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SUPPORTING INFORMATION

A natural polysaccharide-based antibacterial functionalization strategy for liquid and air filtration membranes

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1. Experimental section

1.1 Materials

Guanidine hydrochloride (98%), hexamethylene diamine (99%), dextran (AR), and $NaIO_4$ (AR), were purchased from Energy Chemical (China). Ethylene glycol (AR), methanol (AR), and absolute ethanol (AR) were purchased from Beijing Chemical Plant (China). Polyether sulfone (PES) filtration membranes were purchased from Haiyan New Oriental Plastic Technology Co., Ltd. (China). Polypropene (PP) melt-blown fabrics were gifted by Shandong Chambroad Petrochemicals Co. Ltd. (China). 3-aminopropyl triethoxysilane (APTES, 98%) was purchased from TCI Chemical (China). Lipopolysaccharide was purchased from Solarbio Life Sciences (China). Endotoxin detection limulus kit (test tube quantitative chromogenic matrix method) and endotoxin-free water were purchased from Xiamen Bioendo Technology Co. Ltd. (China). Live/dead staining kit was purchased from Thermo Fisher Scientific (USA).

1.2 Synthesis of polyhexamethylbiguidine chloride (PHMG)

In a 250 mL three-necked flask, 14.3 g (0.15 mol) of guanidine hydrochloride and 18.3 g (0.1575 mol) of hexamethylene diamine were added. The rotation speed of the stirrer was kept as 350 rpm. The mixture was heated to 100 °C and kept for 1 h. Then, the temperature was raised to the polycondensation temperature (170 °C) and the reaction was continued for 4 h. A funnel was connected with the flask and inverted on the surface of water to absorb the by-product NH_3 as an anti-suction device. After reaction, the product was harvested and grounded into powder. The detailed synthesis

route was shown in Fig. S1.

1.3 Chemical structure characterization of PHMG

The chemical structure of PHMG was characterized by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. In a 1 mL centrifuge tube, 6-8 mg of PHMG was dissolved into 600 μL of deuterated dimethyl sulfoxide (DMSO-d6). After complete dissolution, the sample was transferred to a clean nuclear magnetic tube, and the product was subjected to ^{1}H NMR analysis using Bruker NMR AMX 400M ^{1}H NMR at room temperature.

1.4 Determination of minimal bactericidal concentration of PHMG

Centrifuge tubes, pipette tips and other consumables were autoclaved at 121 °C for 20 minutes, and then cooled and dried for use. The Luria-Bertani (LB) liquid culture media were prepared with 1 g of tryptone, 0.5 g of yeast extract and 0.5 g of sodium chloride into 100 mL of deionized water. After dissolution, the media were sterilized by autoclave at 121 °C for 20 min. After sterilization, the LB culture media were cooled to room temperature and then transferred to a 4 °C refrigerator for storage for use.

The solid culture media were prepared by 32 g of tryptone soy agar (TSA) into 800 mL of deionized water. The mixture was put into an autoclave and sterilized at 121 °C for 20 min. Then, the media were taken out, and poured into 90 mm sterile plastic petri dishes. The dishes were closed and cooled.

In a 15 mL centrifuge tube containing 10 mL of LB liquid media, 10 μL of thawed *S. aureus* strain was added. The bacterial suspension was vortexed and placed in a constant temperature shaker with a rotating speed of 150 rpm at 37 °C for 12 h. *E. coli*

was expanded in the same way, and the culture time was 8 h. The absorbance of the bacterial suspension was measured by a spectrophotometer (NanoDrop ONE, Thermo Fisher, USA) to ensure that the OD 600 value was between 0.6 and 0.8. The bacterial density was measured by plate colony counting method. In a 10 mL centrifuge tube, 5 mL of the bacterial suspension was added, and centrifuged at 4000 rpm for 3 min. The supernatant was removed, and then sterile phosphate buffer saline (PBS) was added to dilute the bacterial density into 2×10^8 CFU/mL.

The MBC of PHMG against *E. coli* and *S. aureus* was determined by two-fold dilution method. PHMG was dissolved in PBS into a solution of 8, 4, 2, 1, 0.5, 0.25, and 0.125 μg/mL. In each centrifuge tube, 500 μL of PHMG solution with different concentration was added. The control group was 500 μL of sterile PBS, and each group had three replicates. In each centrifuge tube, 500 μL of bacterial suspension with a bacterial density of 2×10^8 CFU/mL was added. The final PHMG concentrations were 4, 2, 1, 0.5, 0.25, 0.125, and 0.0675 μg/mL, and the final bacterial density in each tube was 10^8 CFU/mL. Then, the samples were cultured in a constant temperature shaker at 37 °C with a rotating speed of 150 rpm for 12 h. After culture, 50 μL of bacterial suspension was removed and spread on a solid plate medium, and incubate at 37 °C for 18 h. Finally, the colonies on the solid media were counted.

1.5 Synthesis of oxidized dextran (ODex)

The ODex was synthesized according to a previous study.[S1] In a 250 mL round-bottom flask, 5 g (0.031 mol) of dextran (M_w= 20,000 Da) was added into 100 mL of deionized water and stirred for 4 h at room temperature until it was completely

dissolved. In another 100 mL round bottom flask, 5.94 g (0.028 mol) of sodium periodate ($NaIO_4$) dissolved in 50 mL of deionized water in the dark. Then, the $NaIO_4$ solution was slowly added into the dextran solution. After reacting at room temperature for 24 h in the dark, 3 mL of ethylene glycol was added to the flask to react for 1 h for oxidation reaction termination. The raw product was dialyzed in deionized water with a dialysis bag with a cut-off molecular weight of 3500 Da at room temperature for 3 d, during which the water was changed every 8 hours. The product, ODex, was freeze-dried and stored in a refrigerator at 4 °C under the protection of nitrogen.

1.6 Determination of hydroformylation degree of ODex

The hydroformylation degree of ODex was determined according to a previous study.[S2] First, hydroxylamine hydrochloride solution at the concentration of 0.25 mol/L was prepared. Then, 0.1 g of the ODex was added into a 50 mL flask, and 25 mL of the above 0.25 mol/L hydroxylamine hydrochloride solution was also added. The reaction was conducted for 24 h under nitrogen protection. After 24 h, the solution was titrated by 0.1 mol/L NaOH solution. The degree of hydroformylation of ODex was calculated by potentiometric titration according to the following formula:

$$\text{degree of hydroformylation} = \frac{\Delta V \times 0.001 * n}{2 * m/162.02} \times 100\% \quad \text{Formula 1}$$

where ΔV was the consumed volume of NaOH solution at the turning point of titration (mL); n was the concentration of NaOH (mol/L); and m was the mass of ODex (g).

1.6 Preparation of amino-functionalized surfaces

The PES membranes and PP melt-blown fabrics were cut into round samples with

the diameter of 50 mm. The samples were ultrasonically cleaned for 3 times in deionized water, methanol, and ethanol alternately (1 minute each time), and then taken out and dried at 50 °C.

Similar with our previous work, the amino-functionalized surfaces were prepared by APTES.[S3] To prepare a 10% APTES solution, 5 mL of APTES was added into 45 mL of a mixed solvent of ethanol and deionized water ($V_{ethanol}$: V_{water} = 9 : 1). After treating the samples in an oxygen plasma instrument (90 W, 3 min, double-sided), we put the samples into a 60 mm glass crystallization dish containing 50 mL of the above-mentioned APTES solution. Then, the samples were reacted in an oven at 60 °C for 6 h. The samples were taken out and washed three times in deionized water to remove unreacted APTES, and then cured in an oven at 60 °C for 2 h to prepare PES-APTES and PP-APTES, respectively.

1.7 Preparation of aldehyde-based surface by Schiff base reaction

First, the carbonate buffer solution (pH=9.6) was prepared. In 1 L of deionized water, 2.94 g (0.035 mol) of sodium bicarbonate and 1.59 g (0.015 mol) of sodium carbonate were dissolved.

In 50 mL of carbonate buffer solution (pH=9.6), 1 g of ODex was dissolved to prepare a 20 mg/mL ODex solution. Then, 50 mL of ODex solution was transferred to a clean glass petri dish, and PES-APTES and PP-APTES was soaked in the ODex solution, respectively. The petri dishes were shaken at 37 °C for 12 h with the rotation speed of 150 rpm. The prepared samples were washed for three times with carbonate buffer solution (pH=9.6) after the reaction to remove unreacted ODex. Then, the

samples were naturally air-dried at room temperature. PES-ODex and PP-ODex with the aldehyde-functionalized surface were obtained, respectively.

As a control group, 1 g of glutaraldehyde was blended with 50 mL of carbonate buffer solution (pH=9.6) to form a 20 mg/mL glutaraldehyde solution. By the same method, PES-APTES samples were soaked in the glutaraldehyde solution. The reaction was shaken at 37 °C for 12 h. After the reaction, the samples were washed for three times with carbonate buffer solution (pH=9.6) to remove unreacted glutaraldehyde. After air drying at room temperature, the PES surface with aldehyde group was obtained (PES-GA).

1.8 Grafting PHMG onto the surface by Schiff base reaction

In 50 mL of carbonate buffer solution (pH=9.6), 1 g of PHMG was dissolved to prepare a 20 mg/mL PHMG solution. In a 60 mm glass petri dish, PES-ODex, PP-ODex and PES-GA samples were immersed in PHMG solution, respectively. The samples were shaken at 37 °C for 12 h. After reaction, the samples were rinsed by carbonate buffer solution (pH=9.6) for three times to remove unreacted PHMG.

In 50 mL of carbonate buffer solution (pH=9.6), 0.25 g of sodium cyanoborohydride was dissolved to prepare a 5 mg/mL sodium cyanoborohydride solution. The aforementioned samples were immersed in sodium cyanoborohydride solution at 37 °C for 2 h to reduce the Schiff base bond to C-N bond. After reaction, the samples were washed for three times with deionized water to remove excessive sodium cyanoborohydride. PES-ODex-PHMG, PP-ODex-PHMG and PES-GA-PHMG samples were obtained, respectively.

1.9 Physical and chemical characterizations

X-ray photoelectron spectroscopy (XPS, AXIS-His, Kratos, UK) was used to characterize the chemical element content on the surface. The zeta potentials were tested and characterized by a surface potential analyzer (Surpass^{TM} 3, Anton Paar, Austria). Through scanning electron microscope (SEM, JSM-7500F, JEOL, Japan), the morphological changes on the surface were observed. The porosity was measured by the weighing method. The water flux was measured under the driving force of gravity.

1.10 Bacteria filtration experiment of PES membranes driven by gravity

PES, PES-ODex-PHMG and PES-GA-PHMG samples were used, and three duplicates were set for each group. The above-mentioned samples with the diameter of 50 mm were installed into the filter membrane fixture. A 20 mL disposable syringe without the plunger rod was connected to the filter membrane fixture. In the syringe barrel, 15 mL of *E. coli* suspension at the density of 4×10^5 CFU/mL was added. The filtration process was driven by gravity, and the filtrate was collected.

The filtrate collected from each group of samples was shaken evenly on a vortex shaker, and then diluted at 100 times with sterile PBS. On each solid culture medium, 50 μL of the diluted filtrate was evenly spread. After culturing in a biochemical incubator at 37 °C for 18 h, the petri dishes were photographed and the numbers of colonies were counted. The filtration efficiency was calculated by the following formula:

$$filtration\ efficiency\ (\%) = \left(1 - \frac{c}{c_0}\right) \times 100 \quad \cdots\cdots\cdots\cdots \text{Formula 2}$$

where c_0 was the initial bacterial density, and c was the bacterial density in the filtrate.

1.11 Cyclic bacteria filtration experiment of PES membranes

The cyclic bacteria filtration experiment was performed by a peristaltic pump. The peristaltic pump flow rate was set to 60 mL/min. The volume of the bacteria suspension in the centrifuge tube was 40 mL, and the bacterial densities were10^5, 10^6, 10^7, and 10^8 CFU/mL, respectively. After circulating and filtering for 6 min, the final filtrates were collected. The filtrate of each group was diluted at 25-fold, 250-fold, 2500-fold, and 25000-fold with sterile PBS. On the TSA solid culture media, 50 μL of the diluted filtrate was evenly spread, and cultured at 37 °C. After 18 h of incubation, the petri dishes were photographed and the numbers of colonies were counted.

In the circulating filtration experiment with the bacterial density of 10^8 CFU/mL, the circulation time was extended to 1 h. The filtrate was collected and diluted at 25,000 times. And then, the filtrate was cultured, and the number of colonies was counted by the aforementioned method.

1.12 Stability evaluation of PES-ODex-PHMG

PES and PES-ODex-PHMG samples with the diameter of 6 mm were autoclaved for use. *E. coli* and *S. aureus* were used as representative strains. The bacteria were cultured according to the aforementioned method. The bacteria suspension was diluted with sterile PBS to the bacterial density of 10^5 CFU/mL, and then 50 μL of the bacteria solution was spread on the solid culture media and incubated at 37 °C for 18 h. Then, the samples were photographed.

In order to verify the stability of modified PES membrane, the PES-ODex-PHMG samples were immersed in deionized water for 60 days, and the water was changed

once a week. Then, the soaked PES-ODex-PHMG samples were subjected to a cyclic filtration experiment according to the method of section 1.11. Similarly, the PES-ODex-PHMG samples after ultrasonic washing were also tested.

1.13 Algae filtration experiment of PES-ODex-PHMG

Chlorella vulgaris was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, and was expanded with BG11 medium. The absorbance at 450 nm measured by NanoDrop ONE. The OD 450 of algal suspension was diluted to 0.6 for use. PES and PES-ODex-PHMG MF membranes with the pore size of 10 μm were selected and cut into samples with the diameter of 25 mm. The algal filtration experiment against chlorella algae suspension was performed according to the method of section 1.11. The volume of the algae suspension was 15 mL. The flow rate of the peristaltic pump was set as 30 mL/min, and the cyclic filtration was performed for 5 min. The filtrates of each group were collected, and the OD 450 of the filtrates were measured.

1.14 Endotoxin adsorption test

First, the standard curve was determined. In 1 mL of endotoxin-free water, the bacterial endotoxin working standard sample containing 15 EU (endotoxin unit) was dissolved to prepare 15 EU/mL endotoxin standard solution. Then, the standard solution was diluted into 1 EU/mL, 0.5 EU/mL, 0.25 EU/mL, and 0.1 EU/mL. In each test tube, 0.1 mL of the endotoxin standard solution of the above-mentioned four concentrations or endotoxin-free water (negative control) was added. Then, 0.1 mL of limulus reagent was added into each tube. The test tubes were covered with aluminum

foil to prevent contamination of external endotoxin. The test tubes were incubated in a 37 °C water bath for 8 min. Then 0.1 mL of the chromogenic matrix solution was added to the test tube. The tubes were gently shaken for mixing, and then incubated in a 37 °C water bath for 6 minutes. Then, 0.5 mL of azo reagent 1, azo reagent 2 and azo reagent 3 solutions were added into each tube in turn. Finally, the samples were reacted for 10 min and the absorbance at 545 nm was measured. In the standard curve, the ordinate was the absorbance value at 545 nm, and the abscissa was the concentration of the corresponding endotoxin standard solution.

The 10 mg/mL LPS solution was prepared by adding 10 mg of LPS into 1 mL of bacterial endotoxin-free water. Then, the LPS solution was diluted into 0.1 mg/mL and 0.1 μg/mL with endotoxin-free water. The endotoxin absorption experiment was performed by the similar method and device in section 1.11. PES and PES-ODex-PHMG samples were used for filtration experiment. For cyclic absorption experiment, 30 mL of 0.1 μg/mL LPS solution was circulated through PES and PES-ODex-PHMG samples for 1 h, and then the filtrates were collected for testing. A total of three samples to be tested were 0.1 μg/mL LPS solution, PES filtrate, and PES-ODex-PHMG filtrate. The concentrations of the filtrates were measured by the aforementioned method of determination of standard curve.

1.15 Bacteria filtration efficiency test of melt-blown fabrics

Microbial aerosol generator was used to generate microbial aerosol, and eight-level aerosol particle size distribution sampler was used to sample bacterial aerosol. According to the ASTM F2101-01, bacteria filtration efficiency was tested by *S. aureus*

suspension at the density of 5×10^5 CFU/mL. *S. aureus* with the bacterial density of 5×10^5 CFU/mL was used to the aerosol generator. The PP and PP-ODex-PHMG samples were clamped between the sampler and the aerosol chamber, where the control group did not contain any sample. The pump was turned on, and then turned off when the air flow rate was adjusted to 28.3 LPM. The aerosol generator was set to 8 s per cycle, where the pump is turned on for 1 s. Then, the aerosol generator and the sampler were turned on at the same time. The aerosol generator was turned on for 1 minute, during which the aerosol generator was turned on 7 times, and the aerosol was generated for 7 s. After 2 min, the sampler was closed. The sampling plates are collected according to the label of each level, and placed in a 90 mm petri dish with solid culture media. The petri dishes were cultured at 37 °C for 24 hours, and then photographed and counted. The BFE was calculated using formula 2, where c was the colony counts on the sampling plate of the experimental group, and c_0 was the colony counts on the sampling plate of the control group.

1.16 Live/dead staining of the intercepted bacteria

The live/dead staining was to stain the surface of the PES MF membranes and PP melt-blown fabrics after filtering the bacteria. The samples after cyclic bacteria filtration experiment of PES membranes and bacteria filtration efficiency test of melt-blown fabrics were stained by a live/dead staining kit (Thermo Fisher, USA) following the instruction of the manufacturer. On each sample, 20 μL of propidium iodide (PI) solution and 20 μL of SYTO 9 solution were added and stained for 15 minutes in the dark. The stained samples were observed by a laser scanning confocal microscope

(CLSM, SP8, Leica, Germany). The numbers of live and dead bacteria were counted according to the CLSM images, where live bacteria showed green fluorescence and dead bacteria were red.

2. Supporting figures and tables

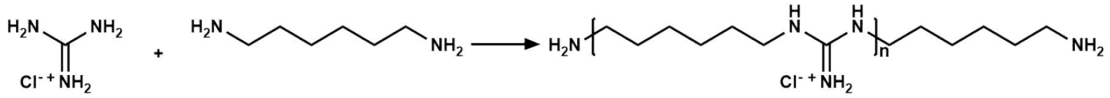

Fig. S1 The synthesis route of PHMG

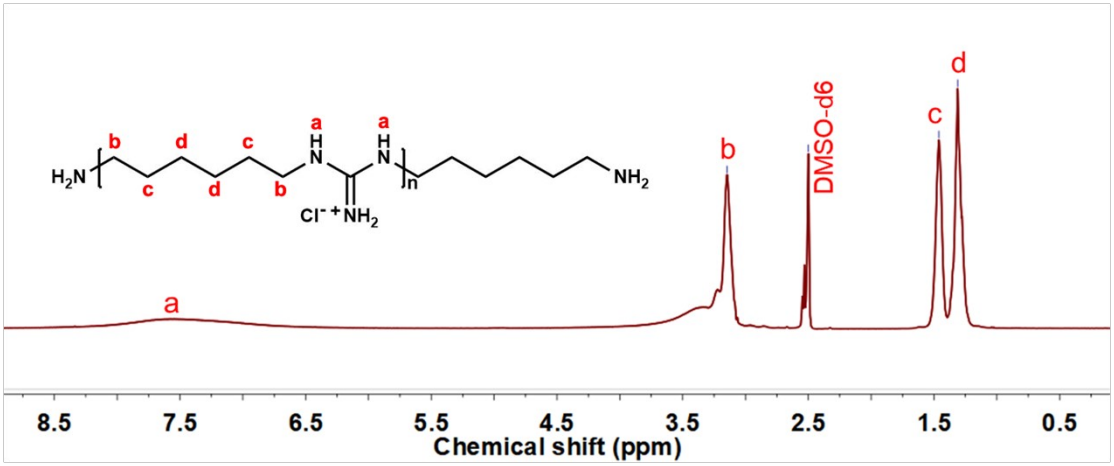

Fig. S2 The ^{1}H NMR spectrum of PHMG

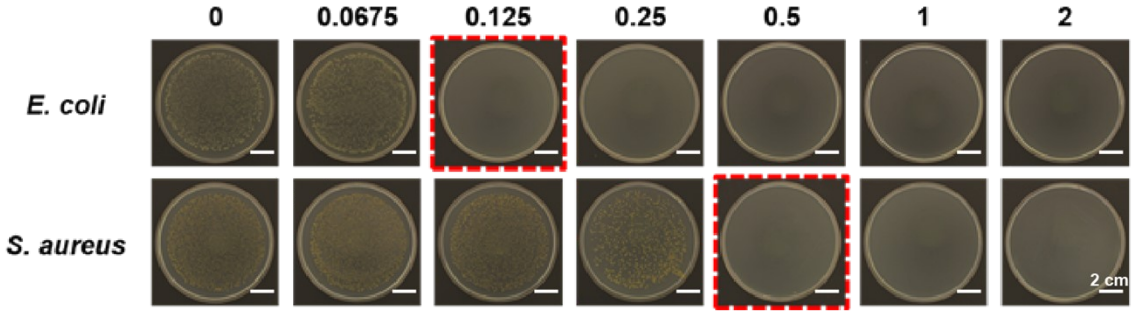

Fig. S3 The antibacterial efficiency of PHMG (unit: μg/mL)

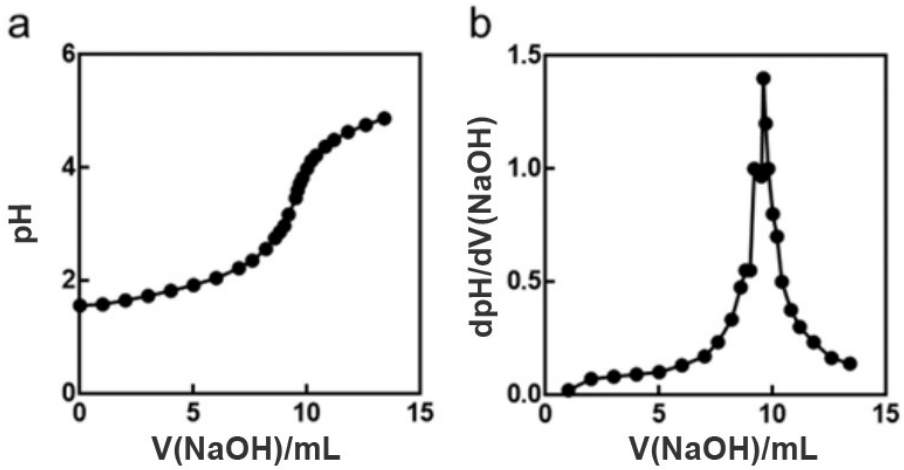

Fig. S4 (a) Hydroxylamine hydrochloride-potentiometric titration curve of ODex; (b) the first order differential curve

Table S1 Element ratios of the pristine and modified PES MF membranes

	C	O	N	S	Si
PES	72.1	15.31	7.05	3.49	2.05
PES-ODex	62.24	28.14	2.98	3.24	3.39
PES-ODex-PHMG	64.83	19.72	8.03	3.38	4.04

Table S2 Element ratios of the pristine and modified PP melt-blown fabrics

	C	O	N	Si
PP	94.05	1.46	2.77	1.72
PP-ODex	81.13	9.15	7.58	2.14
PP-ODex-PHMG	72.5	7.9	17.46	2.14

Table S3 Bacterial filtration efficiency of PES-ODex-PHMG against *E. coli* with different density

Bacterial density（CFU/mL）	10^5	10^6	10^7	10^8
Filtration efficiency（%）	100	99.09	77.5	57.2
Filtered number of bacteria（CFU）	3.72×10^6	3.69×10^7	2.88×10^8	2.12×10^9

3. References

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