Effective Antibacterial Glass Fiber Membrane Prepared by Plasma-Enhanced Chemical Grafting

Min Guo,† Fanke Meng,*† Guoping Li, Jiyue Luo, Yiwen Ma, and Xue Xia

Shenzhen Angel Drinking Water Industrial Group Corporation, Angel Industrial Park, Baoan District, Shenzhen, Guangdong 518108, China

ABSTRACT: This paper reports a novel glass fiber membrane with an effective antibacterial performance by chemical grafting of quaternary ammonium salt (QAS) which is enhanced by a plasma bombardment technique. Plasma bombardment as a pretreatment of the membrane can increase the QAS anchored on the membrane from 0.8 to 1.3 wt %. The chemical grafting technique can increase the membrane zeta potential from negative values to positive values in aqueous solutions at various pHs. Furthermore, the plasma-enhanced chemical-grafting membrane has more positive zeta potentials (49.0 mV at pH = 7) than the chemical-grafting membrane without the plasma bombardment technique (38.9 mV at pH = 7). In the antibacterial performance evaluation, the Escherichia coli survival rate decreased from 127.0% of the pristine membrane to 4.1 and 11.3% of the plasma-enhanced chemical-grafting membrane and the chemical-grafting membrane, respectively. In addition, the plasma-enhanced chemical-grafting membrane shows durable antibacterial activity against E. coli with copious water rinsing as much as 3 L·cm⁻².

INTRODUCTION

In the past decades, many water purification techniques are investigated and applied to provide clean and safe drinking water, among which the antibacterial technique is one of the most studied methods. For example, heavy metal cations (Ag⁺, Cu²⁺, etc.) and ultraviolet (UV) are currently widely used for disinfection. The heavy metal cations can inhibit bacteria reproduction by damaging the cytomembrane. In fact, the antibacterial properties of silver and silver compounds have been used since antiquity. Silver cations can be adsorbed onto the negative bacterial cytomembrane due to the Coulombic force and then react with thiol groups (−SH) to destroy membrane proteins, leading to bacterial lysis. The UV sterilization is another scenario. With high photon energy, the UV light can kill the bacteria by breaking the double helix structure of DNA or inactivating proteins. However, the above two typical techniques have weaknesses apparently. First, although the heavy metal cations with a low content (ex. several ppb of Ag⁺) can effectively inhibit bacteria, the heavy metal cations in drinking water are also toxic to human cells. Second, although the UV sterilization is quick and efficient, special protections should be equipped to avoid UV leakage. In addition, the bacterial dwellings inside water cleaning facilities, for example, activated carbon and filtration membranes cannot be disinfected because of UV blockage by the materials.

Because the benzalkonium chloride showed a performance of skin disinfection, the antibacterial role of the cationic surfactants was discovered, among which the quaternary ammonium salt (QAS) was a typical member. As one of the QAS, the 3-(trimethoxysilyl)propyldimethyloctadecyl ammonium chloride (TPMMC) was used in this research and the molecular structure is shown in the Scheme 1. As the core cation of the QAS molecule, N⁺ is covalently bonded with two methyl groups, one octadecyl group and one 3-(trimethoxysilyl)propyl group, exhibiting a positive Coulombic
property. The antibacterial mechanism of the QAS is proposed as follows. First, due to negative charging of the bacterial cytomembrane, the bacteria can be adsorbed onto the QAS molecules driven by Coulombic interaction. Second, the cations pin the QAS molecules to the bacterial cytomembrane, and then the hydrophobic and lipophilic group of the QAS, as the octadecyl group in the TPMMC, can disrupt the cytomembrane and penetrate into the bacteria for releasing potassium ions and other cytoplasmic inclusions to lead cell death.\textsuperscript{1,18} Theoretically, a single QAS molecule can disrupt a bacterium. However, a number of QAS molecules anchored onto a substrate can function as a polycationic agent to improve the antibacterial performance for two reasons. First, the polycations facilitate the adsorption of the bacteria onto the QAS antibacterial agent because of a higher positive charge density of the agent than that of the monomeric cations.\textsuperscript{1,18} Second, the nonfixed small QAS molecules can raise health concerns.\textsuperscript{1,19} Usually, the QAS molecule has a jointing group on the N\textsuperscript{+} for binding the QAS molecule to the substrate. For example, the 3-(trimethoxysilyl)propyl group in the TPMMC work as a linkage to graft the QAS organic molecule to a polymer or inorganic substrate by forming O−Si covalent bonds. Therefore, the substrate with the TPMMC antibacterial agent can both strengthen Coulombic attraction to the bacteria and reduce the concern of dissolving small QAS molecules into drinking water. Currently, many techniques for grafting the QAS molecules onto a suitable substrate are intensively studied.\textsuperscript{2,9,13,18} For typical examples, surface activation by acid and alkaline is normally used to clean inorganic substrates and increase active sites by acid and alkaline etching;\textsuperscript{18} ozone surface activation is more apt to be used for polymer substrates because of the generation of the surface active groups by ozone oxidization.\textsuperscript{9} However, either of the two methods has an obvious weakness of environment pollution.

In this research, we used a glass fiber membrane to carry QAS molecules (TPMMC) as a polycationic antibacterial agent, and the plasma bombardment technique as a pretreatment to enhance the chemical grafting between the glass fiber membrane and the QAS antibacterial agent. The glass fiber membrane has many merits in the water purification domain than other materials, such as excellent chemical and physical stability, and low material cost. Compared with other QAS-grafting methods, this plasma-enhanced chemical-grafting (PCG) technique is more environmentally friendly, for example, no acid or alkaline usage, or no ozone releasing, and more easy for operation. In addition, the PCG technique can be used to activate both inorganic and polymer substrates, indicating broader applications than other QAS-grafting methods. As far as we know, it is the first time to use the PCG technique to improve antibacterial performance of the glass fiber membrane. The antibacterial experiments clearly showed that \textit{Escherichia coli} had a lower survival rate by contacting to the membrane with plasma bombardment pretreatment than that with contacting to the membrane without plasma bombardment pretreatment. Therefore, this PCG technique paves a novel way of chemically grafting QAS for surface modification of a substrate to improve antibacterial performance, which can not only be used in water treatment, but also expanded to a whole antibacterial industry.

\textbf{RESULTS AND DISCUSSION}

Scheme 1 shows a brief process of PCG technique. The air plasma broke surface Si−O−Si bonds of the glass fiber to form dangling silanol groups (Si−OH) with moisture (the room relative humidity was around 60%), which was a surface activation process of increasing active Si−OH for chemically grafting QAS.\textsuperscript{7} The TPMMC was hydrolyzed in an ethanol aqueous solution to form Si−OH at 30 °C for 4 h. The Si−OH groups in the TPMMC and dangling on the glass fiber membrane were dehydrated and formed siloxane groups (Si−O−Si), building up a linkage between the TPMMC and the membrane.\textsuperscript{14,16} As shown in the Figure 1, the PM, plasma-bombarded membrane (PBM), chemical grafting membrane (CGM), and PCG demonstrated fibrous structures with an axial dimension larger than 100 μm and a radial dimension less than 5 μm. The pore formed between the fibers was around 1−5 μm. This fibrous structure was beneficial for bacteria filtration, which improved the contact to the surface-anchored antibacterial agent. Both the PM and PBM in Figure 1a,b showed clear fiber boundaries, which however, was indistinct on CGM and PCGM in Figure 1c,d. In Figure 1d, some fibers were sticky to each other, as shown in the red-circled areas. The glue-like areas was due to anchored TPMMC.

Thermal gravimetric analysis (TGA) was used to unravel thermal durability and QAS content. The TGA plots of the four membranes were shown in the Figure 2. From ambient temperatures to 150 °C, all the four samples showed a weight loss of less than 5%.
loss about 2.5 wt %, which was attributed to the moisture adsorbed on the fibers. An extra weight loss of about 1.0% from 150 to 380 °C was likely because of the vaporization of acrylic acid as a small molecule binder. Most of the weight loss was occurred from 380 to 450 °C, which was a mixed process including thermal decomposition of the binder and TPMMC, small molecules vaporization, and so forth. Then, polymerization occurred mostly between 450 and 650 °C, which resulted from plasma etching. While, the weight loss of the samples became almost flat from 650 to 800 °C because of the eventual mineralization. By comparison between the PM and PBM without chemical grafting of TPMMC, it is clear that the plasma bombardment led to a weight loss of 0.6 wt % (PM and PBM plots indicating sample weights of 90.2 and 90.8 wt %, respectively, at 650 °C), which resulted from plasma etching. While, the CGM and PCGM retained less weights of 89.4 and 89.5 wt %, respectively, owning to the weight loss of the surface-grafted antibacterial agent. Clearly, the weight difference between the PM and CGM resulted from the antibacterial agent, namely, the TPMMC grafted onto the PM was 0.8 wt %. Identically, the TPMMC grafted onto the PBM was 1.3 wt %. The 0.5 wt % more TPMMC grafted onto the PCGM than that onto the CGM was probably because the plasma bombardment increased active dangling Si–OH groups on the glass fiber to anchor more TPMMC by hydrolysis.

The Fourier transform infrared (FTIR) spectra were shown in Figure 3 to analyze typical functional groups of the membranes. Because all the four membranes contained an acrylic acid binder, the C=O stretching peaks were shown at 1727 cm⁻¹ in the four FTIR plots. Identically, the glass fiber substrate containing silicon oxides as the main ingredient, therefore, two strong peaks at 458 and 1008 cm⁻¹ were attributed to Si–O–Si bending vibration and Si–O–Si asymmetric vibration respectively. The peaks at 2850 and 2920 cm⁻¹ corresponded to the C–H stretching, and the peaks at 1452 and 1490 cm⁻¹ were related to the C–H bending. The C–H groups of PM and PBM were resulted from an acrylic acid binder, while for the CGM and PCGM, the groups were resulted from both the binder and the TPMMC. While, as grafted onto the glass fiber, the TPMMC only existed in the samples of CGM and PCGM. Therefore, only the two membranes demonstrated two fingerprint peaks of the C–N deformation at 1564 and 1534 cm⁻¹, respectively, in the inset of Figure 3 with detailed plots from 1520 to 1580 cm⁻¹.

The element composition and chemical status of the nitrogen were analyzed by X-ray photoelectron spectroscopy (XPS) in Figures 4 and 5. In Figure 4, all the four materials contained Si 2s, Si 2p, O 1s, and C 1s peaks, indicating silicon oxides and polymer binder in the substrate glass fibers. The detailed scanning of N 1s was shown in Figure 5. Nitrogen was not contained in the PM because of no chemical grafting of TPMMC or air plasma bombardment pretreatment. With XPS peaks deconvolution, the N 1s peak of the PCGM was divided into two peaks at 399.7 and 402.3 eV, which indicated two different types of N status. The peak at 399.7 eV was due to interstitial N doping into the glass fiber substrate without forming either N–O or N–Si bonds. In the plasma bombardment, the N in air was ionized and doped into the glass fiber, therefore, both the PCGM and PBM with the air plasma bombardment showed interstitial N 1s peaks resulting from the N dopant, which however, were not shown in either CGM or PM. It should be noted that the interstitial N 1s peak of the PCGM was slightly stronger than that of the PBM, which might be probably because the TPMMC that was covered on the surface of the PCGM partially, hindered some Al Kα characteristic X-ray photons penetrating into the substrate to excite electrons in the N 1s orbitals because the X-ray photons can only affect the surface materials in a depth of about 2 nm. The peaks at around 402.3 eV only existed in the PCGM and CGM, which was a typical N 1s peak position of the QAS. Remarkably, the larger TPMMC N 1s peak area of the PCGM than that of the CGM was because of more
TPMMC grafted on the PCGM, which was also proved in the TGA results.

In Figure 6, the surface zeta potentials of the PM, PBM, CGM, and PCGM in a pH range from around 4–10 were obtained. Without chemical grafting of TPMMC on the surface of the membrane, the zeta potentials (−50 to −60 mV) of the PM and PBM were similar to each other in the test pH range. While grafted with TPMMC, the zeta potentials of the membranes increased significantly, indicating that N⁺ of the TPMMC can increase surface positive charge. It distinctly shows that the zeta potential of the PCGM was higher than that of the CGM in the whole pH test range, for a typical example, the zeta potential was 49.0 and 38.9 mV of the PCGM and CGM, respectively, at pH = 7. The higher zeta potential was derived from larger TPMMC molecules coverage of the PCGM than that of the CGM, which was consistent with both the TGA and XPS results.

Overall, according to the materials characterization data, it clearly demonstrates that with plasma bombardment, the membrane can carry more QAS molecules, leading to a higher positive charge density on the surface, which can definitely strengthen Coulombic attraction to the bacteria. Figure 7 showed survival rates of the E. coli with contact to the membranes for 20 min. Without membranes in the solution, the E. coli survival rate increased up to 163.7% in 20 min, indicating a fast rate of reproduction. With the PM and PBM in the solution, the mean survival rates of the E. coli were 127.0 and 121.9%, respectively. Although the survival rate of the E. coli decreased by comparison with the blank test, more E. coli were still existed in the solution after 20 min vibration than the initial solutions, indicating neither of the membranes can inhibit E. coli reproduction. As shown in the scanning electron microscopy (SEM) images, the membranes have a fibrous structure with a pore size between fibers similar to the E. coli, therefore some E. coli might be retained in the pores by the fibers to remain less E. coli in the solution. With TPMMC grafted on the membranes, the mean survival rates of E. coli were decreased significantly to 11.3 and 4.1% for the CGM and PCGM, respectively. The survival rates dropping obviously resulted from antibacterial TPMMC. Moreover, with plasma bombardment as a pretreatment, the PCGM showed a lower E. coli survival rate than that the CGM did, which was attributed to more TPMMC grafted onto the membrane. For one thing, more TPMMC can lead to a higher positive charge density of the membrane to strengthen Coulombic attraction to E. coli. For the other, more lipophilic octadecyl groups were given to penetrate the E. coli cytomembrane, release the cytoplasmic inclusions and kill E. coli. In addition, it should be clarified that the plasma bombardment was only a process for surface activation without altering the fibrous structure which was also demonstrated in the SEM images, therefore the improvement of the antibacterial performance excluded the possibility of less E. coli remaining in the PCGM solution because of more E. coli retained in the membrane than those of the CGM. For a practical application, we measured the durability of antibacterial performance of the PCGM in the Figure 8. Before the durability test, the membranes were already rinsed several times by copious warm water in a Buchner funnel with a wash-pump method. Consequently, the PCGM still showed stable E. coli survival rates of 4.1, 3.0, 5.5, and 3.7% with water rinsing quantity from 0 to 3 L·cm⁻² with 1 L·cm⁻² as an interval. Therefore, the stable survival rate indicated strong linkage of antibacterial TPMMC onto the membrane which also indicated less antibacterial TPMMC releasing and dissolving in the water to raise health concerns.

CONCLUSIONS

In conclusion, chemical-grafting method can anchor the QAS molecules onto a glass fiber membrane, which was first enhanced by an easy-operation and environmentally friendly plasma bombardment pretreatment. Although the direct
chemical-grafting method can also anchor antibacterial QAS on the surface of the glass fiber membrane, the plasma bombardment pretreatment as a surface activation technique can further enhance the chemical-grafting process. Compared with the direct chemical-grafting method, the PCG can anchor more QAS on the glass fiber membrane resulting in a higher positive charge density on the membrane surface, which is beneficial for both bacteria attraction and bacterial lysis. In addition, the PCG membrane still demonstrated a durable antibacterial performance after copious water rinsing. Therefore, the plasma bombardment pretreatment for enhancement of QAS chemical-grafting provides a new way to boost QAS antibacterial performance and facilitate practical antibacterial application of the QAS in drinking water purification industry.

## EXPERIMENTAL SECTION

**Materials Synthesis.** The pristine glass fiber membrane (PM) was obtained from FeiBo Inc., Guangdong, China. The glass fiber membrane contains silicon oxides as a main ingredient and a small part of acrylic acid as a binder. As shown in the Scheme 1, the whole synthesis process was divided into three main steps, antibacterial chemical preparation, plasma bombardment pretreatment, and chemical grafting. The antibacterial chemicals were prepared as four steps: first, AEM5700 (Microban Inc., Huntersville, NC, United States) with an effective ingredient of TPMMC was mixed with tetraethyl orthosilicate (>99.0%, Aladdin Inc., Shanghai, China) with a ratio of 1:3 as a homogenous solution A; second, the ethanol (>99.5%, Aladdin Inc., Shanghai, China) was mixed with deionized (DI) water (18.2 MΩ·cm) with a ratio of 9:1 as a homogenous solution B; third, 95 g of the solution B was mixed with 5 g of the solution A in a beaker; fourth, the beaker was placed in a water bath at 30 °C for 4 h for TPMMC hydrolysis.

Then, the plasma bombardment pretreatment was conducted in a radio frequency (RF) plasma setting (type model: CRF-APO-RP1020, CRF Inc. at Shenzhen, China) with a power of 750 W and a frequency of 13.6 MHz. The compressed and dried air with a flux of 2 L·s⁻¹ was fed for a plasma generator. The PM was plasma-bombarded for about 1 min in the RF plasma setting. After plasma bombardment, the membrane was named as the PBM. It should be mentioned that the PM should be placed in ambient conditions with a preferable relative humidity about 40~70% at this experiment, the relative humidity was about 60% to form dangling silanol groups (Si−OH) by reaction between the broken Si−O bond and H₂O molecule.

The PBM was saturated in the prepared antibacterial liquid for several minutes and then baked at 120 °C for 2 h in an oven for reaction of chemical grafting. After the reaction, the membrane was rinsed with copious warm DI water (50 °C) in a Buchner funnel with a wash-pump method for several times to remove extra unlinked TPMMC molecules and other organic monomers, and then dried at 120 °C for 0.5 h in an oven. The dried membrane was named as plasma bombardment-enhanced CGM (PCGM). As a comparison, the PM directly soaked in the antibacterial liquid without plasma bombardment was named as CGM.

**Materials Characterization.** The morphologies of the membranes were obtained by a SEM (Hitachi SU8010) with a magnification of ×500 and an electron acceleration voltage of 1.0 kV. To obtain distinct images, Au was coated on the membrane samples to increase electronic conductivity. The FTIR spectra (attenuated total reflection mode) were obtained by a Thermal Nicolet is50 spectrometer (Thermo Scientific, Waltham, MA) for vibration analysis of the chemical groups. The chemical composition and status of the elements were analyzed by a XPS (PHI 5000 Versa Probe System II, Physical Electronics, MN) with Al Kα (1486.6 eV) as the X-ray source. The standard C 1s peak at 284.8 eV was used as the reference to calibrate the binding energies of all detailed elemental spectra of the XPS. The contents and thermal stability of the materials were analyzed by a TGA (Mettler TGA2, Mettler-Toledo, Switzerland) in a nitrogen atmosphere from ambient temperatures to 800 °C with a temperature ramping rate of 10 °C·min⁻¹. The surface zeta potential of the membranes was measured at a pH range between 4 and 10 with a zeta potential analyzer (SurPASS 3, Antoon Paar, Austria).

**Antibacterial and Durability Tests.** The antibacterial tests included three main processes: reagents preparation, vibration reaction, and gericulture. The reagent preparation can be divided into four steps. First, 3.3 g of nutrient agar (Guangdong Huankai Microbial Sci. & Tech. Co. Ltd., Guangzhou, China) was mixed with 100 mL of DI water as the gericulture media. Second, 0.03 M of phosphate buffer saline (PBS) was prepared by blending 7.16 g of disodium hydrogen phosphate dodecahydrate (>99.0%, AR, Na₂HPO₄·12H₂O, Xilong Scientific Co., Guangdong, China) and 1.36 g of potassium dihydrogen phosphate (>99.5%, AR, KH₂PO₄, Guangdong Chemical Reagent Engineering Technological Research and Development Center, Guangdong, China). Third, *E. coli* (Guangdong Huankai Microbial Sci. & Tech. Co. Ltd., Guangzhou, China) solution was prepared with a bacterial concentration between 3 × 10⁵ and 4 × 10⁵ CFU·mL⁻¹. Fourth, the PM, PBM, CGM, and PCGM were cut into small pieces (each piece was about 5 × 5 mm) and every 0.75 g of each membranes pieces were sampled for the following tests. After sterilization of the membrane samples and containers, four groups of 0.75 g of the membrane samples were placed into the containers, each of which was added into 70 mL of the 0.03 M of PBS solution. An extra container with PBS but no membrane samples was prepared for a control test to obtain the *E. coli* reproduction performance. Each of the five containers was then added with 5 mL of *E. coli* solution. After gently shaking the containers for about 1 min to homogenize the solutions, then 1 mL of each solution was sampled. The five containers were sealed and placed in a vibration system (type: ZHWY-211C, Shanghai Zhicheng Inc., Shanghai, China) for slightly swaying with a frequency of 120 rpm at 35 °C for 20 min. This vibration process was used to evaluate the *E. coli* growth with and without contact to the membranes. After vibration, the five *E. coli* solutions were sampled again. Thereafter, total 10 samples (five before and the other five after vibration) were individually diluted with PBS solution and cultured identically in Petri dishes with nutrient agar for gericulture at 37 °C for 48 h. After gericulture, the bacterial colony was numbered. Then, the survival ratio of each sample can be calculated by the following equation

\[
\text{Survival ratio} = \frac{\text{number of colony after vibration}}{\text{number of colony before vibration}} \times 100\%
\]

The PCGM was fixed in a homemade membrane test mold for water flushing with a flow-through method for the
durability test. Namely, the membrane edges were sealed and water was flushed through the membrane. Copious DI water was flushed through the PCGM with a flux rate of 0.05 L-min⁻¹-cm⁻². The membrane samples flushed for 0, 20, 40, and 60 min, respectively, were used for the durability test to simulate the membrane antibacterial activities in a long term usage. The E. coli survival ratio was obtained as the same way in the above description.

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: frank.meng@126.com. Phone: +86-755-29574552-8608.

ORCID

Fanke Meng: 0000-0001-7961-4248

Author Contributions
M.G. and F.M. contributed equally.

Notes
The authors declare no competing financial interest.

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