Supporting Information

Anti-superbug cotton fabric with excellent laundering durability

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Experimental details

Materials

Cotton fabric was purchased from Shanghai Textile Industry Institute of Technical Supervision. BVIM was purchased from Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences. The monomer was used without any further purification. Deionized water was used throughout this work.

Preparation of anti-superbug cotton fabric

Water solution of 1-butyl-3-vinyl imidazole chloride (BVIM) with concentration vary from 20% to 60% (v/v) was prepared. Cotton fabric samples were immerged into the solutions. After full absorption of BVIM solution (about 30 min), the cotton fabric samples were padded and squeezed with stainless steel rods to keep the liquid rate on the cotton fabric around 100% (w/w). Then the cotton fabric samples were put into tubes and vacuum pumped for 5 min to remove oxygen. The tubes were sealed and then irradiated by γ-ray in a ^{60}Co source to the absorbed dose of 30 kGy at room temperature. After that, the grafted cotton fabric samples were extracted with hot-water to remove the residual monomer and homopolymer. Finally, the modified cotton fabrics (denoted as cotton-g-PBVIM) were vacuum-dried for further measurements.

The degree of grafting (DG) of the BVIM on cotton fabric was measured by gravimetric method and calculated by eq. (1):

$$DG = (W_g - W_0)/W_0 \times 100\% \quad (1)$$

Where: W_0 is the weight of the pristine cotton fabric, W_g is the weight of the cotton fabric after irradiation induced grafting.

Characterization of the anti-superbug cotton fabric

Fourier transform infrared (FT-IR) spectra were used to analyze the chemical structure of the cotton fabric. The FT-IR analysis was taken on a BRUKER TENSOR 27 FT-IR spectrometer. The pristine and functionalized cotton fabrics were measured under attenuated total reflection (ATR) method.

SEM analysis was performed on a JEOL JSM-6700F SEM instrument. The pristine cotton fabric and functionalized cotton fabrics were deposited of gold by sputtering. The SEM voltage was set at 10 kV.

The moisture permeability of cotton fabric was evaluated by measuring the water vapour transmission rate (WVTR) according the standard of GB/T 12704.2-2009. About 34 mL distilled water was put into an aluminium cup with a diameter of 7.4 cm and weighed after being balanced for 1 h at 38 ℃ and 50% humidity to mimic the conditions under which the human body starts to sweat. The fabric samples were placed on the amuminium cup. WVTR values were calculated from the change in the weight in the 2 h period using the following equation:

$$WVTR = \Delta W / At \quad (2)$$

Where: ΔW is the change of the weight of the water contained in the cup, A is the test area of the sample, t is the time duration (in this case, t is 2 h).

The softness of cotton fabric was evaluated by measuring the stiffness of the fabric according to the standard of GB/T 18318.1-2009. The lower stiffness means the fabric

is softer. The stiffness of the pristine and the grafted cotton fabric was measured in a YG (B) 002D automatic textile stiffness testing instrument (Wenzhou Darong Textile Instrument Co., Ltd).

Antibacterial efficacy test of the anti-superbug cotton fabric

Antibacterial properties of the functionalized cotton fabric to *E. coli* (ATCC 8739), *S. aureus* (ATCC 6538), *MRSA* (ATCC 43300), *E. faecium* (CICC 20537), vancomycin-resistant *Enterococci faecium* (*VREF*, CICC 21605), and *C. albicans* (ATCC 10231) were tested by TüV SüD Products Testing Co. Ltd. (Shanghai, China). Antibacterial properties of the functionalized cotton fabric to *Acinetobacter calcoaceticus* carrying *blaNDM-1* gene were tested by Academy of Military Medical Sciences, China. The procedures of these tests were according to the AATCC (American Association of Textile Chemists and Colorists) test method 100-2012. Circular swatches 4.8±0.1 cm in diameter from the test cotton fabrics and control sample were cut and stacked in 250 mL wide-mouth jars with screw caps, respectively. 1 mL bacteria solution with the bacteria density of 1~2 × 10^5 cfu/mL (colony formatting units/mL) is put onto each sample. As soon as possible after inoculation (0 h), 100 mL neutralizing solution was added to each of the jars. The jars were shaked vigorously for 1 min. Then serial dilutions (such as 10, 10^2, 10^3) were made with water and plated on nutrient agar. The same inoculation procedures to another test sample and control sample were repeated, and the inoculated cotton fabrics were cultured at 37 ^oC for 24 h. 100 mL neutralizing solution was added to the jars. After shaking the jars vigorously for 1 min, serial dilutions with water were made and

plated on nutrient agar. All plates were incubated at 37 ^{o}C for 48 h. The bacteria counts were counted as the bacteria numbers per sample. The reduction ratio of bacteria by the specimen was calculated by the following equation:

$$\text{Reduction} = (C_{0h}\text{-}A_{24h})/C_{0h} \times 100\% \quad (3)$$

Where: A_{24h} = the number of bacteria recovered from the inoculated test specimen swatches in the jar incubated after 24 h, C_{0h} = the number of bacteria recovered from the inoculated control sample in the jar incubated after inoculation for 0 h.

Antibacterial properties of the functionalized cotton fabric to *K. pneumoniae* (ATCC 4352), *S. aureus* (ATCC 6538), *MRSA* (ATCC 33592), *A. calcoaceticus* (ATCC 23055) were tested by Hohenstein Laboratories GmbH Co. KG. The test procedures were according to the standard of ISO (International Organization of Standardization) 20743: 2013. The procedures were similar to the AATCC standard mentioned above. The calculation of the reduction efficacy was according to the following equation:

$$\text{Germ reduction} = \log_{10}C_{18h} - \log_{10}T_{18h} \quad (4)$$

Where: C_{18h} and T_{18h} are the bacteria numbers recovered from the inoculated control sample and the test sample in the jar incubated after 18 h.

In this article, for easy to compare the results based on ISO standard to the results based on AATCC standard, the differences of logarithm values were calculated and converted into reduction ratio values, therefore, equation 2 was turned into the following equation:

$$\text{Reduction} = (C_{18h}\text{-}A_{18h})/C_{18h} \times 100\% \quad (5)$$

Laundering durability test

A laundering durability evaluation was carried out according to AATCC Test method 61−2006, condition 2A. The cotton fabrics were cut into 50 mm × 150 mm patches and washed in a rotating closed canister containing 150 mL aqueous solution of an AATCC standard WOB (without optical brighteners) detergent (0.15%, w/w) and 50 stainless steel balls in a thermostatically controlled water bath at 49 ^{o}C, 40 ± 2 rpm. One accelerated laundering circle equals to 5 times domestic laundering.

Test of animal skin irritation

The animal skin irritation test was conducted based on the requirements of ISO 10993-10:2010: Biological evaluation of medical devices Part 10: Test for irritation and skin sensitization. Male rabbits were closely clipped the fur on the back of the animals. Two 2.5 cm × 2.5 cm antibacterial cotton fabrics and two negative controls were directly applied to the clipped region on each rabbit for 24 h, 48 h, and 72 h. The tissue reaction for erythema and oedema were graded according to the classification system. Irritation score was from 0 (no erythema or no oedema) to 4 (severe erythema or severe oedema). Observations after 1 h, 24 h, 48 h, and 72 h contact time were used for calculation. For each animal, the score both erythema and oedema at each time point were added together separately for each test sample and the negative control.

Test of the acute oral toxicity of antibacterial cotton fabric

The acute oral toxicity test was conducted based on GB 15193.3-2014. 20 g antibacterial cotton fabrics were immerged into 60 mL distilled water and extracted at 60 ^{o}C for 2 h. Ten male and ten female mice were given extracted fluid 10 g kg^{-1} by

gavage. Toxic symptom was observed every 2 days and the weight of each animal was measured every 1 week. The dead animals and the survivals after 14 days were anatomized for general pathological observation.

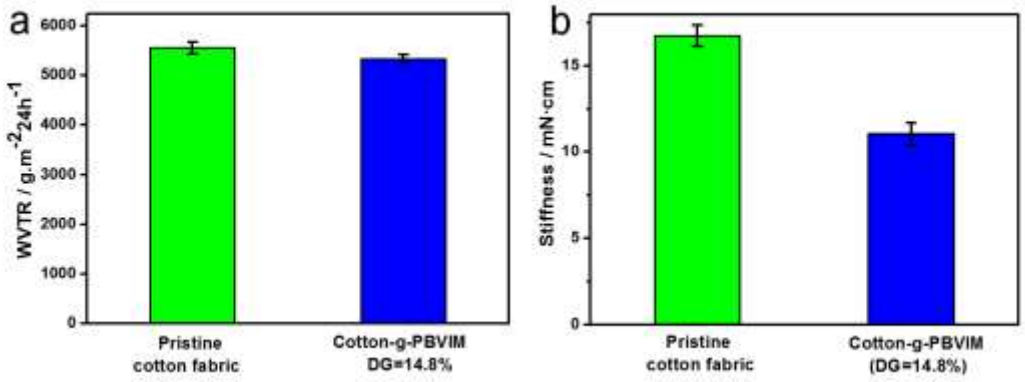


Figure S1. (a) The water vapor transmission rate (WVTR) of the pristine cotton fabric and cotton-g-PBVIM with a DG of 14.8%; (b) the stiffness of the pristine cotton fabric and cotton-g-PBVIM with a DG of 14.8%.

Table S1. The averaged colony count of various bacteria cultured on the control sample and the cotton-g-PBVIM with the DG of 14.8% for 24h according to the standard of AATCC-100: 2012.

Bacteria	Colony count of bacteria on control sample at 0 h (cfu/mL) (C)	Colony count of bacteria on control sample after 24 h (cfu/mL) (G)	Colony count of bacteria on cotton-g-PBVIM with DG of 14.8% after 24 h (cfu/mL) (A)	Reduction of bacteria (%) (R)
E. Coli	1.3×10^5	1.4×10^8	<100	> 99.92
E. faecium	1.6×10^5	4.2×10^6	<100	> 99.94
VREF	1.4×10^5	9.5×10^6	<100	> 99.94
A. calcoaceticus carrying *blaNDM-1*	3.6×10^4	6.2×10^6	<1	> 99.99
C. albicans	1.1×10^3	1.1×10^6	<1	> 99.91

Table S2. The averaged colony count of various bacteria cultured on the control sample and the cotton-g-PBVIM with the DG of 14.8% for 18h according to the standard of ISO 20743: 2013.

Bacteria	Control sample		Cotton-g-PBVIM with DG of 14.8%		Germ reduction (log_{10}cfu)
	Absolute cfu	log_{10}cfu	Absolute cfu	log_{10}cfu	
K . pneumoniae	3.92×10^{7}	7.59	6.15×10^{3}	3.79	3.80
A . calcoaceticus	1.23×10^{8}	8.09	1.64×10^{2}	2.21	5.88
S. aureus	8.81×10^{5}	5.94	< 20	≤ 1.28	≥ 4.67
MRSA	2.05×10^{6}	6.31	<20	≤ 1.28	≥ 5.03

a b

Figure S2. The colony count of *S. aureus* (ATCC 6538) cultured on the control sample (a) and the cotton-g-PBVIM with the DG of 14.8% (b) for 18h. The condition of the bacterial culture is according to the standard of ISO 20743: 2013.

Figure S3. The colony count of *Klebsiella pneumoniae* (ATCC 4352) cultured on the control sample (a) and the cotton-g-PBVIM with the DG of 14.8% (b) for 18h. The condition of the bacterial culture is according to the standard of ISO 20743: 2013.

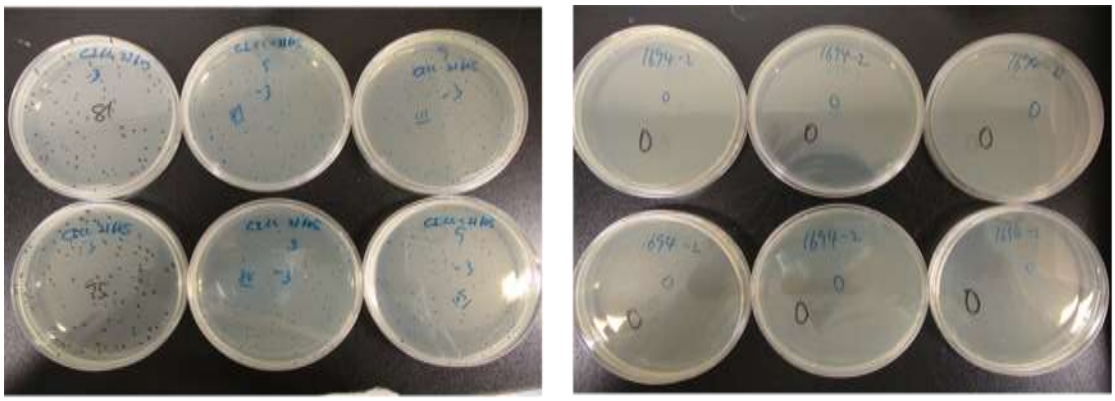
Figure S4. The colony count of *Enterococcus faecium* (CICC 20537) cultured on the control sample (a) and the cotton-g-PBVIM with the DG of 14.8% (b) for 24h. The condition of the bacterial culture is according to the standard of AATCC-100: 2012.

a b

Figure S5. The colony count of *Acinetobacter calcoaceticus* (ATCC 23055) cultured on the control sample (a) and the cotton-g-PBVIM with the DG of 14.8% (b) for 18h. The condition of the bacterial culture is according to the standard of ISO 20743: 2013.

a b

Figure S6. The colony count of *MRSA* (ATCC 33592) cultured on the control sample (a) and the cotton-g-PBVIM with the DG of 14.8% (b) for 18h. The condition of the bacterial culture is according to the standard of ISO 20743: 2013.



a b

Figure S7. The colony count of *VREF* (CICC 21605) cultured on the control sample (a) and the cotton-g-PBVIM with the DG of 14.8% (b) for 24h. The condition of the bacterial culture is according to the standard of AATCC-100: 2012.

a b

Figure S8. The colony count of *Canidia albicans* (ATCC 10231) cultured on the control sample (a) and the cotton-g-PBVIM with the DG of 14.8% (b) for 24h. The condition of the bacterial culture is according to the standard of AATCC-100: 2012.

Table S3. After 30 accelerated laundering cycles, the averaged colony count of various bacteria cultured on the control sample and the cotton-g-PBVIM with the DG of 14.8% for 24h according to the standard of AATCC-100: 2012.

Bacteria	Colony count of bacteria on control sample at 0 h (cfu/mL) (C)	Colony count of bacteria on control sample after 24 h (cfu/mL) (G)	Colony count of bacteria on cotton-g-PBVIM with DG of 14.8% after 24 h (cfu/mL) (A)	Reduction of bacteria (%) (R)
E. Coli	1.1×10^{3}	3.0×10^{6}	<1	> 99.91
S. aureus	8.5×10^{2}	6.9×10^{5}	<1	> 99.88
MRSA	1.4×10^{5}	4.2×10^{6}	<100	> 99.93
VREF	1.4×10^{5}	9.5×10^{6}	<100	> 99.93
A. calcoaceticus carrying *blaNDM-1*	3.6×10^{4}	6.2×10^{6}	<1	>99.99
C. albicans	1.1×10^{3}	1.1×10^{6}	<1	> 99.91

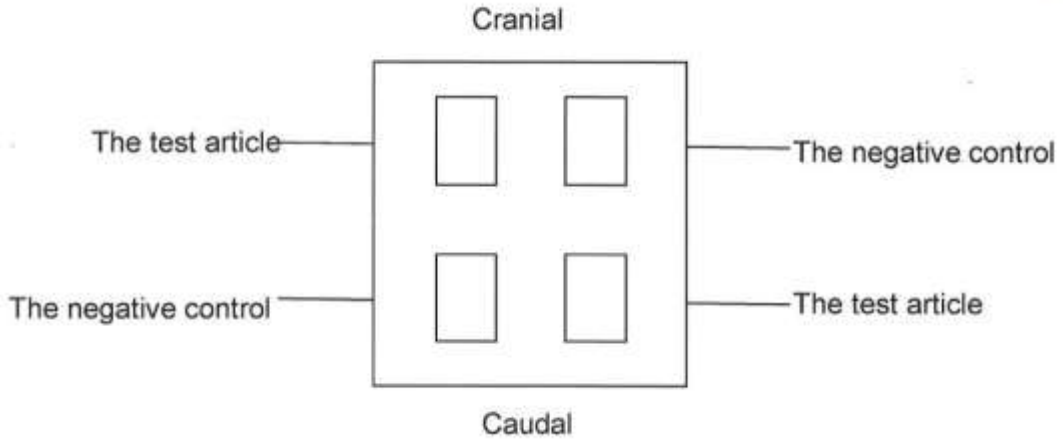


Figure S9. Illustration of the contact region of cotton-g-PBVIM (DG=14.8%) and negative control on a rabbit's back.

Table S4. Primary irritation index categories in rabbit according to the ISO 10993.10-2010 standard.

Mean score	Response category
0--0.4	Negligible
0.5--1.9	Slight
2--4.9	Moderate
5—8	Severe

Table S5. Dermal responses to the rabbit skin contacting cotton-g-PBVIM (DG=14.8%) according to ISO 10993.10-2010 standard.

Rabbit number	Response	Primary irritation index					
		Observe time (24 h)		Observe time (48 h)		Observe time (72 h)	
		Sample	Control	Sample	Control	Sample	Control
1	Erythema	0	0	0	0	0	0
	Edema	0	0	0	0	0	0
2	Erythema	0	0	0	0	0	0
	Edema	0	0	0	0	0	0
3	Erythema	0	0	0	0	0	0
	Edema	0	0	0	0	0	0

Table S6. The acute oral toxicity of cotton-g-PBVIM (DG = 14.8%) in mice. The test was performed according to the GB 15193.3-2014 standard.

Dose (g/kg)	Gender	Number of mice	Weight (g) (mean ± SD)			Dead mice (N)
			0 days	7 days	14 days	
10	Male	10	20.5 ± 0.71	30.2 ± 0.92	31.1 ± 1.79	0
10	Female	10	20.7 ± 0.82	27.7 ± 1.42	33.4 ± 1.45	0