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Supporting Information

A "grafting through" strategy for constructing Janus cotton fabric by mist polymerization

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1. Materials

The cotton fabric (60 ends/cm, 30 picks/cm, 0.42 mm thickness, 120 g/m^2 weight, 35.2 m^2/g specific surface area) was obtained from Shaoxing Qidong Textile Co., Ltd. Before chemical modification, the cotton samples (3.0 cm × 3.0 cm) were cleaned by ultrasonic washing in 1 % sodium laurylsulfonate solution for 30 min, then washing in ethanol (2 h) and deionized water (30 min × 3 times). A bovine serum albumin (ABS) was obtained from Shanghai Aladdin Co., Ltd (China). Albumin from human serum (AHS) was obtained from Hefei Bomei biotechnology Co., Ltd (China). ICR rats were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. The Human hepatocellular carcinoma Huh7 cells (Huh7) were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin solution were purchased from Gibco (CA, USA). The Annexin VFITC Apoptosis Detection Kit was purchased from BD Biosciences (San Diego, CA, USA). The cell counting kit-8 (CCK-8) was purchased from sangon biotech (Shanghai, China). Before assay, the HuH-7 cells were cultured in DMEM, supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C, 5% CO_2.

2. Mist polymerization method

Recently, our group developed a surface modification technology using "mist polymerization" to fabricate functional cotton fabrics. We atomized monomer solutions into fine mist (mist droplet size is smaller than 500 nm) and transport it to the fabric surface for a grafting polymerization. Driven by the interface effect between the mist droplets and the substrate surface, a very thin liquid film is formed on the fiber surface, and finally transferred to a polymer coating. Because the mist polymerization is carried out in a very small volume, it has distinguishable features to conventional liquid phase polymerization, whereas has some characteristics similar to the gas phase polymerization. The mist polymerization has advantages in less damages on fiber structure, very fine grafting layer, and a small dosage of the monomer.

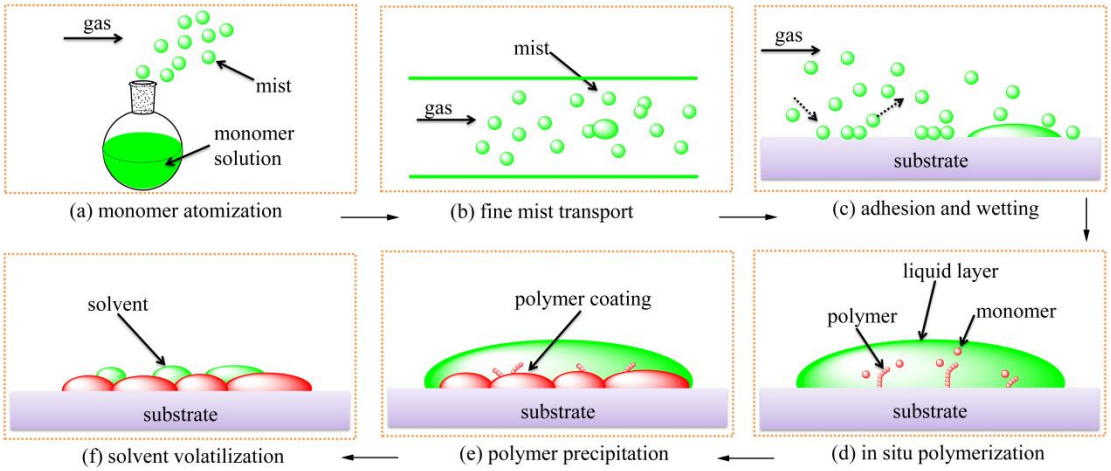

Fig. S1 The main stages of the "mist polymerization".

The “mist polymerization” can be divided into six stages: monomer atomization, fine mist transport, adhesion and wetting of the mist droplets, in situ polymerization, polymer precipitation, and solvent volatilization (Fig. S1).

3. Characterizations

Surface morphology of the cotton fabrics was investigated using an FE-SEM set (Ultra-55, Zeiss, Germany) with an energy dispersive X-ray spectrometer (EDS). Surface roughness was tested by using an atomic force microscope (AFM, Bruker Dimension Icon). Attenuated total reflectance infrared (ATR-IR) spectra were collected utilizing a Nicolet Avatar 370 spectrometer (Nicolet Company, Madison, USA) equipped with an ATR accessory. The X-ray diffraction (XRD) analyses for the modified fabrics were obtained using an X-ray diffractometer (ARL XTRA, Switzerland). The X-ray photoelectron spectroscopy (XPS) analysis was performed using an AXIS multifunctional X-ray photoelectron spectrometer (ULTRA DLD, Shimadzu Ltd., Japan) at a power of 450 W. The method of water absorptivity, water vapor permeability and tensile strength tests of the cotton fabric was described in our previous work. [1, 2]

[1] Q. Xu, L. Xie, H. Diao, F. Li, Y. Zhang, F. Fu, and X. Liu, *Carbohydr. Polym.*, 2017, **177**, 187-193.

[2] Q. Xu, L. Shen, P. Duan, L. Zhang, F. Fu, and X. Liu, *Chem. Eng. J.*, 2020, **379**, 122401.

4. Quantitative estimation of C=C double bonds on the AA-fabric surface

To estimate the quantity of C=C double bonds linking on the cotton fabrics, the concentration decrease of a Br_2 solution that caused by the chemical adsorption of the fabric was measured. The modified cotton fabric (5 cm × 5 cm) was immersed in Br_2 solution (10 mL, 0.15 mol/L) for 10 min. After removing the fabric, the absorbance value at 393 nm of the solution was measured to determine the concentration of Br_2. This test was repeated for three times, and the adsorbed quantity of C=C double bond was calculated according to the following formula:

$$Q=\frac{C\times V}{S}$$

where C (mol/L) is the concentration decrease, V is the solution volume, S is the area of fabric samples (25 cm^2), Q (mmol/cm^2) is the quantity of C=C bonds on the fabric surface.

5. Antimicrobial tests

Escherichia coli (*E. coli*, ATCC 1555) and *staphylococcus aureus* (*S. aureus*, ATCC 547) were used as the model microorganisms according to an improved AATCC 100-1999 method. Before each assay, the test bacteria were incubated in letheen broth (LB) fluid nutrient medium (containing 5 g/L yeast extract, 10 g/L peptone, 10 g/L NaCl and adjust pH to 7.4) at 37 ^oC for 24 h. A standardized density of bacteria was used for the challenge inoculation. The fabric specimens (0.05 g) were cut to about 5 mm piece, sterilized by UV light for 30 min, placed in a sterile container 5.0 μL of activated *E. coli* or *S. aureus* in 4 mL fluid nutrient medium (10^8 CFU/mL) was added into the sterile tubes containing modified cotton fabrics, shaken at 25 ^oC for 18 h at 150 rpm. The supernatant was diluted to an appropriate concentration (diluted to 40^2 times),

dispersed onto LB agar plants, and incubated at 37 ^{o}C for 24 h. The number of survival microorganism was determined by counting the colonies as a colony forming unit (CFU)/mL, and bacteriostatic reduction (BR) rate of microorganisms was calculated as follows:

$$BR = \frac{B\text{-}A}{B} \times 100\%$$

where A and B are the (CFU)/mL of the surviving microorganisms after 24 h for an agar plate containing the modified sample and control sample, respectively.

6. Inhibition zones of cotton fabrics

Firstly, the *S. aureus* and *E.coli* (1.0×10^8 CFU/mL) were diluted 40^2 times, then 100 μl of diluted solution were dispersed onto LB agar plants, and incubated at 37 °C for 24 h. Thereafter, the cotton fabric were cut into circular pieces (diameter, 10 mm), sterilized by UV light, placed on the above agar plate, and continue incubation at 37 ° C for 24 hours. The diameters of the inhibition zones (IZ) were determined. The width of the inhibition zone (W_{inh}) was calculated using the following equation:

$$W_{inh} = \frac{d_1\text{-}d_2}{2}$$

where d_1 is the total diameter of the inhibition zone on the cotton fabric, and d_2 is the length of the cotton fabric (10 mm).

7. The bacterial adhesion test of cotton fabric

Bacterial adhesion property of the modified fabrics was evaluated using SEM observation on the fiber surface that had been experienced a bacterial adhesion experiment. The fabric (one piece, 3 cm × 3 cm) was immersed in 20 mL of a bacterial solution (*S. aureus* or *E. coli*, concentration, 10^7 CFU/mL), incubated at 37 ^{o}C for 2 h, held vertically for 3 min to remove the excess bacterial solution, put into a fresh nutrient broth (20 mL) and incubated at 37 ^{o}C for 24 h with vibration at a 120 rpm. The sample was washed using sterile water (50 mL × 3 times) and sterile PBS (50 mL × 3 times) to remove the free bacteria, treated with 2.5 wt % glutaraldehyde aqueous solution for 1 h to fix the bacteria remained, washed using PBS (50 mL × 3 times) and distilled water (50 mL × 3 times). The fixed bacteria were dehydrated using a series of graded 50 mL ethanol solutions (50, 75, 90, and 100 wt %, for 15 min each), and dried in a dessicator overnight.

8. The protein adhesion test of cotton fabric

To evaluate anti-protein adsorption ability of the cotton fabric samples (3 cm × 3 cm) were immersed into a bovine serum albumin (ABS) solution (10 mL, 20 mg/mL) and stirred for 10 min. The anti-protein adsorption ability was evaluated by measuring the decrease in light transmittance in the remaining ABS solution at 280 nm using the UV–vis spectrophotometer.

Moreover, we chose albumin from human serum (AHS) to further evaluate the anti-protein adhesion performance of the cotton fabric samples. The cotton fabric samples (3 cm × 3 cm)

were immersed into the AHS solution (10 mL, 20 mg/mL) and stirred for 10 min. The anti-protein adsorption ability was evaluated by measuring the decrease in light transmittance in the remaining AHS solution at 278 nm using the UV–vis spectrophotometer.

9. Skin stimulation test

The skin integrity stimulation of original cotton and both side of the DMC-Cotton-3-PEG fabrics were carried out on three rats. Before testing, the hairs (3 cm × 3 cm) on the back were removed with no damage to skin. These rat skins were covered with original cotton and both side of the DMC-Cotton-3-PEG fabrics for 24 h. Then remove the fabric, and take a photograph for these mice skin to observe erythema and edema by the naked eye. For the damaged skin stimulation, the tested rat skins were pierced until exudation occurred. The test method and evaluation were the same as that of the skin integrity stimulation test.

In addition, after the damaged skins of the rats were covered by fabrics for 24 h, remove the fabrics, and the epithelial cells of the skin were extracted for flow cytometry. The specific method is divided into two steps; the first is the acquisition of rats skin epithelial cells, the skin pieces were shaken twice at 250 rpm for 20 min at 37˚C in HBSS medium supplemented with 5% FBS (Gibco) containing 2 mM EDTA. The second is to perform flow cytometry on the epithelial cells, the antibodies used in this study including anti-TCRβ-PE (H57-597) was purchased from BD Biosciences, F4/80 (BM8) was obtained from eBioscience. Data were acquired on a FACSCanto™ II flow cytometer (BD Biosciences) and analyzed with FlowJo (Treestar) software. To analyze morphological changes, rat skin samples were paraffin-embedded, sectioned and stained with hematoxylin and eosin (H&E). The staining tissue slices were observed under the light microscopy (OLYMPUS BX51) for standard histological analysis.

All the experiments in the skin test were performed in compliance with the laws and guidelines by the Department of Science & Technology of Zhejiang Province (China), and their committees have approved the experiments.

10. Cytotoxicity analysis

DMC-Cotton-3-PEG fabric (3 cm × 3 cm) was immersed in a physiological saline solution (10 mL), stirred at 25 ^{o}C for 24 h to obtain a leachate solution. Before vitro analysis, the leachate solution was sterilized in an autoclave (121 ^{o}C, 20 min). The obtained leachate solution was used for cytotoxicity tests. The samples for the cytotoxicity tests are listed in Table S6.

10.1 In vitro cytotoxicity

The in vitro cell cytotoxicity analysis of leachate solution from DMC-Cotton-3-PEG fabric was measured using a cell counting kit-8 (CCK-8) assay. In brief, Huh7 cells were seeded in a 96-well plate at a density of 1×10^{6} cells/mL and cultured for 8 h in 100 μL of 10 % FBS-DMEM. Then the leachate solutions (Table S4) were added in the plates respectively. The plates were not added any solutions as a negative control sample. After incubation for 24 h, the medium was added

with 10 μL CCK-8 solution. Then the plates were further incubated for 1 h, the absorbance at 450 nm was measured using a microplate reader (Epoch2, BioTek).

10.2Cell apoptosis assay

The apoptosis inducing capability of leachate solution were evaluated with the Annexin V-FITC Apoptosis Detection Kit. Huh7 cells were seeded in 12-well plates at the density of 1×10^6 cells/mL and cultured in 1 mL of DMEM containing 10% FBS for 8 h. The plates were divided into two groups, one groups were added 10 μL of physiological saline (control group), and other groups were added 10 μL leachate solution obtained from DMC-Cotton-3-PEG fabric (c group). After continue to cultivate for 24 h, 5 μL of Annexin Ⅴ-FITC and 5 μL of PI solution were added to 100 μL of cell suspension, mixed and incubated at room temperature in the dark for 15 min, and then subjected to flow cytometry detection. Data were acquired on a FACSCanto™ II flow cytometer (BD Biosciences) and analyzed with FlowJo (Treestar) software

In order to observe the apoptosis of cells more directly, Huh7 cells were seeded in 12-well plates at the density of 1×10^6 cells/mL and cultured in 1 mL of DMEM containing 10% FBS for 8 h. The plates were divided into two groups, one groups were added 10 μL of physiological saline (control group), and other groups were added 10 μL leachate solution obtained from DMC-Cotton-3-PEG fabric (c group). Then continue to cultivate for 24 h, the Huh7 cells were stained with DAPI and Annexin V. A fluorescent microscope was used to observe the Huh7 cells.

11. Laundering method

Laundering durability was evaluated by monitoring the antimicrobial ability of the cotton fabric sample periodically after every stringent washing process. The cotton fabrics (15 mm × 15 mm) were washed with 50 mL of an aqueous solution of sodium dodecanesulphonate (2.0%, w/w) in a beaker, (diameter, 50 mm) stirring (300 rpm, magnetic stirrer, 9 mm × 25 mm) at 25 ^{o}C for 10 min, rinsed with deionized water (25 ^{o}C, 10 mL × 4 times), and dried at 60 ^{o}C. For comparison, another washing test process basing on the AATCC test method 61-2006 was further performed. Briefly, the cotton fabrics (15 mm × 15 mm, 10 piece) were washed in an aqueous solution of sodium dodecanesulphonate (200 mL, 2.0 %, w/w), with mechanical stirring (250 rpm) and abrasion effect of 20 stainless steel balls (diameter, 6.4 mm) in a beaker at 45 ^{o}C for 10 min, rinsed with deionized water (25 °C, 100 mL × 4 times), and dried at 60 ^{o}C.

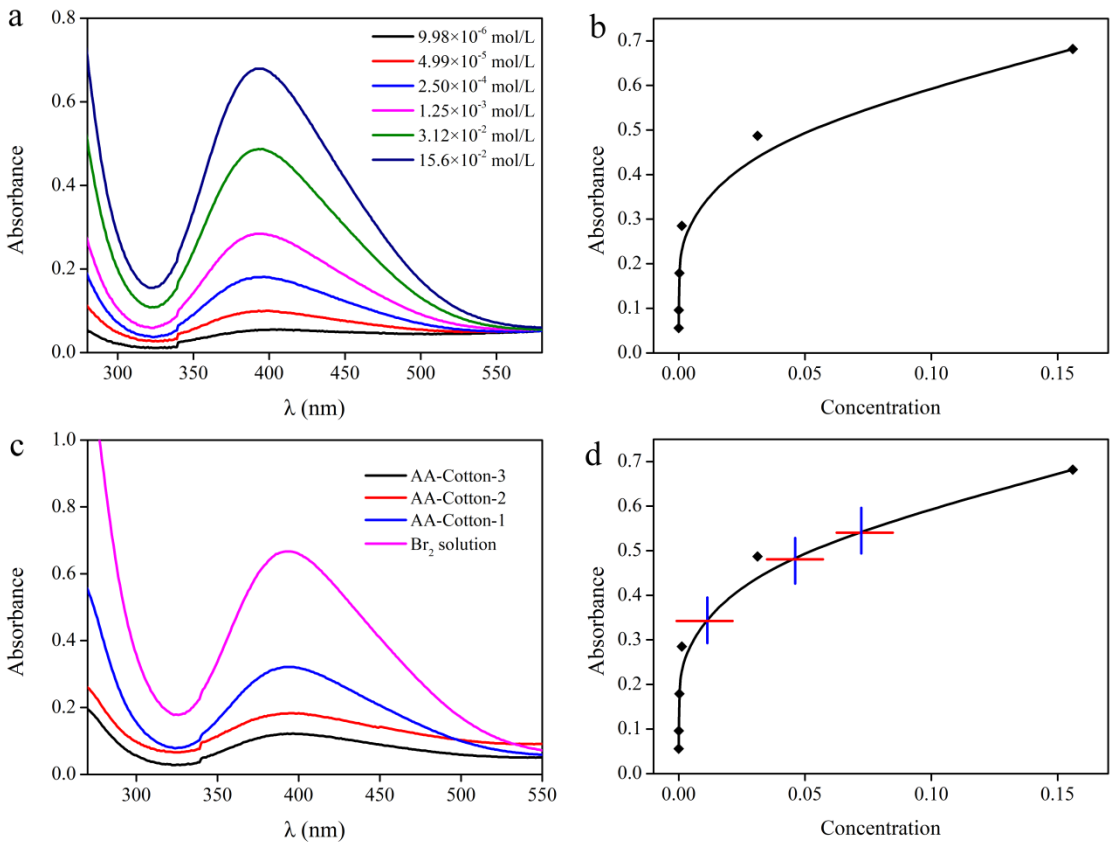

Fig. S2 The UV–vis absorbance spectra of Br_2 solution at different concentrations (a). Linear relationship of absorbance at 393 nm versus Br_2 solution concentration (b). UV–vis absorbance spectra of the Br_2 solutions which absorbed with the fabric samples (c). The amount of Br_2 solution concentration after absorbed with the fabric samples (d).

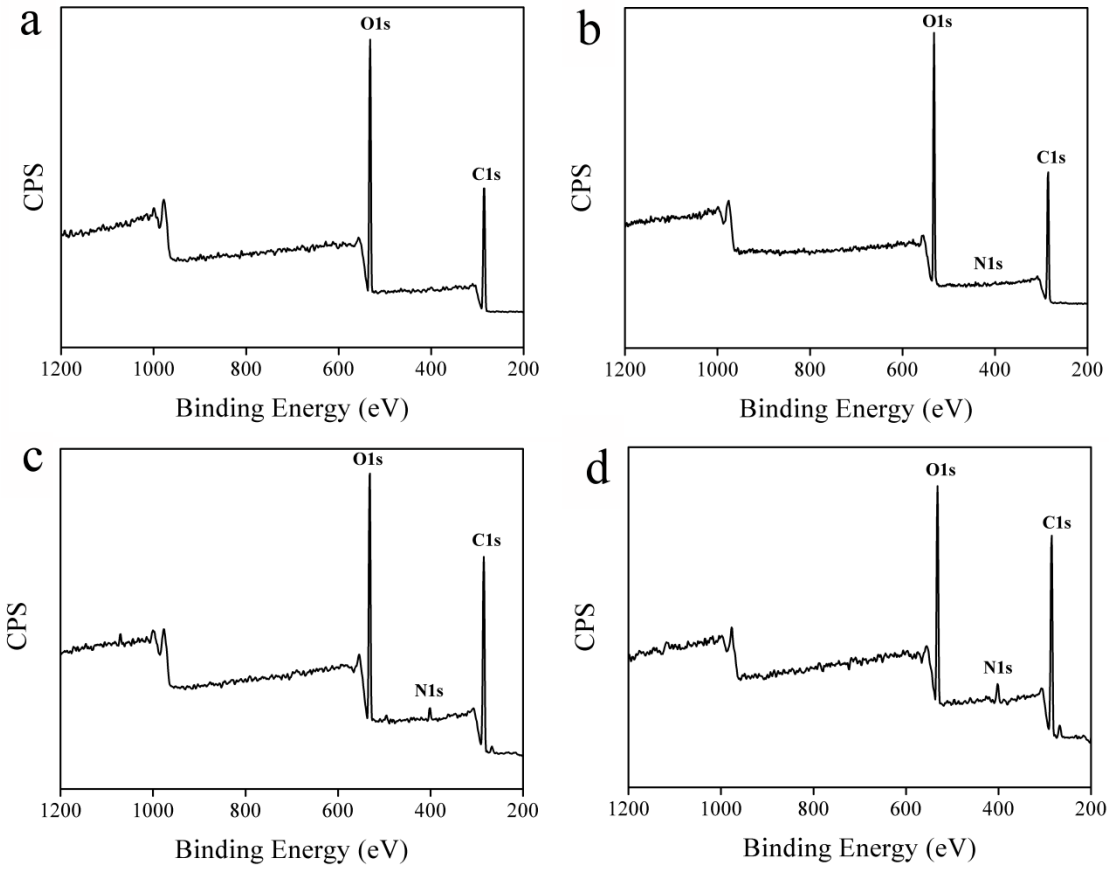

Fig. S3 XPS survey of the original cotton fabric (a), DMC-Cotton-1 fabric (b), DMC-Cotton-2 fabric (c), and DMC-Cotton-3 fabric (d).

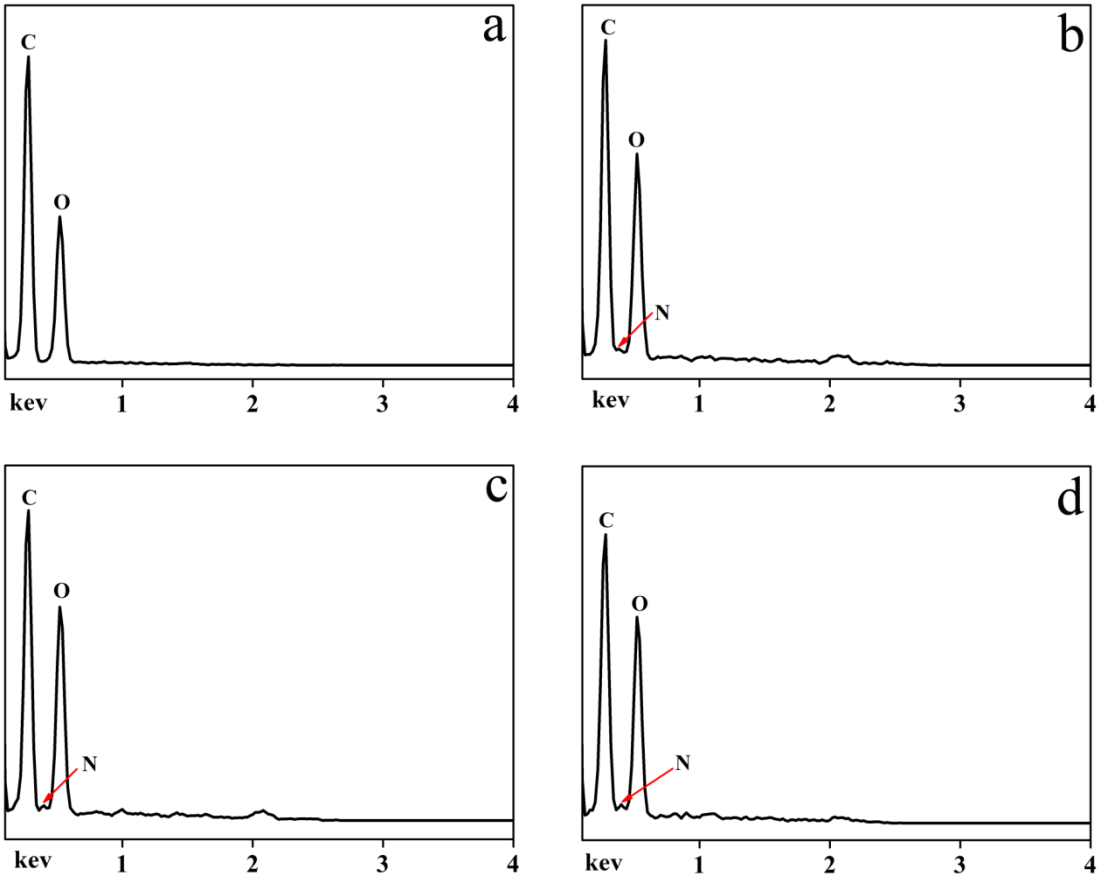

Fig. S4 The EDS spectra of the original cotton fabric (a), DMC-Cotton-1 fabric (b), DMC-Cotton-2 fabric (c), and DMC-Cotton-3 fabric (d).

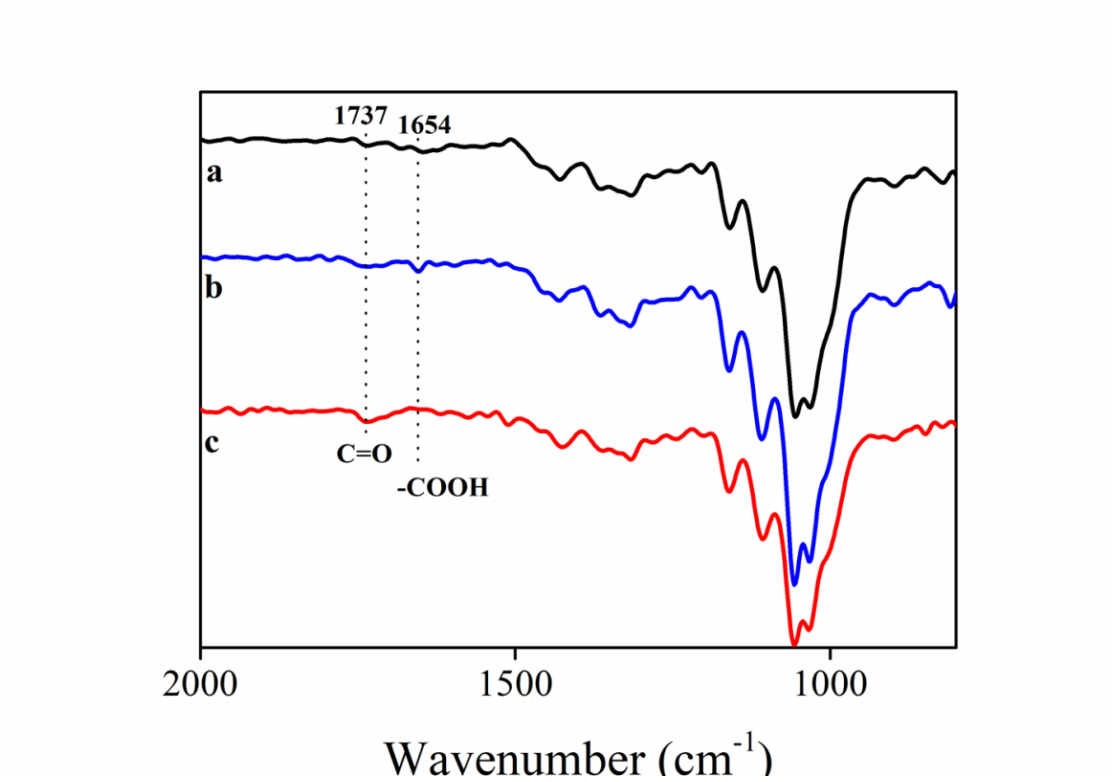

Fig. S5 ATR spectra of original cotton fabric (a), the opposite side of DMC-Cotton-3 fabric polymerized with AA monomer (b), and DMC-Cotton-3-O fabric (c).

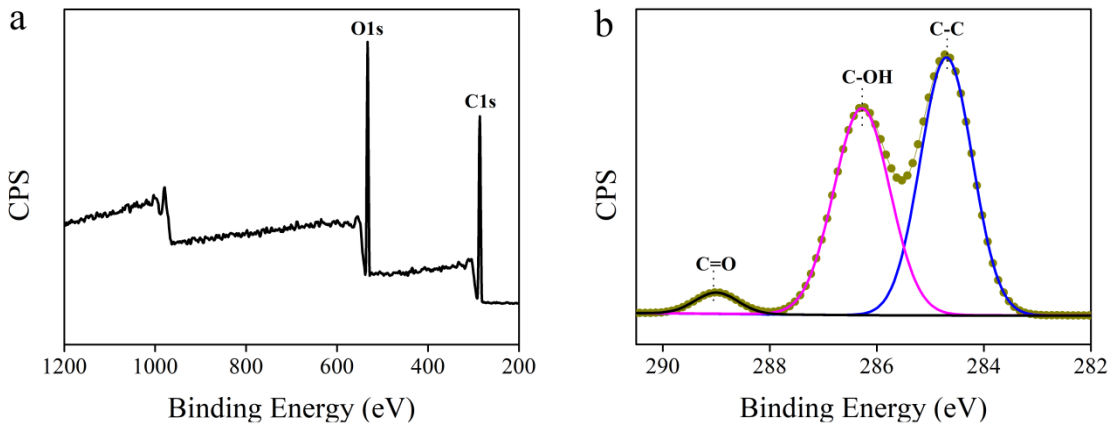

Fig. S6 XPS spectra and deconvolution C1s XPS of the DMC-Cotton-3-O.

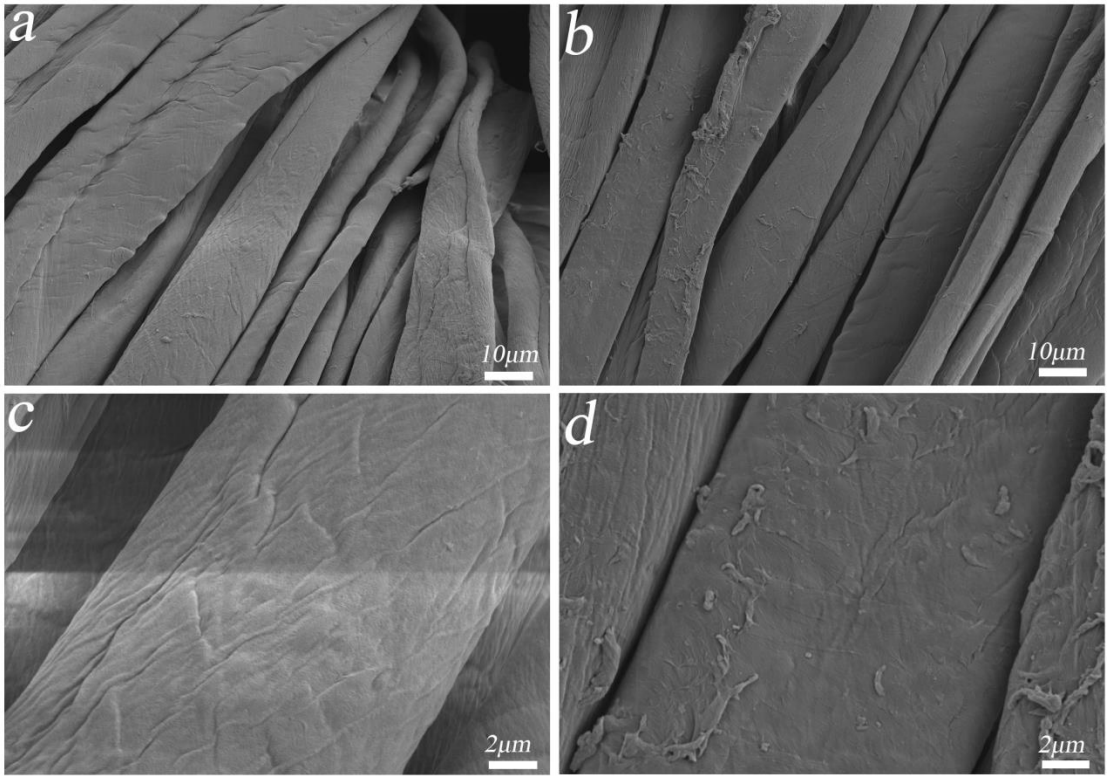

Fig. S7 SEM image of the opposite side of the DMC-Cotton-3 fabric (a, c), and the DMC-Cotton-3-O fabric (b, d).

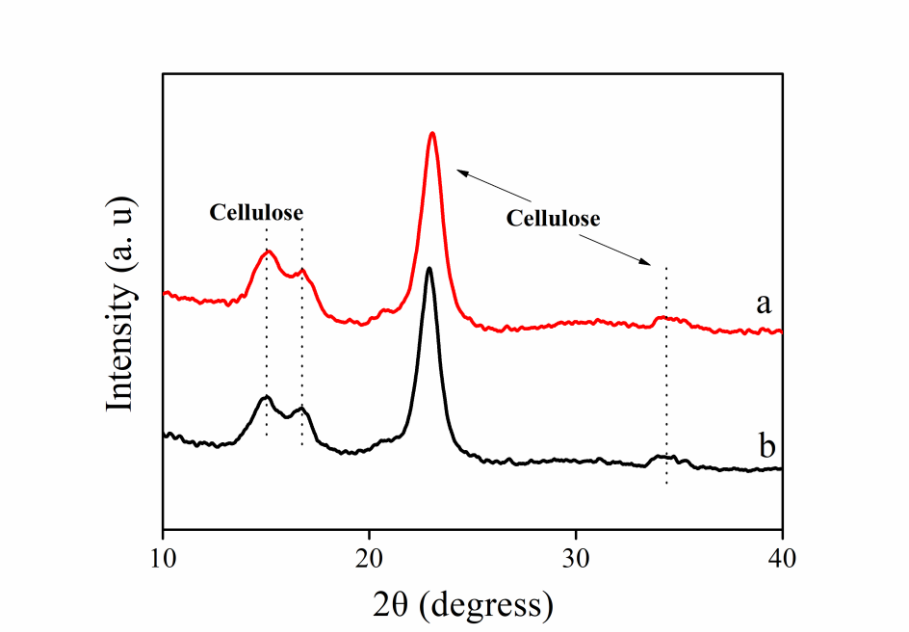

Fig. S8 XRD curves of the DMC-Cotton-3 fabric (a), and the DMC-Cotton-3-O fabric (b).

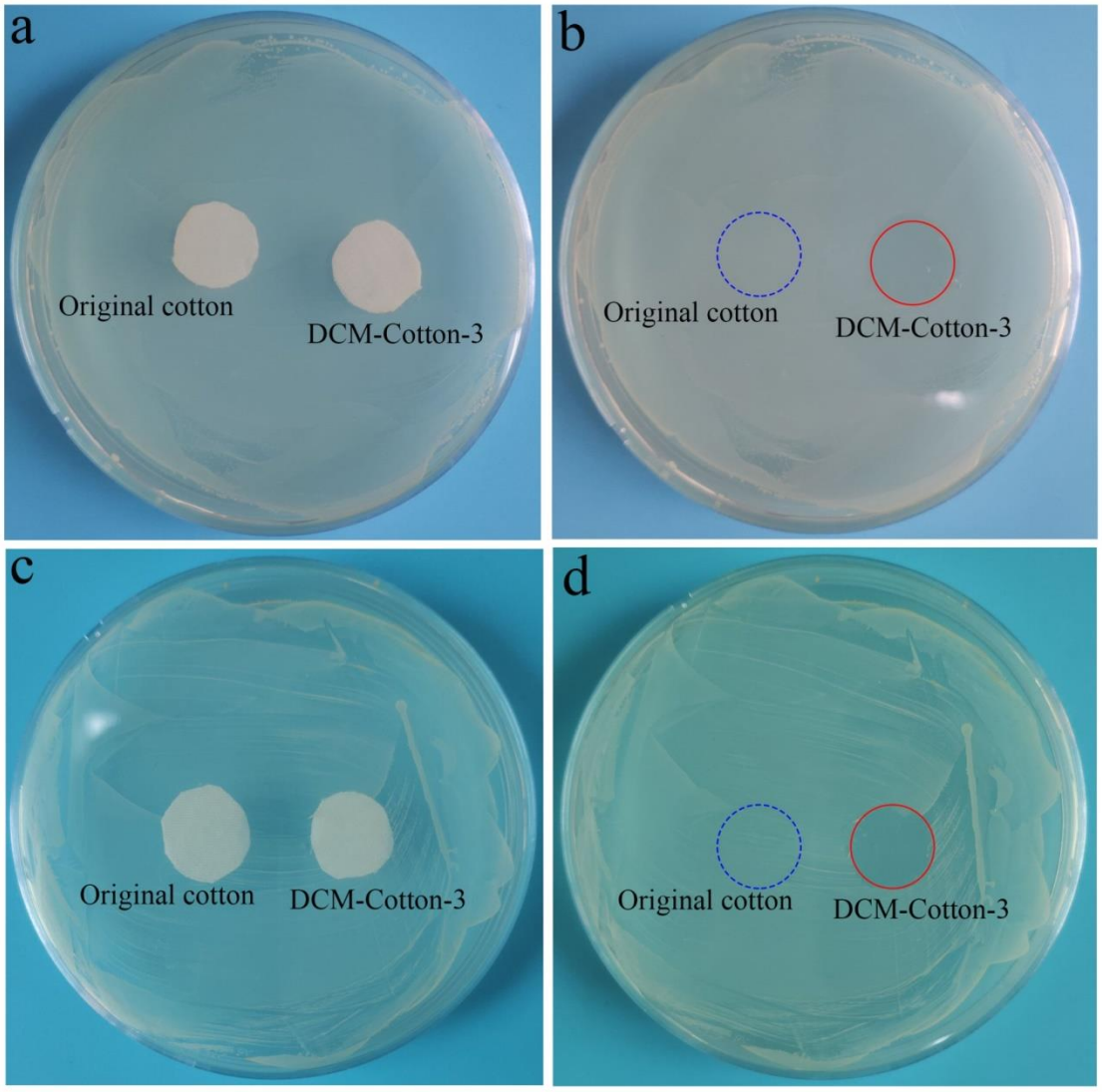

Fig. S9 Photographs of the inhibition zones of fabric samples against *E. coli* (a, b) and *S. aureus* (c, d).

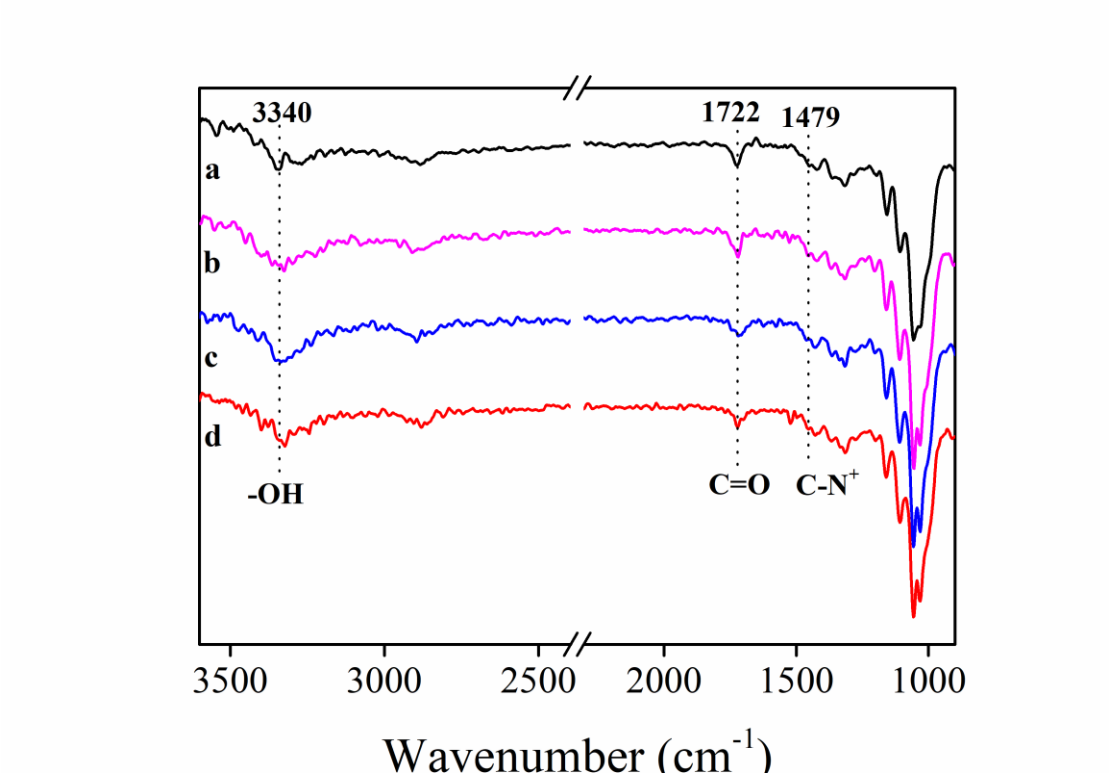

Fig. S10 ATR spectra of the DMC-Cotton-3 surface after withstand 20 (a), 40 (b), 60 (c), and 80 (d) laundering cycles.

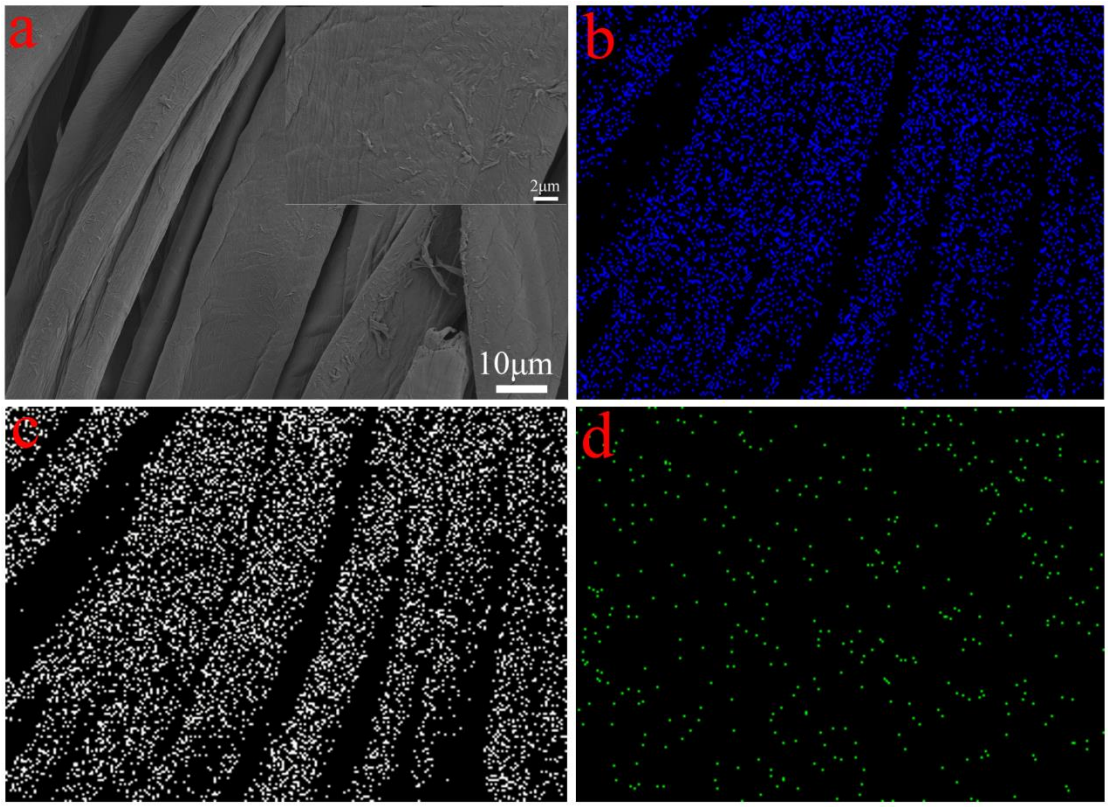

Fig. S11 SEM and C (b), O (c), and N (d) element mapping images of the DCM-Cotton-3 fabric after withstand 80 laundering cycles.

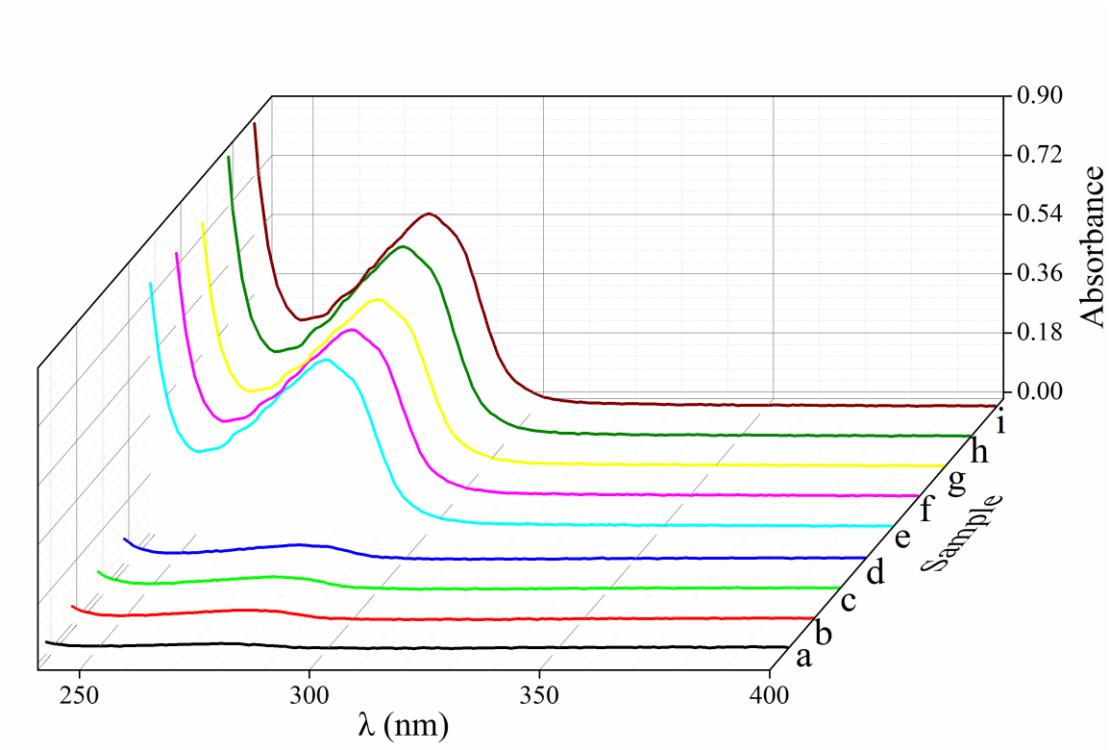

Fig. S12 Anti-protein adhesion behavior of cotton fabric samples. UV–vis spectra of albumin from human serum (AHS) solution after absorption by the original cotton (a), DMC-Cotton-1 (b), DMC-Cotton-2 (c), DMC-Cotton-3 (d), DMC-Cotton-1-PEG (e), DMC-Cotton-2-PEG (f), DMC-Cotton-3-PEG (g), PEG-Cotton-PEG (h), and AHS solution without absorption (i).

Table S1 The information of the modified cotton fabric.

Sample	DMC (mmol/L)	$(NH_4)_2S_2O_8$ (mmol/L)	Na_2SO_3 (mmol/L)
DCM-Cotton-1	53.20	1.07	1.07
DCM-Cotton-2	266.01	5.32	5.32
DCM-Cotton-3	532.02	10.64	10.64

Table S2 The information for the both sides of the modified fabric.

Fabrics	Outside	Inside
Original cotton	--	--
DMC-Cotton-1-PEG	DMC-Cotton-1	DMC-Cotton-3-O
DMC-Cotton-2-PEG	DMC-Cotton-2	DMC-Cotton-3-O
DMC-Cotton-3-PEG	DMC-Cotton-3	DMC-Cotton-3-O
DMC-Cotton-3-DMC	DMC-Cotton-3	DMC-Cotton-3
PEG-Cotton-PEG	DMC-Cotton-3-O	DMC-Cotton-3-O

Table S3 Estimated quantities of C=C double bonds on the AA-Cotton fabrics

Sample	AA-Cotton-1	AA-Cotton-2	AA-Cotton-3
Q (mmol/cm^2)[a]	4.54×10^{-3}	1.83×10^{-2}	2.89×10^{-2}

[a.] The quantities of C=C double bonds on the fabric sample.

Table S4 The grafted amount of both the polymers determined by gravimetric method.

Sample	M_1 (mg)[a]	Q_1(mg/cm^2)	M_2 (mg)[b]	Q_2(mg/cm^2)
DMC-Cotton-1-PEG	2.5±0.1	0.010±0.001	17.6±0.3	0.070±0.001
DMC-Cotton-2-PEG	12.8±1.1	0.051±0.004	15.8±0.6	0.063±0.002
DMC-Cotton-3-PEG	26.1±0.9	0.104±0.003	15.7±0.5	0.062±0.002

[a.] The amount of DMC grafted onto the front surface of modified cotton fabric (ten pieces, 5 cm × 5 cm);

[b.] The amount of PEG-400 grafted on the inside surface of the modified cotton fabric (ten pieces, 5 cm × 5 cm).

Table S5 The elemental contents that measured by the XPS scans on the fabrics.

Sample	Atomic composition (%)			
	C1s	O1s	N1s	Cl2p
Original cotton	58.76	41.24	--	--
DMC-Cotton-1	58.57	39.00	1.85	0.58
DMC-Cotton-2	64.09	30.77	3.12	1.41
DMC-Cotton-3	65.62	27.02	4.58	2.78

Table S6 Sample composition for the CCK-8 test.

Sample	Leachate solution[(1)] (μL)	Physiological saline solution(μL)
control	0	0
a	0	1
b	0.5	0.5
c	1	0

[(1).] The leachate was obtained by incubating DMC-Cotton-3-PEG fabric in physiological saline solution for 24 h.

As shown in Table S7**,** the BR rate of the DMC-Cotton-3 surface remained 100% even after 30 washing cycles. Moreover, the BR rate of DMC-Cotton-3 surface was also above 96.5% even after 50 washing cycles. This result once again proves that the modified fabric has superior antibacterial properties and excellent laundering durability.

Table S7 BR values of the DMC-Cotton-3 fabric after washing

Washing period[a]	BR (%)	
	E. coli	*S.aureus*
10	100.0	100.0
30	100.0	100.0
50	96.8	96.5

[a.] The washing test adopts the AATCC test method 61-2006.