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Surface-independent one-pot chelating copper ions on filtration membranes for antibacterial properties

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One-pot chelating copper ions for antibacterial properties was performed on the filtration membrane surfaces inspired from the dopamine (DOPA) chemistry. This simple, universal, and cost-effective DOPA-Cu²⁺ approach provides an applicable avenue for mitigating long-term biofouling of various surfaces.

Biofilm developed by adhesion and subsequent proliferation of bacteria on solid surface is of great concern in both healthcare and industrial applications.¹ For example, nosocomial infections are the major challenges to patient safety and half of them are device-related due to the bacterial fouling on medical device surfaces.² Similarly, the growing biofilm on filtration membrane surfaces has also become a major obstacle to membrane-based water purification technologies because it can contaminate the purified water and also deteriorate the membrane performance significantly.³ Therefore, there is a critical need for an efficient, facile, and cost-effective method to prevent the biofilms forming on different substrate surfaces.

To address this problem, significant research attentions have been paid to develop antibacterial surface via incorporating biocidal metal nanoparticles or their oxides/ionic forms (*e.g.*, silver, copper and zinc) onto the substrate surfaces.⁴ Copper has been widely used as an effective biocide for surface biofouling mitigation due to its strong antibacterial activity against a broad range of bacterial species and also low costs.⁵ To date, many efforts have been devoted to incorporate copper nanoparticles⁶ or their oxides/ionic forms, including copper hydroxide nanoparticles,⁷ copper oxide nanoparticles⁸ and copper ions,⁹ onto substrate surfaces for biofouling resistance. For instance, polyethyleneimine (PEI)-coated

copper nanoparticles (Cu NPs) were incorporated onto a reverse osmosis (RO) membrane surface through electrostatic interactions, leading to an 80–95% reduction in the number of attached live bacteria.¹⁰ Alternatively, copper ions could be immobilized on the substrate surfaces via anchoring polymer brushes first, and therefore chelating copper ions.^{9b,11} However, the aforementioned approaches for loading copper NPs or their oxides/ionic are dependent on the substrate surface chemistry having certain functionalities or specific charges to promote their strong bonding, and they are not applicable to most of other surfaces without those special physicochemical properties. Therefore, it still remains a tremendous challenge to develop simple and universal strategies for incorporating copper onto substrate surfaces.

Mussel-inspired dopamine (DOPA) chemistry opens a versatile and applicable approach for chelating copper ions to construct biofouling-resistant surfaces.¹² The previous studies have demonstrated a thin and highly hydrophilic polydopamine (PDA) coating could be formed onto a wide range of substrate surfaces via the self-polymerization of DOPA, regardless of the substrate materials and their surface properties.¹³ Furthermore, the formed PDA coating contains many functional groups including catechol, amine, and imine, and serves as the anchors for the firmly loading of copper ions. Very recently, PDA assisted Cu (II) ions immobilization onto titanium surface was reported to achieve outstanding antibacterial performance against Gram-negative *E. coli* and Gram-positive *S. aureus*.¹⁴ However, this approach is relatively complicated and time-consuming since two steps of surface modification are involved including the slow polymerization of DOPA first and the followed copper chelation.

Herein, a simple, rapid and one step method was presented to chelate copper ions on filtration membrane surfaces for antibacterial property via simply soaking filtration membranes in DOPA solution containing copper (II) ions, as shown in Scheme 1. In this process, the deposition of PDA coatings and the chelation of copper ions occurred on the membrane surfaces rapidly and simultaneously, regardless of their membrane surface properties, and copper ions was found to accelerate the polymerization of DOPA at acidic condition. In addition, the copper containing filtration membranes were tested to show great antibacterial performances against *S. aureus*. Our

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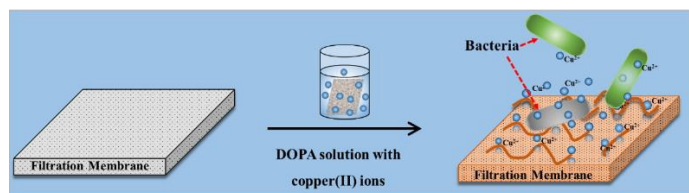
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Electronic Supplementary Information (ESI) available: Detailed experimental procedure, SEM micrographs, EDX mapping analysis, Water contact angles, Cu LMM XP spectra, UV-VIS spectra, Concentration profile of copper ions, Normalized water flux and rejection of membrane, Antibacterial photographs. See DOI: 10.1039/x0xx00000x

research aims to develop a biofouling-resistant membrane surfaces through this simple, universal, cost-effective and applicable DOPA-Cu²⁺ approach.



Scheme 1. One-pot chelating Copper ions procedure on filtration membranes for antibacterial properties

One-pot chelating copper on filtration membranes was performed through immersing the membrane coupons in DOPA solution containing Cu²⁺, and several popular membranes were used as model substrates including polysulfone (Psf) ultrafiltration (UF) membrane, polyvinylidene fluoride (PVDF) UF membrane, Psf hollow fiber UF membrane, thin film composite forward osmosis (TFC-FO) polyamide membrane and polyvinylidene fluoride-co-hexafluoro-propylene (PVDF-HFP) electrospun nanofibrous membrane. To prevent the formation of copper (II) hydroxide precipitation, the DOPA solution containing Cu²⁺ is acidic at pH 4.5, at which DOPA could not polymerize to form PDA from the reported self-polymerization of DOPA only occurring at alkaline condition with air or oxygen as an oxidant.^{12b, 13} However, in the presence of Cu²⁺, DOPA solution turned to dark brown over time even at pH 4.5, and the PDA was deposited on membrane coupons upon immersion in DOPA solution for 60 minutes since their colors all changed to dark, shown in Figure 1 a, regardless of their membrane materials and their forms in flat sheet or hollow fiber. Scanning electron microscopy (SEM) was used to observe the surface morphologies of membrane coupons before and after PDA deposition (Figure S1 and Figure 1 b). Particle-like PDA coatings were clearly found on Psf, PVDF, and TFC FO polyamide membrane surfaces, shown in Figure 1 b, which is consistent with the reported morphologies upon PDA coating.¹⁵ The surface element compositions of the modified membrane coupons were examined using Energy Dispersive X-Ray Spectroscopy (EDX). As shown in Figure 1c, strong signals of copper element were detected on these modified membrane surfaces, indicating the successful immobilization of copper during the deposition process of PDA. The distribution of copper element was mapped on the Psf membrane surface using EDX to be uniform across the scanned area, shown in Figure S2. Static contact angle measurements were also performed on the Psf membrane surfaces to show a rapid decrease from 83.2 ± 2.5° to 53.4 ± 2.4° within 1-hour incubation with DOPA, and then reached to a plateau of 49.6 ± 3.5° even with longer incubation from 1 hour to 12 hours, shown in Figure S3, indicating the improved surface hydrophilicity upon the deposition of PDA.

X-ray photoelectron spectroscopy (XPS) elemental analysis was also investigated to detect the copper and other elements on the PDA modified membrane surfaces including Psf, TFC-FO polyamide layer, and PVDF. The binding energy (BE) peaks at 954 eV and 932 eV were observed and attributed to Cu 2p_{1/2} orbital and Cu 2p_{3/2} orbital respectively, shown in Figure 2A and 2B, further confirming the presence of copper on the PDA deposited surfaces. The molar fractions of copper element on the Psf, TFC-FO polyamide layer, and PVDF were determined to be 1.4%, 2.33% and 1.36%, respectively, which are very close to the reported 2.41% on other

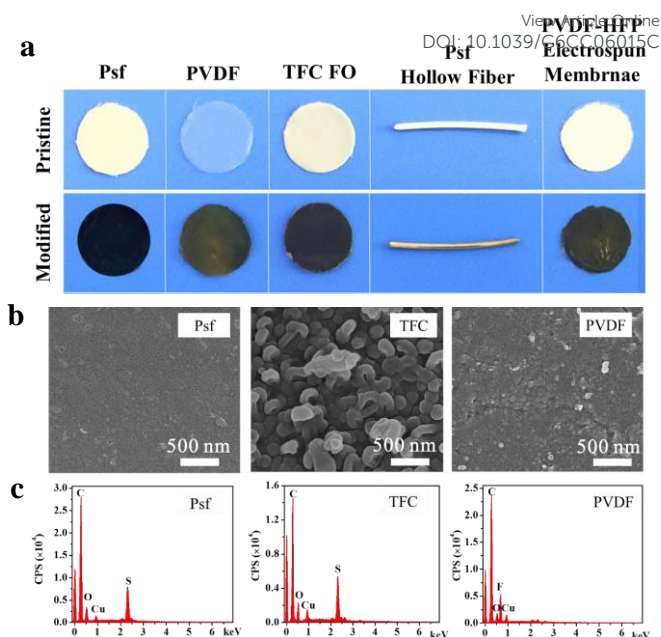


Figure 1. Photographs of pristine and modified membranes via simple immersion of membrane coupons into DOPA solution with Cu²⁺ at pH 4.5 Tris-HCl Buffer (a); SEM micrographs (b) and EDX spectra (c) of the modified Psf UF membrane, TFC FO polyamide layer and PVDF UF membrane.

substrates.¹⁶ In addition, the molar fractions of nitrogen elements were high up to 7.26%, and 5.02% on the Psf and PVDF membrane surfaces, respectively, indicating the thick coatings of PDA formed. The forms of the immobilized copper were also analyzed to see the typical BE peak of Cu²⁺ at 934.4 eV shown in Figure 2C and another characteristic Cu²⁺ satellite peaks around 963 eV and 943 eV shown in Figure 2B.^{5a, 17} The lower BE peak at 932.7 eV suggested the presence of Cu⁺ or Cu⁰.¹⁷ However, Cu 2p_{3/2} XPS could not differentiate between Cu⁺ and Cu⁰. Therefore, the X-ray-induced

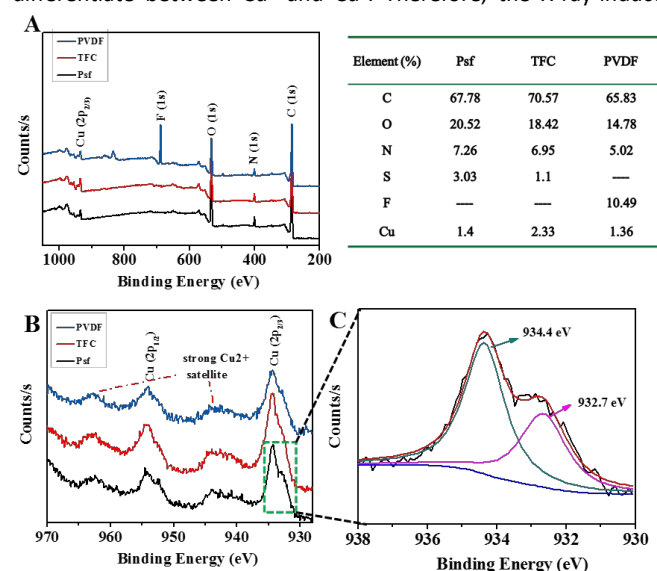


Figure 2. XPS spectrum and elemental compositions of filtration membrane surfaces upon PDA deposition (A), enlarged XPS spectrum from 925 to 970 eV (B) and peak fitting of the Cu 2p_{3/2} spectrum on Psf UF membrane (C).

Auger electron spectroscopy was employed to characterize the valence state of copper. As shown in Figure S4, the peak at 570 eV in the Auger Cu LMM spectra confirmed the presence of Cu^+ and no peak of metallic copper at 568 eV was observed, which proved that the lower BE peak at 932.7 eV in Figure 2C was assigned to Cu^+ .¹⁷ Therefore, the copper chelated on the PDA deposited membrane surfaces was mostly in the form of Cu^{2+} and Cu^+ , and no metallic copper was found, which is consistent with the previous study.¹⁸

To understand the role of Cu^{2+} in the polymerization of DOPA solution, UV-VIS was used to monitor the formation of PDA having a characteristic absorbance peak at 420 nm.¹⁶ When DOPA solution was acidic at pH 4.5, as shown in Figure S5, there was no absorbance detected at 420 nm and no color change observed in air with the absence of Cu^{2+} , which has been reported that DOPA could not self-polymerize at acidic condition. However, the presence of Cu^{2+} made the DOPA solution at pH 4.5 have color change rapidly from pale brown to dark brown, and have increasing strong absorbance at 420 nm in 40 minutes, indicating Cu^{2+} initiated the polymerization of DOPA at pH 4.5. To investigate the role of air on polymerization of DOPA in the presence of Cu^{2+} , the Psf UF membrane coupons were immersed in various DOPA solutions with and without air involved, and the PDA deposition was monitored through their color change. Results shown in Figure S6 illustrated that the color of the membrane coupon was much darker with the presence of Cu^{2+} and air in reaction solution than the one with the presence of Cu^{2+} but under the deoxygenated condition, indicating the formed PDA was much thicker with the presence of Cu^{2+} and O_2 than any other conditions. Therefore, it could conclude that both Cu^{2+} and O_2 together accelerate the polymerization rate of DOPA, and Cu^{2+} alone also make DOPA polymerize slowly at acidic condition. Professor Xu et al also found that the deposition rate of PDA was significantly increased when CuSO_4 and H_2O_2 was present in DOPA solution at pH 8.5.¹⁶ Thus the system of Cu^{2+} and oxidants could expand the reaction conditions of DOPA to broad pH range from alkaline to acidic solution. However, the reaction mechanism of Cu^{2+} has not been understood yet even though Cu^{2+} was reported to work as an oxidant, and no metallic copper was found on the PDA film but mostly in the form of Cu^{2+} .¹⁸ In our study, Cu^+ was also found on the modified membrane surface, confirming Cu^{2+} was reduced to Cu^+ rather than Cu^0 involving the polymerization of DOPA. While, it has been extensively studied that the mechanism of O_2 acting as an oxidant was first to oxidize DOPA into DOPA-quinone, followed by a series of further oxidation to 5,6-indolequinone under alkaline conditions (pH > 7.5).^{12a, 19} As shown in Figure S5, DOPA solution turned its color from light to dark in the air in pH 8.5 Tris buffer and had a growing absorbance at 420 nm with time. Further investigations were needed to understand the reaction mechanism of Cu^{2+} involving the polymerization of DOPA through learning the chemistry of DOPA and O_2 .

The effects of Cu-PDA coating on the transport properties of the membranes were investigated. Results shown in Figure S7 present that the water fluxes of modified Psf UF membrane, PVDF UF membrane and TFC FO membrane decreased by 25.2%, 19.0% and 28.1% compared with pristine membrane, respectively. However, the generation of Cu-PDA layer has benefits on improving the BSA rejection of Psf UF membrane by 36.6% and suppressing the reverse salt flux of TFC FO membrane by 51.6%. The decrease in the water permeability and the increase in the selectivity of modified membrane might be the result of the blocked surface pores upon PDA coating, which has been reported in the previous study.^[20]

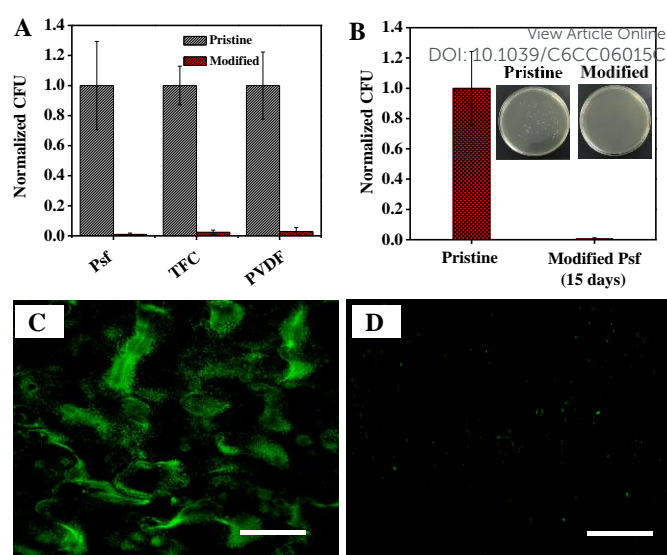


Figure 3. Normalized CFUs of attached live *S. aureus* bacteria on pristine Psf UF membrane, TFC FO membrane and PVDF UF membrane before and after chelating copper ions (A); Normalized CFUs of attached live bacteria and the bacterial culture plate photographs (insert) of pristine and copper containing Psf UF membrane after 15 days of soaking in DI water (copper-containing Psf (15 days), B); Representative fluorescence microscopic images of live *S. aureus* attached to pristine Psf UF membrane (C) and copper-containing Psf UF membrane (D) stained with Syto9 after incubation for 24 h. The scale bars are 200 μm . Data are shown as mean \pm SD from three individual membrane coupons.

Further, we also evaluated the leach of copper from the modified membrane in a dead-end filtration system via quantifying the level of released copper ions in the permeate filtered through the modified membrane. Results shown in Figure S8 found that the concentrations of copper ions in the permeates were 0.015 ppm, 0.012 ppm and 0.013 ppm, respectively, after filtered through Cu-containing Psf UF membranes and collected at 1 h, 12 h and 24 h, all of which were 100 times lower than the maximal contaminant limit of copper in drinking water (1.3 ppm) established by the U.S. Environmental Protection Agency.^[21] These results suggested that the fabricated Cu-containing antibacterial membrane leaked very little Cu into the permeate and had no risk to environment and our health if the modified membrane were to be applied for water filtration.

Finally, the CFU counting method was used to test the antibacterial properties of copper containing membranes against Gram-positive (*S. aureus*) bacteria. The number of attached live *S. aureus* was counted and compared with their pristine membranes to show a significant decrease by $99.0\% \pm 0.9\%$, $97.6\% \pm 1.5\%$ and $97.1\% \pm 2.6\%$ on Psf UF membranes, TFC FO membranes and PVDF UF membranes, respectively, (Figure 3A and Figure S9). Figure 3C and 3D were the representative fluorescence microscopic images of the live bacteria on the pristine and copper containing Psf UF membranes stained with Syto9 after incubation for 24 h. The green fluorescence was from the live bacteria and became stronger with more live bacteria on the membrane surfaces. Thus, the live bacteria were found to be much less on the copper containing Psf UF membranes than on the pristine, since the green fluorescence was much weaker and rarely observed on the copper containing Psf UF membranes than on the

pristine. Furthermore, our results also showed that the copper containing Psf UF still exhibited strong antibacterial activity of $99.4\% \pm 0.6\%$ inactivation efficiency after soaking the membrane coupon in water for 15 days to leach copper (Figure 3B). All of our findings suggested copper containing membranes could have outstanding and long-term bactericidal efficacy against Gram-positive bacteria.

In summary, we presented a facile and universal approach to one-pot chelate copper ions on the filtration membrane surfaces for antibacterial properties. It was also found that Cu^{2+} and O_2 together greatly accelerated the polymerization rate of DOPA. The incorporated copper ions were proven to impart membrane surfaces with outstanding antibacterial properties against *S. aureus*. Given the simplicity and versatility of this approach, combined with the low costs and excellent antibacterial properties of copper, this strategy for one-pot chelating copper ions provides an applicable avenue for mitigating long-term biofouling of various surfaces.

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