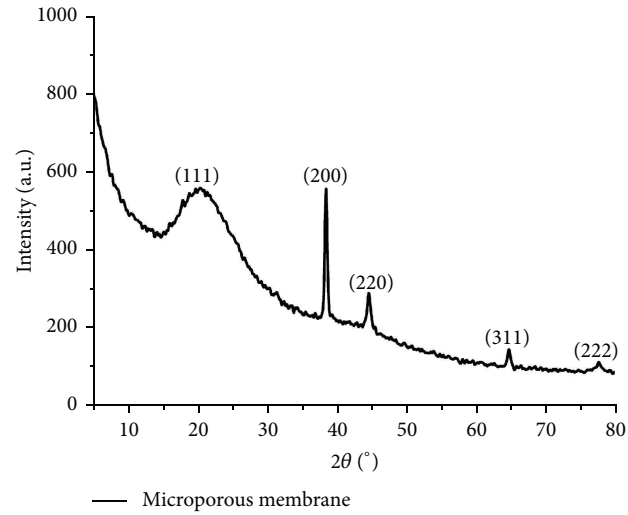


Figure 2: XRD pattern of microporous membrane decorated with silver nanoparticles.

hours after being cleaned 5 times and then dried in vacuo. (2) Preparation of Tollen's reagent: ammonium hydroxide was added to 20 mL $AgNO_3$ (1.5 wt%) until the mixed solution became transparent and pH = 7-8. The corresponding reaction equations are

$$2Ag^+ + 2OH^- \longrightarrow Ag_2O \downarrow + H_2O \quad (1)$$

$$Ag_2O + 4NH_3 + H_2O \longrightarrow 2\left[Ag\left(NH_3\right)_2\right]^+ + 2OH^- \quad (2)$$

$$NH_4{}^+ + OH^- \longrightarrow NH_3 \cdot H_2O \quad (3)$$

A membrane was placed on the filter. As shown in Figure 1, 1.0 mL of Tollen's reagent was added from the top of the filter, and 1.0 mL glucose (1.5 wt%) was added to the opposite side. For adequate reaction of the two solutions, atmospheric pressure (approximately 0.6 N/cm^2) was simultaneously introduced to the top and bottom of the membrane in the filter. Subsequently, the filter membrane was immersed in a 65°C water bath for 1 min. Finally, the membrane was washed with deionized water and dried in a vacuum oven at room temperature.

The reduction equation is

$$C_6H_{12}O_6 + 2Ag\left(NH_3\right)_2 OH \longrightarrow C_5H_{11}O_5COONH_4 + 2Ag \downarrow + 3NH_3 + H_2O \quad (4)$$

2.2. Morphology of Surface Enhanced Raman Scattering Substrate and the Time of Its Effectiveness. Scanning electron microscopy (SEM, Hitachi S3400) was used to investigate the porous membrane decorated with silver nanoparticles. The SEM images were recorded in a 15 kV accelerating voltage. Then, we investigated the effective time of the decorated porous membrane using crystal violet (CV, from China Sinopharm Group Company Limited) as Raman probes. For comparison, 1.0 mL of Tollen's reagent and 1.0 mL of glucose (1.5 wt%) were added on a glass slide (without pores), which was heated to 65°C. Then, the glass substrate and porous membrane with the silver nanoparticles were exposed to air. Every 24 h, CV (1×10^{-5} M) was deposited on the two substrates (the glass substrate and porous membrane with silver nanoparticles), and the corresponding Raman signals were collected.

2.3. Surface Enhanced Raman Scattering Measurement. After the sample (25 μL CV) was deposited on the silver nanoparticles porous membrane, the membrane was dried in a vacuum oven at room temperature. For the biomedical application, the bacteria (*S. aureus* ATCC 27217, purchased from China Center of Industrial Culture Collection) were trapped in the pores of membrane to increase the contact area with the silver nanoparticles. First, a solution of *S. aureus* was cleaned five times with deionized water, and the porous membrane decorated with silver nanoparticles was divided into five pieces of equal size. Then, the parameter B_{in}, which is the ratio of *S. aureus* trapped inside the micropores, was introduced. Subsequently, the five pieces of the silver nanoparticles porous membrane were immersed in the solution of *S. aureus* with turbidity (2.7 MCF), and then, the mixtures were centrifuged at different rotation speeds (12000, 10000, 8000, 6000, and 4000 rmp) for 3 min. Different values of B_{in} were obtained due to the different rotation speeds. After being dried in a vacuum oven at room temperature, the membranes were examined by an Advantage Near Infrared Raman Spectrometer provided by DeltaNu Corporation. Here, the laser source for excitation was a 785 nm diode laser with 100 mW of power. The integration time was 5 s, and a resolution of 8 cm^{-1} was used. NuSpec software was used to analyze the spectra.

3. Results

3.1. Morphology of the SERS Substrate. Figure 2 shows the X-ray diffraction pattern of microporous membrane decorated with silver nanoparticles. It is shown that there are some

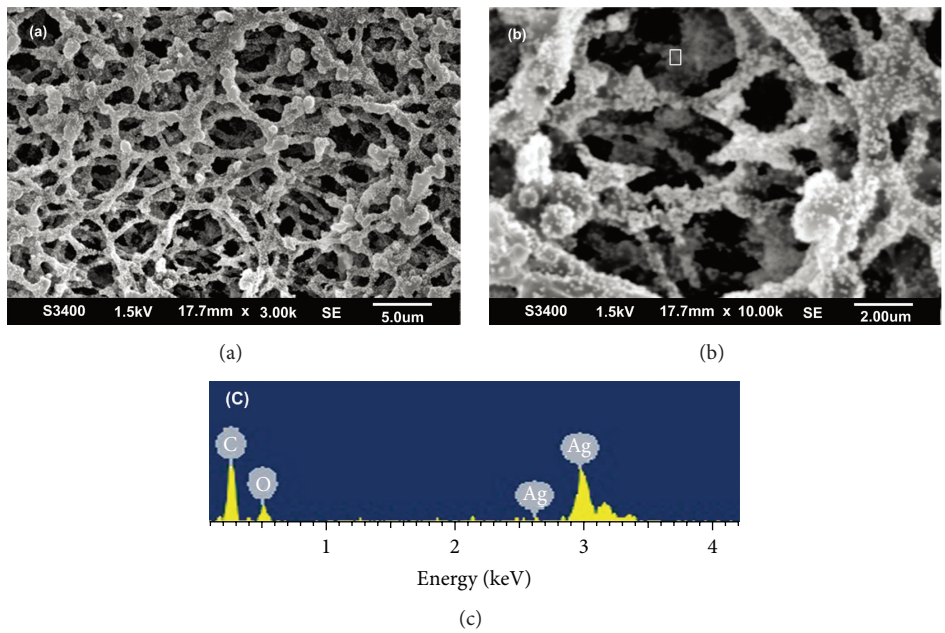


Figure 3: Representative scanning electron microscopy images of the membrane: (a) silver nanoparticles reduced membrane; (b) magnification of (a); (c) X-ray energy dispersive spectroscopy of the rectangle in (b).

amounts of silver nanoparticles here and there in the membrane.

The morphology of the SERS substrate was observed using SEM, as shown in Figure 3. Figures 3(a)-3(b), which show the SEM images of the decorated porous membrane under different magnifications, indicate that the silver nanoparticles have been immobilized in an isolated state in the membrane. X-ray energy dispersive spectroscopy (EDX) results (Figure 3(c)) indicate that the silver nanoparticles were uniformly distributed inside the pores.

Based on the porous structure, the silver nanoparticles were dispersed in the skeleton of the membrane and in the pores. The gaps between closely spaced silver nanoparticles, which are known as "hot spots," were densely dispersed on the membrane.

It is well known that the SERS effect of silver nanoparticles is outstanding without considering their stability. Therefore, it is important to prolong the effective usage time of silver nanoparticles. The micropores in the membrane allow for the protection of the silver nanoparticles from direct exposure to the air. The Raman spectra of CV (1×10^{-5} M) in the two substrates (i.e., silver nanoparticles on glass and silver nanoparticles in a porous membrane) were investigated daily. The Raman shift at 1165 cm^{-1} was chosen for the characteristic peaks of crystal violet. The Raman signal of it is shown in Table 1. For the silver-coated glass substrate, the Raman signal at 1165 cm^{-1} decreased to less than 500 after 10 days. However, this peak was still observed after 15 days for the silver-coated porous membrane, supporting that the effective usage time of the decorated porous membrane is longer than that of glass substrate. The comparison of the Raman spectra for the glass substrate and membrane on the fifth day is shown in Figure 4. Comparing with the glass substrate for Raman signal at 1165 cm^{-1}, the percentage coefficient of variation for the spectra is about 10.2%.

Table 1: The comparison of two substrates (microporous membrane and glass) for Raman signal at 1165 cm^{-1}.

Time (day)	Microporous membrane	Glass
1	5769	5760
2	5287	5211
3	5051	4664
4	4945	4558
5	4439	4027
6	4108	3982
7	3805	3343
8	3609	2484
9	3120	1280
10	2800	523
11	2510	<500
12	2325	<500
13	2008	<500
14	1385	<500
15	920	<500

Microporous membrane is the porous substrate coated with Ag nanoparticles.
Glass is the glass substrate coated with Ag nanoparticles.

The silver nanoparticles on glass substrate were more easily oxidized compared to those in the porous membrane. The pores of the membrane increased the specific surface areas of the substrate and simultaneously prevent AgNP from

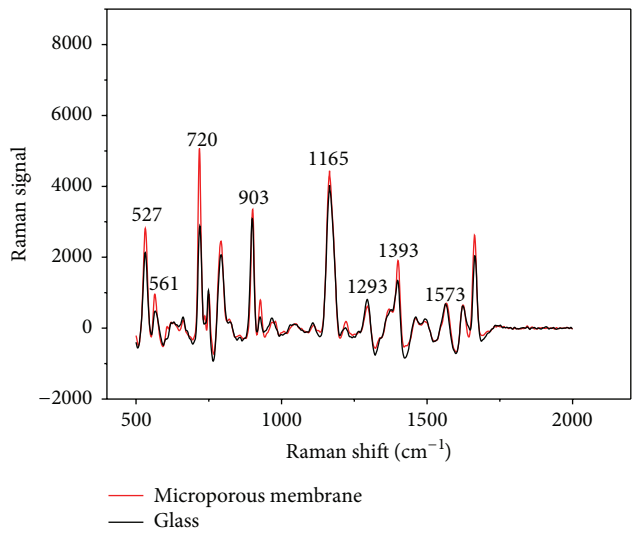
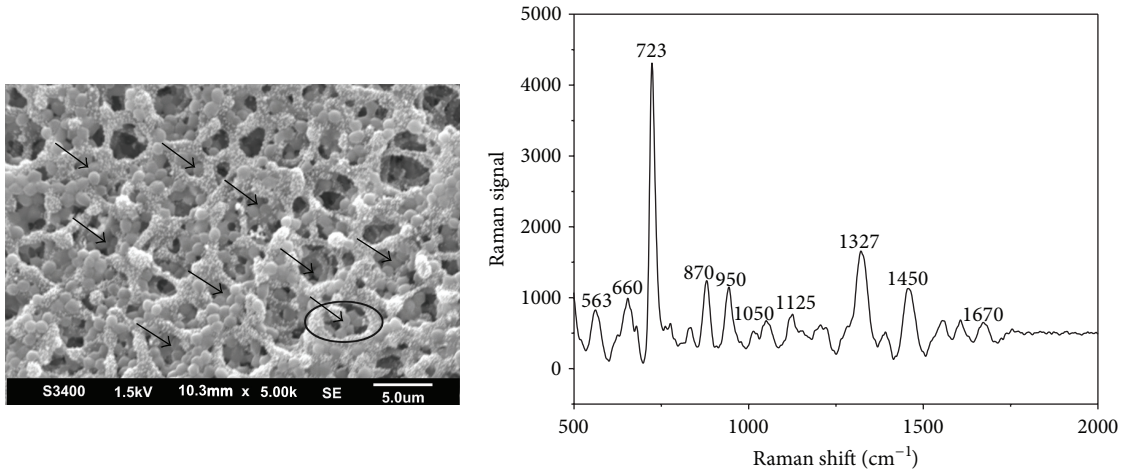


FIGURE 4: Surface enhanced Raman scattering spectra of 25 μL crystal violet molecules (1×10^{-5} M) on the silver nanoparticles coated porous substrate and glass substrate on the fifth day.



(a) (b)

FIGURE 5: (a) Representative scanning electron microscopy image of *Staphylococcus aureus* trapped in the porous silver nanoparticles membrane; (b) surface enhanced Raman scattering spectra of *Staphylococcus aureus* when $B_{in} = 0.683$ (B_{in} is the ratio of *Staphylococcus aureus* trapped inside the micropores).

being oxidized, which is the main reason for enhancing the Raman signal of the porous membrane.

3.2. Biomedical Applications. Another important effect of the pores was investigated. We determined the effect of the percentage of *S. aureus* in the micropores B_{in} on the Raman intensity. Herein, each value of B_{in} was obtained by an average of nearly 20 SEM images (approximately 971 bacteria in each piece of the silver nanoparticles membrane), and the results are shown in Figure 5 and Table 2.

When $B_{in} = 0.683$, many bacteria were imprisoned in the pores (Figure 5(a)). The corresponding SERS spectrum is shown in Figure 5(b).

The Raman peak at 723 cm^{-1}, which corresponds to the ring breathing of the adenine ring, was chosen as the characteristic peak for *S. aureus*. For different values of B_{in}, the Raman signal at 723 cm^{-1} was investigated in Table 2. The decorated microporous membrane can hold enough

TABLE 2: Raman signal of *Staphylococcus aureus* at 723 cm^{-1} in different values of B_{in} (B_{in} is the ratio of *Staphylococcus aureus* trapped inside the micropores).

B_{in}	Raman signal
0.683	4315
0.664	2464
0.642	1951
0.623	1763
0.605	1558

S. aureus cells to enhance the interaction between the bacteria and the silver nanoparticles or hot spots. Therefore, the Raman signal at 723 cm^{-1} increases as the number of bacteria trapped in the pores increases.

4. Discussion

SERS is an abnormal surface optical phenomenon resulting in strongly increased Raman signals for molecules adsorbed on nanostructured coinage metals. SERS has thus been an object of great interest in many areas of including chemical analysis, corrosion, lubrication, heterogeneous catalysis, biological sensors, and molecular electronics [3–6]. However, it is still a challenge in controlling the size and morphology of nanoparticles and their aggregates, the packing degree of assemblies, and the interparticle gap [10, 12]. Therefore, the fabrication of a reliable, stable, and uniform SERS signal substrate remains demanded until now.

In this paper, we present a rapid and simple method for the fabrication of a silver porous membrane, in which the silver nanoparticles are distributed in the membrane, especially in micropores. The nanoparticles outside the pores are oxidized more easily than those inside the pores. Therefore, the micropores can prevent silver nanoparticles from being oxidized resulting in a substrate that can be used many times due to its long-term effectiveness.

The hot spots, which are the gaps between two silver nanoparticles that are dispersed on the three-dimensional porous membrane, make it possible for biomedical application. The special property of the substrate is investigated in a biomedical application. The pores in the membrane can trap *Staphylococcus aureus* (*S. aureus*) cells, and the Raman signal becomes stronger as more *S. aureus* cells are trapped.

5. Conclusions

In summary, we proposed a simple and low-cost method for fabricating a porous membrane decorated with silver nanoparticles, which is applied to a biomedical application. Two functionalities of the pores in the membrane are investigated, including (i) preventing the oxidation of silver nanoparticles and (ii) trapping bacteria to enhance the Raman signal.

Disclosure

Li Yuan and Tian Xu are co-first authors.

Conflict of Interests

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

Acknowledgments

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