REGULAR ARTICLE

Durable Antimicrobial Cotton Fabrics Prepared by Oxygen Plasma Treatment and Adsorption of Guanidine Salts

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Abstract

Cotton fabrics with durable antimicrobial ability are in high demand in the market. However, most current methods for preparing such fabrics involve complex processes and the use of various organic solvents, resulting in high energy consumption. In this study, a simpler and more environmentally friendly method using oxygen plasma treatment to enhance the surface properties of cotton fabric was proposed. By introducing the antimicrobial agent poly(hexamethylene guanidine hydrochloride) (PHMG) through impregnation, to obtain durable antimicrobial cotton fabrics. Surface analysis confirmed the successful adsorption of PHMG on the cotton fabrics. The antimicrobial cotton fabrics demonstrated better sweat stain management and a strong bactericidal effect against E. coli and S. aureus. Plasma-treated antibacterial cotton fabrics demonstrate a remarkable ability to retain strong antibacterial properties even after undergoing 50 simulated washing cycles. The antibacterial rate remains consistently above 90%. In addition, the cytocompatibility and skin irritation of the antimicrobial cotton fabrics were further investigated. The results indicated that the antimicrobial cotton fabrics demonstrated a favorable safety profile. This straightforward and environmentally friendly method for preparing antimicrobial cotton fabrics holds promise for applications in the textile and medical industries.

Keywords Antibacterial · Cotton fabric · Oxygen plasma treatment · Poly(hexamethylene guanidine hydrochloride)

1 Introduction

Cotton fabrics are widely used for their excellent moisture absorption, comfort, breathability, and compatibility with human skin [1]. However, cotton fabrics with porous structures can easily become contaminated by human secretions, such as sebum and sweat, which serve as nutrients for the rapid growth of microorganisms. This creates an ideal environment for the proliferation of pathogenic bacteria, leading to the formation of bacterial biofilms. The presence of these biofilms not only affects the comfort of wearing cotton fabrics and the overall lifespan of the fabric but also poses a risk of cross-infection, particularly in public environments like hospitals [2–5]. Consequently, there is a significant demand for antibacterial cotton fabric in various fields [6].

Finishing methods for the preparation of antimicrobial cotton fabrics can be categorized into physical and chemical methods [7]. Zhou et al. employed the physical method of impregnation to coat the prepared Ag-PMANa onto cotton fibers, which solved the problem that the fabrics would be dyed black-brown when Ag was coated on the fabrics and exposed to air and/or light atmospheres. However, after subjecting the fabrics to 15 simulated washes, the antimicrobial effect seemed to be noticeably diminished [8]. Guo et al. employed a chemical modification technique to treat cotton fabrics with chloroacetic acid, thereby introducing carboxyl groups to the surface of cotton fibers. Subsequently, they grafted poly(hexamethylene bisguanidine) onto the cotton fibers through an amide reaction. This approach resulted in the development of durable antimicrobial cotton fabrics, with the antimicrobial activity remaining above 99.99% even after undergoing multiple washings, reuse, UV irradiation,

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dyeing, and high-temperature treatment [9]. As mentioned above, although the physical method is simple and convenient, the interaction force between the antimicrobial agent and the cotton fabric is weak, and there is the risk of antimicrobial component leakage within the process of use [10]. On the other hand, the chemical grafting method enhances the durability of antimicrobial function. However, it involves the use of various organic solvents during the modification process, which complicates the post-processing and increases the cost. Additionally, it becomes challenging to meet the requirements of green environmental protection. Therefore, improving the force between antimicrobial agents and fabrics to reduce the leakage of antimicrobial agents is a great challenge in developing a green and convenient preparation method of durable antimicrobial cotton fabrics [11].

Recent studies have pointed out that plasma treatments offer significant advantages over the physical or chemical treatments mentioned above [12]. Plasma treatment can accurately deliver energy or energetic particles to the target surface, which greatly improves the treatment efficiency and improves the surface polarity of the material in a short period of time. The physical and chemical reactions during the treatment process do not require solvents and the whole modification process does not produce pollution, which has significant advantages in terms of environmental protection, time and energy saving. Moreover, plasma treatment affects only the outermost layer of the material and does not alter the inner layer, making it ideal for surface modification [13, 14]. Kramar et al. conducted a study on the application of plasma treatment for cleaning cotton fibers. The study revealed that the plasma treatment primarily impacts the epidermal and primordial layers of cotton fibers, while having little effect on the internal lamellar structure [15]. Plasma treatment of cotton fabrics has been successfully used to enhance or replace wet finishing processes [16–18], endowing cotton fabrics with functional properties such as antibacterial [19], hydrophobic [20], dyeable [21, 22], sound absorption [23], crease-resistant [24], flame-retardant [25] and UV resistant properties [26, 27].

Polyhexamethylene guanidine (PHMG) possesses a high intrinsic positive charge, which enables its adsorption to the surface of cotton fabrics through electrostatic interaction. Additionally, its good water solubility reduces the need for organic reagents. Guanidine salts, when present in polar solvents, remain protonated across a wider pH range. This characteristic enables them to effectively neutralize bacteria over a broader pH range, exhibiting a broad-spectrum efficient bactericidal activity. Moreover, the bactericidal mechanism of PHMG prevents the development of drug resistance and ensures minimal toxicity [28–30]. Therefore, the utilization of PHMG for modifying cotton fabrics shows great promise.

In this work, a way to prepare durable antimicrobial cotton fabrics was proposed, which is a simple, inexpensive,

environmentally friendly and solvent-free method. Durable antimicrobial cotton fabrics were obtained by surface modification of cotton fabrics using oxygen plasma treatment technology and then adsorption of PHMG onto cotton fabrics by the impregnation method. The antimicrobial cotton fabric prepared by this method needs to be capable of stably adsorbing PHMG to the extent that it maintains a satisfactory antimicrobial effect after multiple washes. At the same time, comfort and excellent safety are indispensable features of antimicrobial cotton fabrics.

2 Experimental

2.1 Materials

Cotton fabrics (100% cotton, 240 g/m^2) were taken from ready-to-wear garments from Uniqlo, a subsidiary of Fast Retailing Co., Ltd.; glutaraldehyde was purchased from Shanghai Titan Co., Ltd.; Eosin was purchased from Shanghai Bohr Chemical Reagent Co., Ltd.; and polyhexamethylene guanidine hydrochloride (PHMG, Mw = 720 Da) was prepared in accordance with the previous work [31].

2.2 Plasma Treatment

Experiments were conducted using a Femto A plasma surface treatment system from Diener electronic. Both sides of 18 × 2 cm (warp direction) cotton fabrics were treated with oxygen plasma for various durations (30 s, 60 s, 180 s, 300 s) under the parametric conditions of 70 W power and 0.6 mbar pressure. The treatment time was limited to 300 s based on the initial experimental findings, as extending the treatment time did not have a significant impact on the wettability.

2.3 Preparation of Antimicrobial Cotton Fabrics

The treatments and sample names of the samples in the experiment are shown in Table 1. A is raw cotton fabric. B is the cotton fabric that has only been treated with oxygen plasma which is used to investigate the effects of oxygen plasma treatment on cotton fabrics. C was prepared using a physical impregnation method. Specifically, cotton fabrics measuring 2 × 2 cm were impregnated with different

Table 1 Samples notations

Sample name	Treatment of cotton fabric
A	Nontreated cotton fabric
B	Plasma-treated cotton fabric
C	Cotton fabric@PHMG
D	Plasma-treated cotton fabric@PHMG

concentrations of PHMG solution (0.1 g/L, 0.5 g/L, 1 g/L, 1.5 g/L, and 3 g/L). The impregnated fabrics were then shaken in a shaker for 3 h at a medium gear. Afterward, the fabrics were rinsed three times with deionized water and dried at 70 °C. This process resulted in the production of cotton fabrics Cn. D was prepared using the plasma treatment impregnation method. Specifically, the cotton fabric (2×2 cm) was treated with plasma and then impregnated with different concentrations (0.1 g/L, 0.5 g/L, 1 g/L, 1.5 g/L, and 3 g/L) of PHMG solution. The impregnated fabric was shaken in a shaker at medium gear for 3 h. Afterward, it was rinsed three times with deionized water and dried at 70 °C to obtain cotton fabric Dn. The 'n' denotes the concentration of PHMG.

2.4 SEM Analysis

The moisture of cotton fabrics was removed under vacuum. After gold spraying for 60 s, the surface morphology of all cotton fabrics, fixed with conductive adhesive, was obtained by SEM (S-3400, Hitachi, Tokyo, Japan).

2.5 Wettability Analysis

The wettability of plasma-treated and antimicrobial cotton fabrics were evaluated using the standard method FZT/T 01071 (2008) with slight modifications [32]. This method allows for the measurement of liquid absorption/capillary permeation in the pore structure, as well as liquid absorption on the fiber surface and inside. An 18×2 cm fabric sample (in the warp direction) was immersed in a 0.1% aqueous solution of eosin. The red color of the solution provided a clear indication of the liquid height while a ruler was assembled along the sample. The samples were submerged to a depth of 1 cm, and the rise of the liquid (h) was measured over time (t). Three measurements were taken for each sample, and the average of these measurements was calculated as the final result.

Based on the experimental data, the wettability parameter can be determined using the following equation [15]:

$$h^2 = D \times t \tag{1}$$

where D (mm^2 s^{-1}) is the capillary diffusion coefficient.

2.6 XPS Analysis

Changes in chemical bonds of plasma-treated cotton fabrics modified by PHMG were detected under vacuum by XPS (ESCALAB 250Xi, Thermo Fisher Scientific, Britain). The XPS data were produced by Kratos Axis Ultra furnished with a hemispherical electron energy analyzer. The energy of the incident photon was 1486.6 eV from monochromatic

Al Kα X-ray. With an analyzer passing energy of 100 eV, an energy step size of 1 eV, and a dwell time of 50 ms, survey scans were acquired (1200−0 eV). With an analyzer passing energy of 30 eV, an energy step size of 0.05 eV, and a dwell time of 250 ms, the high-resolution scans of C 1s were also obtained.

2.7 ATR-FTIR Analysis

The chemical bonding structures and functional groups of each group of cotton fabrics were analyzed using total reflection Fourier transform infrared spectroscopy (ATR-FTIR, Nicolet iS50, Thermo Fisher Scientific, Britain). Spectra were recorded in the frequency range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1}.

2.8 Zeta Potential Analysis

The cotton fabrics were crushed into staple fibers resembling cotton wool using a ball mill and configured as a 0.1 wt % suspension using deionized water as a dispersant. The zeta (ζ) potential of each group of cotton samples was determined using the electrophoretic light scattering (ELS) method us, employing an electrochemical workstation (Malvern Panalytical, UK). Three measurements were conducted for each sample and the average of these measurements was calculated as the final result.

2.9 Mechanical Properties Testing

The mechanical properties of cotton fabrics with different oxygen plasma treatment times were characterized by tensile experiments, according to the slightly modified ISO 13934-1:2013. The tensile experiments were carried out using an electronic universal testing machine of type ED26-502, at a tensile rate of 100 mm/min, a gauge length of 100 mm. The size of the cotton fabrics in each group of 140×35 mm, and each group of samples was tested three times.

2.10 Water Contact Angle Measurement

The wetting behavior of cotton fabrics was observed by measuring the contact angle. In this experiment, 1 μL of water droplets was placed on the surface of cotton fabrics and the state of the droplets was recorded by taking 500 photographs every 24 s. Each experiment was repeated at least three times.

2.11 Water Absorption Determination

The water absorption within 60 s of all cotton fabrics was determined by a gravimetric approach with slight modification [33]. In brief, the lyophilized samples were soaked in the

normal saline solution under 37 °C for 10 s, and the samples were transferred. Afterward, excess normal saline on the samples were absorbed with the filter paper. The percentage of water absorption (WAR) of all samples was evaluated by the following equation:

$$WAR\ (\%) = \frac{w_1 - w_0}{w_0} \times 100\% \quad (2)$$

where w_0 and w_1 were weights of the cotton fabrics before and after soaking, respectively.

2.12 Water Vapor Transmission Determination

The water vapor transmission rate (WVRT) of each cotton fabric was investigated by weighing method. Cotton fabrics were cut into appropriate sizes with scissors and then covered on top of a 5 mL glass test tube filled with 3 mL of deionized water and placed in an oven at 37 °C for 24 h. The WVRT of each cotton fabric was calculated as follows:

$$WVRT\ (\%) = \frac{w_0 - w_1}{AT} \times 100\% \quad (3)$$

where w_0 and w_1 were the weights of water in the test tubes before and after the test, respectively, and A and T are the contact areas of the cotton fabric with the test tubes and 24 h, respectively.

2.13 Antibacterial Activity Assay

Antibacterial activity against *E. coli* (BNCC336953, Bei Na Chuanglian Biotechnology Co., Ltd., China) and *S. aureus* (BNCC340652, Bei Na Chuanglian Biotechnology Co., Ltd., China) of antimicrobial cotton fabrics was evaluated by the colony-counting method [34]. Specifically, cotton fabrics (1×1 cm^2) were sterilized in 48-well plates with 75 wt % ethanol aqueous solution under UV light. The cotton fabrics were subsequently washed three times each with normal saline solution and standard liquid medium. Next, antimicrobial cotton fabrics were incubated with 400 µL bacterial suspension solution in the electric heating constant temperature incubator (Yuejin Medical Apparatus Factory, China) for 8 h under 37 °C. Afterwards, the cultured bacterial suspension was diluted 10^4 fold with the normal saline solution. Finally, 30 µL of the diluted bacterial suspension solution was evenly coated on the agar plate and the agar plate was cultured for 12 h in the above incubator. The antibacterial rate was obtained by Eq. 4 as follows:

$$AR\ (\%) = \frac{n_0 - n_1}{n_0} \times 100\% \quad (4)$$

where n_0 and n_1 were colony numbers of the co-cultured with cotton fabric A and antimicrobial cotton fabric on the agar plate, respectively.

The micrographs of *E. coli* and *S. aureus* after co-cultivation with cotton fabric A and antimicrobial cotton fabric were characterized by SEM [35]. Cotton samples (1×1 cm^2) were co-cultured with *E. coli* and *S. aureus* at 37 °C for 8 h. Then, *E. coli* and *S. aureus* that were not attached to the cotton fabrics were removed with saline solution. Bacteria attached to cotton fabrics were fixed with 2.5 wt % glutaraldehyde aqueous solution. Dehydration was carried out using a series of graded concentrations of ethanol (50%, 60%, 70%, 80%, 90%, 100%) and the treated samples were dried under vacuum.

Furthermore, the bacteriostatic circle method was used to test whether the antimicrobial cotton fabrics were performing antimicrobial activity by releasing PHMG. The cotton fabrics of each group, which had been pre-sterilized, were washed three times each with saline solution and standard liquid culture medium. 100 µL of undiluted bacterial suspension was uniformly applied on an agar plate and the groups of cotton fabrics were flatly adhered to the agar plate. The agar plates were inverted and incubated in a constant temperature incubator for 24 h to observe the production of bacteriostatic circles.

2.14 Antibacterial Durability Against Washing Assay

Antibacterial durability against washing of cotton fabrics was evaluated using AATCC 61-2007 with slight modifications [36]. Cotton fabrics (2×2 cm^2) were immersed in a 200 mL washing solution with a concentration of 0.37 wt % at a temperature of 40 °C. The solution was stirred using a magnetic stirrer for 45 min, simulating one washing cycle. One wash cycle is equivalent to 5 analog washes. Dry the cotton fabric after 50 washes. The antimicrobial persistence performance was evaluated by the trace method; briefly, washed cotton fabrics (1×1 cm^2) were sterilized in 48-well plates with 75 wt % ethanol aqueous solution under UV light. The cotton fabrics were subsequently washed three times with normal saline solution and standard liquid medium. The cotton fabrics were incubated with 400 µL of bacterial suspension in an electrically heated thermostatic incubator at 37 °C for 8 h. The incubated bacterial suspension was added to a sterile 96-well plate in a volume of 100 µL per well, and the absorption value of the bacterial suspension co-cultivated with the cotton fabrics was detected by an enzyme marker at 600 nm. The antibacterial rate after washing (ARW %) was calculated from the following equation:

$$\text{ARW \%} = \frac{\text{OD}_0 - \text{OD}}{\text{OD}_0} \times 100\% \qquad (5)$$

where OD_0 is the absorption value of pure cotton fabrics after co-culture with bacterial suspension and OD is the absorption value recorded for different number of washes for each antimicrobial cotton fabric.

2.15 Cytotoxic Evaluation

The cytotoxicity was detected via measuring the viability of mesenchymal stem cells (MSCs) co-incubated with wound dressing by CCK-8 kit (Dojindo, Molecular Technologies Inc., Tokyo, Japan) [37]. Cotton fabrics (1×1 cm^2) were sterilized in 48-well plates with 75 wt% ethanol aqueous solution under UV light. The cotton fabrics were washed three times with saline to remove the residual ethanol, and then immersed in 600 μL DMEM medium for 48 h to obtain the cotton fabric extract. MSCs were inoculated in 48-well plates containing 300 μL of DMEM medium at a density of 1×10^4 cells/well and pre-cultured in a sterile environment of 5% CO$_2$, 37 ℃ for 24 h. Each experimental group was added with 0.2 mL of the corresponding cotton fabric extract, and the blank group was added with 0.2 mL of the DMEM medium, and co-cultured with the MSCs for 24 h. MSCs were stained using CCK-8 and measured at 450 nm by an enzyme marker. The OD values of the cell suspensions of the blank control group and each experimental group were determined at 450 nm using an enzyme marker, and the relative cell activity was calculated according to formula 6

$$\text{Cell viability} = \frac{\text{OD}_e}{\text{OD}_b} \times 100\% \qquad (6)$$

where OD_e is the absorbance of the cell suspension of the experimental group with the addition of cotton fabric extract and OD_b is the absorbance of the cell suspension of the control group with the addition of DMEM medium.

The cell morphology of MSCs co-cultured with antimicrobial cotton fabric extract was observed by inverted microscope (Leica DMi8, Leica Microsystems). The antimicrobial cotton fabrics were sterilized with 75 wt % ethanol and UV light, and then the extract was prepared. MSCs (1.0×10^4 cells/well) were inoculated in 24-well plates and co-cultured with the extract in a biochemical incubator at a CO$_2$ concentration of 5% and a temperature of 37 °C for 24 h. The cell morphology of MSCs co-cultured with the antimicrobial cotton fabric extract was observed using an inverted microscope.

2.16 PH Measurement of Cotton Fabric Water Extraction Solution

The pH of the aqueous extract of cotton fabrics was determined according to GB/T7573-2009. Cotton fabric (2×2 cm^2) and 100 mL of water were added to a 250 mL flask, and the aqueous extracts of three parallel samples were obtained by shaking at room temperature for 1 h. A calibrated pH meter was used to measure 10 mm below the surface of the extract, and the pH value was held steady until the pH indication was stable.

2.17 Skin Irritation Measurement

The animal skin irritation test was performed according to ISO 10993-10:2021 with some modifications [38]. After shaving the backs of mice (4–5 W) with a razor, antimicrobial cotton or regular cotton (1×1 cm^2) was applied to their bare backs using a transparent 3 M dressing. Following 5 days of direct contact between the cotton fabric and the mice's skin, the cotton fabrics were removed and the skin areas were examined for signs of erythema and edema. Subsequently, the area of skin in contact with the cotton fabric was excised, preserved in paraformaldehyde, and sectioned for hematoxylin and eosin (H&E) staining. Finally, the inflammatory signals in the stained tissues were visualized by microscope.

3 Results and Discussion

3.1 Morphology and Wettability of Cotton Fabrics

To investigate the effect of plasma treatment on the morphology of cotton fabrics and the adsorption effect of PHMG, the surface morphology of each cotton fabric was observed using SEM, and the SEM images are shown in Fig. 1a. Figure 1a–A shows that the untreated cotton fabric sample has smooth fiber surfaces with intact fibrils. A slight increase in the roughness of the fiber surface was observed when cotton fabrics were treated in oxygen plasma for 300 s (Fig. 1a–B). And no significant presence of large cracks on the surface of the samples was observed, and the fiber shapes of the cotton fibers could still be clearly distinguished. After impregnating the not plasma-treated cotton fabrics with PHMG solution, as shown in Fig. 1a–C, the fiber surface roughness was similar to that of the original cotton fabrics, with smooth fiber surfaces, and the fiber surfaces with a little substance deposition could be seen. As shown in Fig. 1a–D, after impregnating the cotton fabric treated in oxygen plasma for 300 s with PHMG solution, the fiber morphology of the cotton fibers could only be barely seen, the surface roughness increased significantly, and a thick coating was formed on the cotton
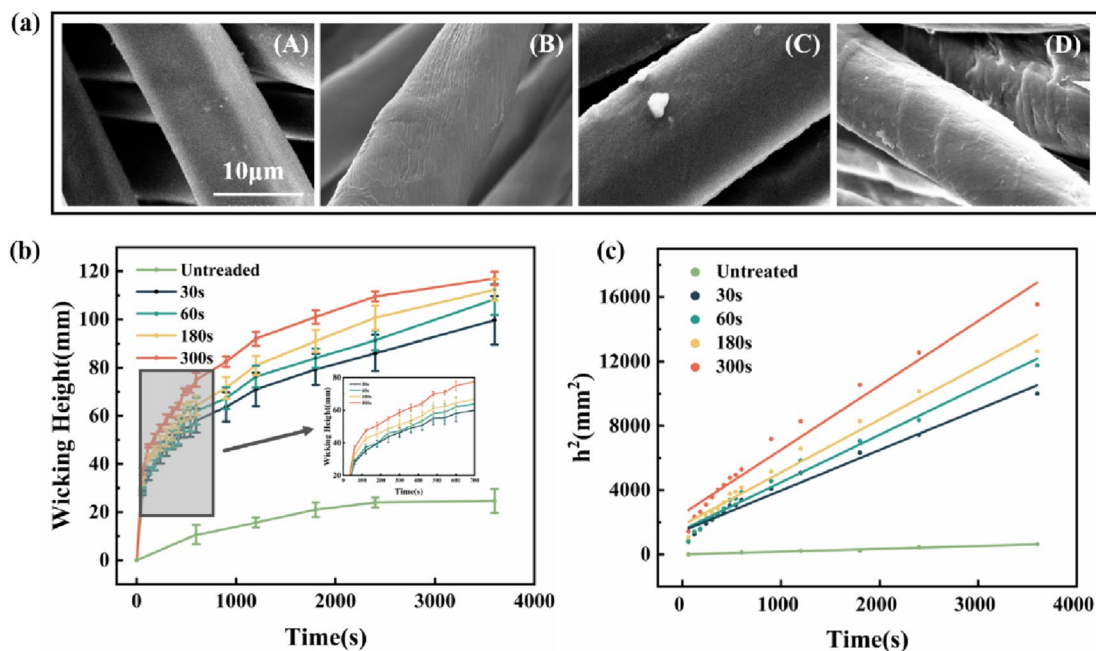
Fig. 1 **a** SEM images of cotton fabrics; **b** Wicking height rise as a function of time for plasma treated cotton; **c** Dependency of h^2 as a function of time for plasma treated cotton

fibers. The results indicate that the oxygen plasma treatment caused differences in the morphology of cotton fibers and differences in the adsorption effect of PHMG.

Wicking height provides a clear and intuitive way to observe the changes in the cotton fabric after treatment. It allows for a comprehensive understanding of the wetting phenomenon in fibers and textile materials, indicating the alterations in surface chemistry and the formation of new pores and capillaries. In Fig. 1b, wicking height increases as the oxygen plasma treatment time is prolonged, reaching its maximum value after 300 s of treatment. The diffusion coefficient D was calculated according to Fig. 1c, and the slope of the curve increased with the oxygen plasma treatment time, which was obviously affected by the plasma treatment. R^2 was above 0.95, which indicated a high degree of fit. The diffusion coefficient of the cotton fabric without oxygen plasma treatment was 0.17 mm^{2}s^{-1}, and the diffusion coefficient of the cotton fabric with oxygen plasma treatment for 300 s was 4.00 mm^{2}s^{-1}, which was improved by about 22 times. The specific data are shown in Table S1. The experimental results showed that the hydrophilicity of cotton fibers could be significantly improved by the oxygen plasma treatment. The increase in hydrophilicity also indirectly indicated that the plasma treatment improves the polarity and surface energy of cotton fabrics [32]. Therefore, the oxygen plasma treatment time was fixed at 300 s and the concentration of PHMG solution was tentatively set at 1 g/L in the subsequent antimicrobial treatment. Fig. S1a shows that the core suction heights of C and D increased relative to the suction height of

A. R^2 and diffusion coefficients D calculated from Fig. S1b were listed in Table S2. The diffusion coefficient of C was 0.44 mm^{2}s^{-1}. This increase can be attributed to the hydrophilic nature of PHMG, which enhances the cotton fabric's ability to capture droplets. On the other hand, the diffusion coefficient of D, was measured to be 3.01 mm^{2}s^{-1}. This value is lower than the diffusion coefficient before impregnation with the PHMG solution, but it is much higher than the diffusion coefficient of A. The decrease may be attributed to the adsorption of a significant amount of PHMG, leading to a decrease in the surface energy of the cotton fabric.

3.2 Chemical Composition of Cotton Fabrics

To further investigate the surface chemical composition of the cotton fabrics, their surfaces were analyzed using XPS and ART-FTIR. The relative surface elemental compositions of each cotton sample are given in Table S3. There is a significant difference between the O/C ratio of the original cotton fabric and the theoretical O/C ratio of cellulose. Compared with the cotton fabric A, the percentage of oxygen increased, the percentage of carbon decreased, and the O/C ratio increased from 0.44 to 0.74 in the cotton fabrics after 300 s of plasma treatment. The full XPS spectra of each cotton sample in Fig. 2a show typical O1s (533 eV) and C1s highly correlated with cellulose (285 eV) peaks, and cotton fabric@PHMG at 400 eV, showed a distinct N1s peak, which indicated the successful adsorption of PHMG. Moreover, there was important information in the

Fig. 2 a Typical XPS survey scan spectra of each cotton fabric; **b** Narrow scan XPS spectra of the C1 s of the raw cotton fabric; **c** Narrow scan XPS spectra of the C1 s of the plasma treated cotton fabric; **d** Narrow scan XPS spectra of the C1 s of the plasma treated cotton fabric@PHMG
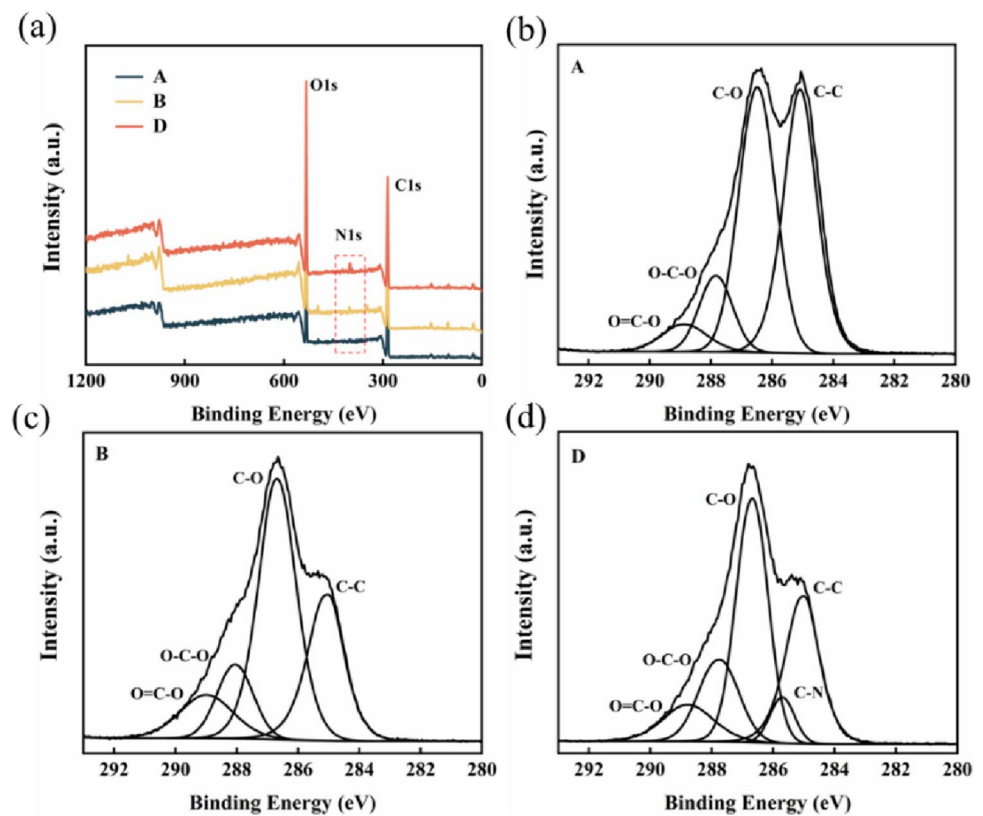
difference in the intensity of the high-resolution C1s peaks, which indicated the successful adsorption of PHMG. Figure 2b–d show the C1 s of high-resolution scanning of cotton samples, in which 284.8 eV corresponds to the C–C bond, 286.3 eV corresponds to the O–C–O bond, 287.7 eV corresponds to the O–C–O bond, and 288.8 eV corresponds to the O=C–O bond in cellulose [15]. Compared with the cotton fabric A (Fig. 2b), the proportion of C–O peaks in cotton fabric B (Fig. 2c) was significantly elevated, and the percentage of C=O peaks also appeared to be increased to some extent, which indicated that polar oxygen-rich groups were introduced to the surface of the material. Moreover, the decrease in the percentage of C–C peaks also indicates that the plasma treatment destroys the non-fibrous components such as pectin and waxes that make up the surface layer of the cotton fibers, so that more hydroxyl groups on the internal cellulose are revealed, and the oxygen plasma oxidizes some of the hydroxyl groups to carboxyl groups. Compared to the control, cotton fabric D (Fig. 2d) showed a new peak at 285.7 eV, which can be attributed to C–N, indicating that PHMG was indeed present on cotton fabrics.

In addition to XPS, ATR-FTIR also was used to characterize each cotton fabric. As shown in Fig. S2, the main characteristic bands of cellulose are clearly shown in all the spectra, i.e., the OH-bond stretching vibration band at 3600–3000 cm^{-1}, the CH-bond stretching vibration band at 2900 cm^{-1}, the OH-bond bending vibration band at 1640 cm^{-1}, the CH_2 bending vibration at 1430 cm^{-1}, the CH-bending vibration band at 1371 cm^{-1}, and the stretching vibration band of the C–O–C bond at 1164 cm^{-1} [8]. Surprisingly, no matter whether plasma treatment or impregnation with PHMG solution was carried out, there were even no subtle differences among cotton samples except for the main characteristic bands of cellulose. This is due to the fact that the sampling depth of ATR-FTIR is 1–2 μm, which characterizes part of the internal cellulose composition of cotton fibers, while the sampling depth of XPS is shallower, within 10 nm, which characterizes part of the primary and secondary cellulose of cotton fibers [15].

In short, the plasma treatment will only affect the surface layer of cotton fibers to improve the polarity and surface energy of the cotton fabric surface, without affecting its internal cellulose composition, so as to achieve the introduction of antimicrobial agents PHMG at the same time, to maintain the basic good properties of the original material.

3.3 Zeta Potential of Cotton Fabrics

The character of functional groups on the surface of fibers and their accessibility directly impact the surface charge of materials. Therefore, plasma treatment affects the surface charge of cotton fabrics. In this study, cotton fabrics were crushed into short fiber form and dispersed using deionized water. The results for zeta potential at the same concentration

are shown in Fig. 3. The potential of the pure cotton fabric was around − 10.3 mV and the potential of the cotton fabric treated with plasma for 300 s was around − 30.0 mV, with a significant increase in negative charge, and after impregnating both of them with PHMG solution, the potentials both increased to near − 3 mV. This can be explained by the presence of glyceraldehyde carboxyl groups and polar hydroxyl groups on the cellulose, which render the surface of cotton fabrics negatively charged in water. PHMG, being a cationic antimicrobial agent, can adsorb onto the surface of cotton fabrics through electrostatic action, neutralizing the surface charge and bringing the zeta potential close to neutrality. This phenomenon is consistent with the aforementioned characterization results. Plasma treatment disrupts the non-cellulose components of the surface layer of cotton fiber, thereby exposing more glycolaldehyde carboxyl groups and polar hydroxyl groups. Additionally, some of the hydroxyl groups are oxidized to carboxyl groups. As a result, the surface of plasma-treated cotton fabric carries more negative charges in water, which enhances the electrostatic force between the fabric and PHMG. This may lead to excellent durable antibacterial properties.
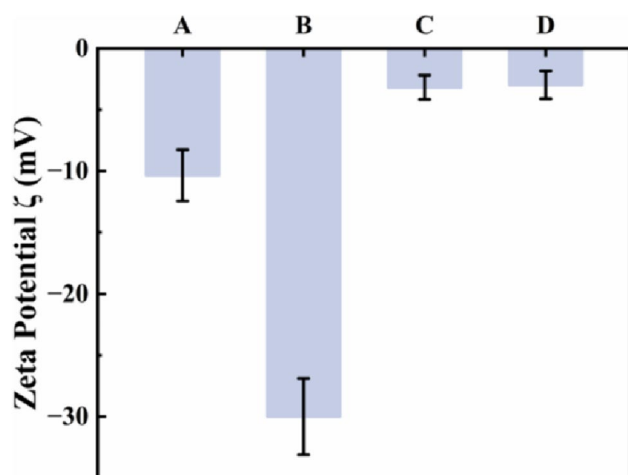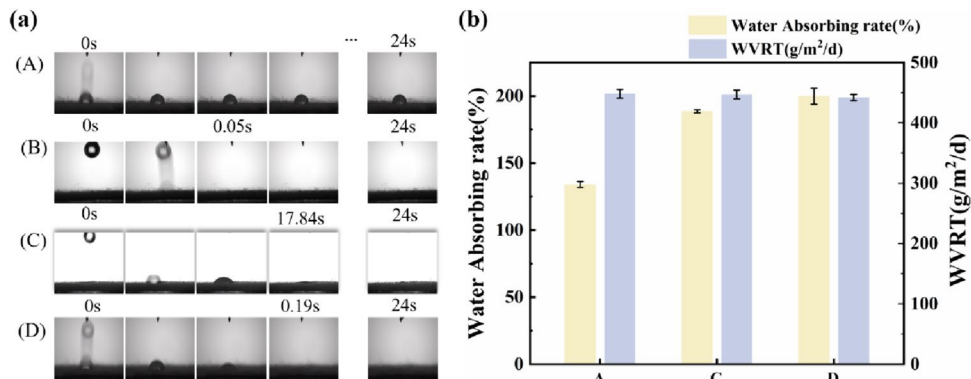

Fig. 3 Zeta potential of each cotton fabric

3.4 Mechanical Properties of Cotton Fabrics

The plasma surface treatment will etch and somewhat oxidize the surface of the cotton fibers, so it is necessary to determine the effect of plasma treatment on the mechanical properties of cotton fabrics. In Fig. S3, it can be seen that the elongation at break of cotton fabrics gradually decreased with the increase of oxygen plasma treatment time. The elongation at break of the cotton fabric without plasma treatment was 105.47%, and the elongation at break of the cotton fabric treated in plasma for 300 s decreased to 94.38%, which was 10.51% lower compared to the original cotton fabric, and the specific data are shown in Table S4, so the time of the oxygen plasma treatment should not be too long. As for the tensile strength, the tensile strength of each group of cotton fabrics basically remained around 21 MPa. On the whole, after plasma treatment, the performance of extension has a little decrease and the tensile strength of cotton fabrics is basically unchanged. Considering the application scenarios of cotton fabrics in life, the elongation at break of more than 90% can basically cope with a variety of application environments.

3.5 Comfort Evaluation of Antibacterial Cotton Fabrics

The enhanced hydrophilicity of cotton fabrics, with improved moisture absorption and core-absorption properties, can improve the comfort of cotton fabrics. Specifically, when the body sweats from exercise, hydrophilic cotton fabrics can quickly absorb sweat from the skin, thus keeping the skin dry and enhancing wearing comfort. The wettability of the cotton fabrics was evaluated visually using a contact angle meter. It can be observed in Fig. 4a that the measurement droplets were still able to maintain an intact droplet morphology after contacting the original cotton fabrics for 24s; cotton fabrics B treated with oxygen plasma for 300 s absorbed the measured droplets at the very instant of contact with them, with an absorption time within 0.15 s; the measured droplets were gradually wetted in contact with

Fig. 4 a Water contact angle of all kinds of cotton fabrics; **b** The water vapor permeability and water absorption within 1 min of the prepared cotton fabrics

the cotton fabric C and were completely absorbed by the cotton fabric after about 18 s; and cotton fabric D took about 0.2 s to absorb the droplets, the absorption time was slightly increased compared to cotton fabric B, but it still showed extreme hydrophilicity. The presence of non-cellulose components (e.g., pectin and wax) on the fiber surface of cotton fabric A caused hydrophobicity; whereas, the plasma treatment destroyed the non-cellulose components on the fiber surface, exposing the oxygen-rich hydrophilic groups, leading to a substantial increase in hydrophilicity. The deposition of hydrophilic PHMG on the surface of the cotton fabric resulted in a slight increase in the ability of the cotton fabric to capture droplets; at the same time, the large amount of adsorbed PHMGs also resulted in a decrease in the surface energy of the plasma-treated cotton fabric, which led to an increase in droplet absorption time. This is consistent with the results of the wicking height experiments conducted earlier.

At the same time, the water vapor transmission rate and the fast water absorption capacity of the cotton fabrics were evaluated to simulate the assessment of air permeability and sweat stain absorption capacity. As shown in the Fig. 4b, the water vapor transmission rates of treated samples C and D showed a small decrease, but were basically the same as those of A, remaining around 450 g/m^2/d. Compared with the water absorption rate of 134% in 1 min of the pure cotton fabric, the those of both samples C and D were increased, 189% for sample C and 200% for sample D. In conclusion, cotton fabric C has better hydrophilicity and water absorption than A, while D has superhydrophilicity and excellent water absorption, but the water vapor transmission rates of C and D remain essentially invariable. In the absence of heavy perspiration, cotton fabric D absorbs sweat from the body surface quickly, thus improving wearing comfort.

3.6 Antibacterial Assessment of Cotton Fabrics

Hygroscopic cotton has a tendency to become contaminated with bacteria, which can reduce the lifespan of cotton fabrics and even have negative effects on the wearer's health. In accordance with GB/T 20944.3-2008, the antibacterial performance of cotton fabrics was studied using the colony counting method with E. coli and S. aureus. All the treated cotton fabrics exhibited antibacterial effects, and the effectiveness of these effects increased with higher concentrations of PHMG. This can be visually observed in Fig. 5, a significant antibacterial effect was observed when n reached 1. The antibacterial rate for both Escherichia coli and Staphylococcus aureus exceeded 98%, effectively inhibiting bacterial proliferation and resulting in a significant reduction in the number of colonies on the agar plate.

To accurately measure the amount of PHMG adsorption, the elemental content of each group of cotton fabrics were analyzed using an elemental analyzer, and the specific data are shown in Table S5. Calculated according to Eq. S1, the amount of PHMG adsorbed by cotton fabric C1 was 3.19


Fig. 5 Visual pictures of antibacterial effect of the prepared cotton fabrics
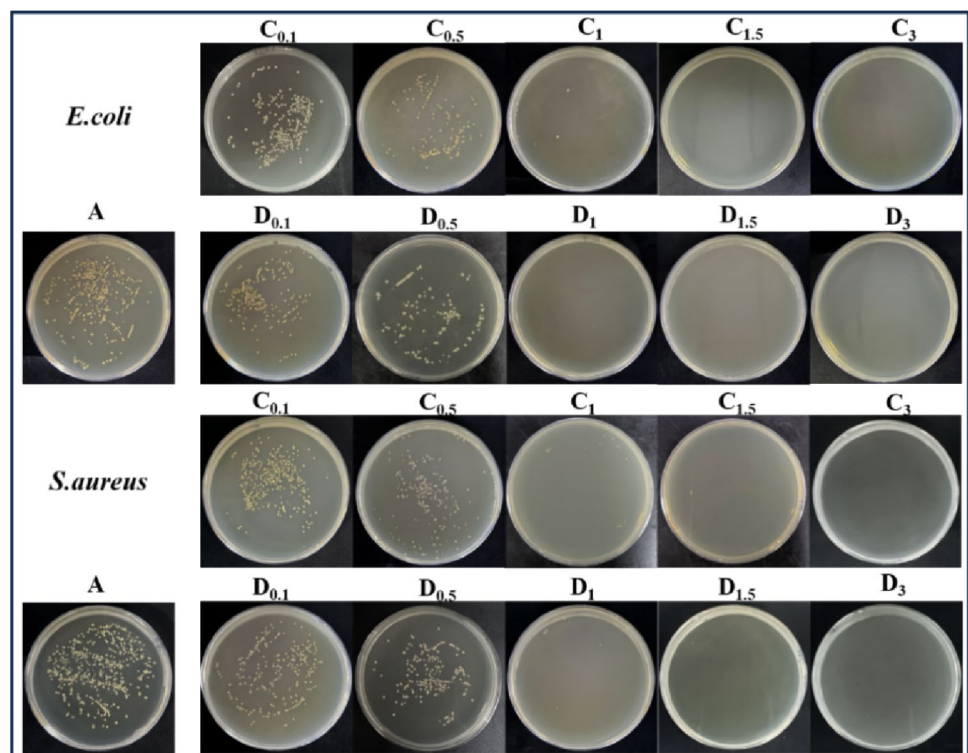
mg/g, and the amount of PHMG adsorbed by cotton fabric D1 was 5.88 mg/g.

To further elucidate the antimicrobial mechanism, Fig. 6 displays micrographs of cotton fabric A, C_1, and D_1 co-cultured with *E. coli* and *S. aureus*. The images reveal that the membranes of *E. coli* and *S. aureus* co-cultured with A remained intact, and the bacteria maintained their basic morphology, indicating normal growth. However, when co-cultured with cotton fabrics C_1 and D_1, the membranes of *E. coli* and *S. aureus* exhibited irregular shapes, rough surfaces, and breakage, suggesting that the bacteria had died. The results of SEM showed that the antibacterial mechanism of cotton fabrics C_1 and D_1 is the presence of PHMG on the surface of cotton fabrics, which leads to membrane damage under the electrostatic action of guanidinium cationic antimicrobial agents. The cations of PHMG disrupt the ζ-potential of the bacterial surface and impair the integrity of the bacterial membrane.

The adhesion stability of antimicrobial agent PHMG on cotton fabrics directly affects the durability of antimicrobial effect and the safety of utilization. Therefore, bacteriostatic circle experiments were performed to verify the solubility of PHMG and whether cotton fabric has antimicrobial activity by releasing PHMG. It can be observed in Fig. S4 that antimicrobial cotton fabric C produced small bacteriostatic circles, while antimicrobial cotton fabric D did not produce a bacteriostatic circle, indicating that there was a slight release of PHMG on cotton fabric C, and the cotton fabric D basically does not release PHMG when performing antimicrobial activity. This is due to the fact that PHMG mainly exists in the form of sedimentation on the surface of antimicrobial cotton fabric C, and the interaction force between PHMG and the surface of cotton fabric is weak; while after the cotton fabric D was treated with oxygen plasma, the number of oxygen-containing polar groups on the surface was increased, and the surface energy was increased, which strengthened the interaction force between PHMG and the surface of the cotton fabric D, and greatly inhibited the release of PHMG.
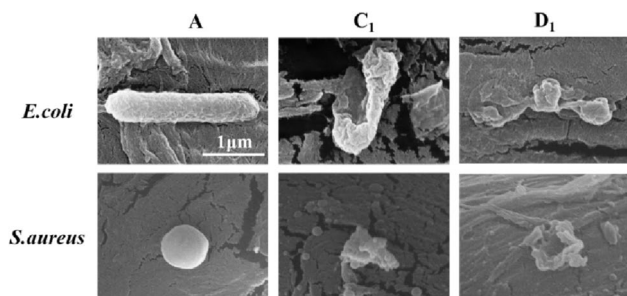
Fig. 6 Images of *E. coli* and *S. aureus* incubated with each cotton fabric

## 3.7 Antibacterial Durability Against Washing Assessment

Samples C and D were both subjected to 50 simulated washes and then dried, and the antimicrobial persistence of samples C and D was evaluated by the trace method. The antimicrobial rates of samples with PHMG concentrations below 1 g/L were not discussed, and the other samples, specifically, are shown in Fig. 7. The antibacterial rates of C_1, $C_{1.5}$, and C_3 against *E. coli* were 94%, 94%, and 98% before cyclic washing, and decreased to 31%, 33%, and 37% after cyclic washing, respectively; Before cyclic washing, the antibacterial rates of C_1, $C_{1.5}$, and C_3 against S. aureus were 96%, 97%, and 98%, respectively, and after cyclic washing, they decreased to 43%, 44%, and 49%, respectively; Before cyclic washing, the antibacterial rates of D_1, $D_{1.5}$, and D_3 against *E. coli* were 94%, 96%, and 98%, respectively. After cyclic washing, they decreased to 89%, 93%, and 9 5%, respectively; Before cyclic washing, the antibacterial rates of D_1, $D_{1.5}$, and D_3 against *S. aureus* were 97%, 97%, and 99%, respectively. After cyclic washing, they decreased to 95%, 94%, and 97%, respectively.

The antimicrobial data showed that although sample C without oxygen plasma treatment was also able to adsorb PHMG, thus achieving good antimicrobial effects on *E. coli* and *S. aureus*, the antimicrobial rate decreased significantly after multiple washing. This is due to the weak interaction force between the cotton fabric surface and PHMG, resulting in a significant decrease in PHMG after washing. On the contrary, the antimicrobial rate of sample D after oxygen plasma treatment was only slightly reduced after multiple washing, and still maintained a good antimicrobial effect, showing stable and durable antimicrobial performance. This may be that the plasma treatment has significantly enhanced the interaction force between PHMG and the surface of cotton fabrics, and PHMG was still stably adsorbed on the surface of cotton fabrics after many washes. The antimicrobial grade of washed fabrics is divided in FZ/T73023-2006, as shown in Table S6. According to Table S6, samples D_1, $D_{1.5}$, and D_3 all meet the AAA level standard.

3.8 Safety Evaluation of Antimicrobial Cotton Fabrics

The cytocompatibility of antimicrobial cotton fabrics was assessed by co-culturing mesenchymal stem cells (MSCs) with extracts from the fabrics and measuring their relative survival using a CCK-8 kit. Figure 8a showed that all the cotton fabrics did not have a significant cytotoxic effect on MSCs compared to the blank control group, where MSCs were cultured in DMEM medium. The cytotoxicity of cotton fabrics A, C_1, and D_1 was 98%, 74%, and 94%, respectively. D_1 has good cytocompatibility like A. Furthermore,

Fig. 7 Antimicrobial activity of antibacterial cotton fabrics with or without cycle washing (*E. coli* (**a**), *S. aureus* (**b**))
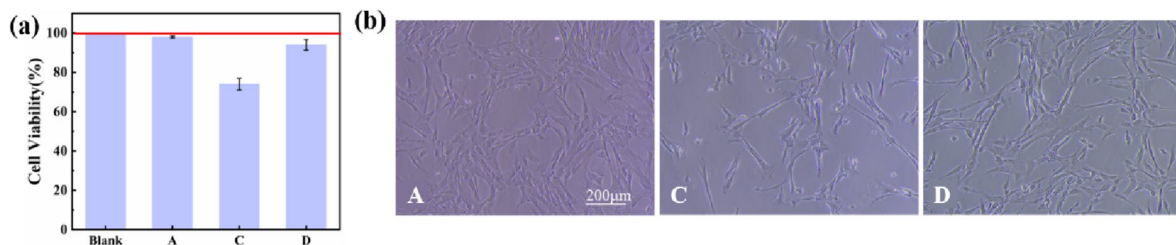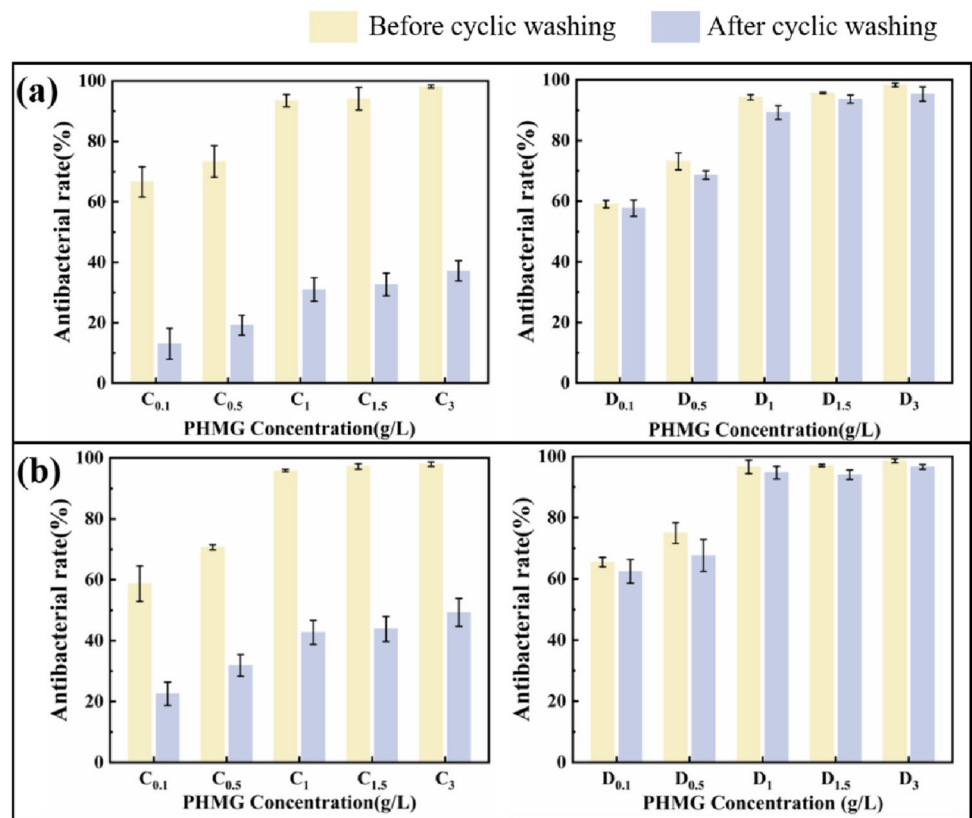

Fig. 8 The cell compatibility assay in vitro of cotton fabrics. **a** The MSCs viability co-cultured with the extraction solution of the prepared cotton fabrics for 24 h; **b** Representative microscopy images of MSCs cultured with the prepared cotton fabrics

an inverted microscope was used to observe the morphology of the MSCs co-cultured with each group of cotton fabric extracts. Figure 8b revealed that cells in the experimental groups with the addition of cotton fabric A and D_1 extracts exhibited robust growth, with a large number of cells surviving and regular cell morphology observed under the microscope. However, in the group with cotton fabric C_1, relatively few cells survived and the cytocompatibility was slightly poor, mainly due to the presence of some dissolution of PHMG. Therefore, the cotton fabrics D_1 showed no obvious toxicity and met the requirements for cell survival, ensuring the normal biological process of cells.

The human skin maintains a weakly acidic pH level between 4.5 and 6.5, which is influenced by various substances such as water-soluble substances in the skin's stratum corneum, excreted sweat, water–lipid emulsifying substances on the surface, and carbon dioxide released through respiration. If the skin's pH value is changes overly due to certain activities, it can cause significant harm. This includes weakening the skin's barrier function, reducing its ability to retain moisture and resist external substances, resulting in dryness, itchiness, contact dermatitis, and even inflammation or exacerbation of certain skin diseases. Therefore, it is crucial to test the pH of the cotton fabric extract being prepared. The different grades of cotton fabrics classified by GB 18401-2010 according to the pH of the extracts of cotton fabrics are shown in Table S7. As shown in Fig. 9a, the pH values of each group of cotton textile extracts were within the light-colored region, indicating a pH range between 4.0 and 7.5. This pH range meets the standard for

Fig. 9 Skin tolerance assessment. **a** The pH of the extraction solution of cotton fabrics; **b** The visual pictures of mice back before and after covered by the cotton fabrics at day 0 and day 5; **c** H&E staining pictures corresponding to the back skin covered by cotton fabrics of mice at day 5
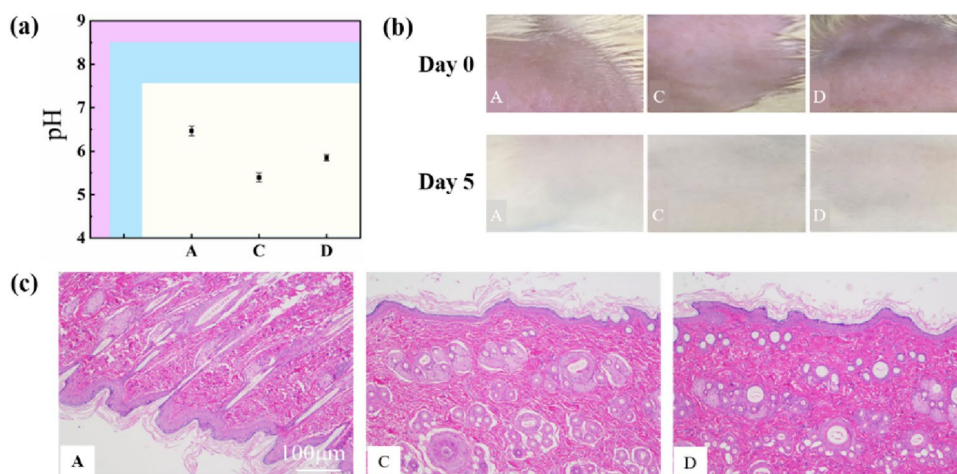
baby textiles. This is primarily attributed to the use of pure cotton fabrics as the base material, which possess excellent skin-friendliness. Therefore, the plasma surface treatment does not compromise the material's skin-friendliness.

Skin irritation evaluation is a mandatory test for cotton fabrics to determine their suitability for direct skin contact. In this study, antimicrobial cotton fabrics and pure cotton fabrics (1×1 cm^2) were applied to the exposed backs of mice using transparent 3M dressings. After 5 days of contact, the cotton fabrics were removed. As shown in Fig. 9b, it was observed that there were no signs of erythema or edema on the backs of the mice exposed to the antimicrobial cotton fabrics. This suggests that all cotton fabrics tested were free from skin irritation, highlighting the good biocompatibility and low irritation of cotton as a substrate material. Furthermore, skin areas in contact with the cotton fabrics were excised and preserved in paraformaldehyde. Histological analysis using H&E staining revealed intact tissue and undamaged cells, with no apparent abnormalities observed [Fig. 9c]. These results demonstrate the excellent dermal compatibility of each prepared cotton fabric.

4 Conclusions

In this study, cotton fabrics with durable antimicrobial function were successfully prepared by adsorbing PHMG onto the surface of oxygen plasma-treated cotton fabrics through strong electrostatic interactions. No additional chemical reagents (except oxygen and PHMG) were needed throughout the process. The plasma treatment destroyed the noncellulosic components of the surface layer of cotton fibers, exposed the hydroxyl groups of the internal cellulose, and oxidized some of the hydroxyl groups to carboxyl groups, which increased the polarity and wettability of the cotton fabric surface, and caused the surface of the cotton fibers to carry more negative charges, which strengthened the

interaction force between the PHMG and the surface of the cotton fabrics, thereby inhibiting the leakage of antimicrobial agents. Modified cotton fabrics are more absorbent and much more hydrophilic. Sweat stain management is improved without heavy perspiration. The antimicrobial effect of the modified cotton fabrics was enhanced with the increase of PHMG concentration, and when the concentration reached 1 g/L, the antimicrobial rate for *E. coli* and *S. aureus* was more than 98%, and the antimicrobial activity was maintained at more than 90% after 50 simulated washes with detergent solution, showing excellent antibacterial durability against washing. The safety of antimicrobial cotton fabrics was further explored. Since the plasma treatment does not change the internal properties of the material, the antimicrobial cotton fabrics retained the original skin-friendliness of cotton, with no skin irritation and no cytotoxicity. In summary, oxygen plasma treatment enhances the interaction force between the surface of cotton fabric and PHMG, which provides an effective idea for the preparation of antimicrobial cotton fabrics.

Author Contributions Jiabao Shi: writing, original draft, investigation and methodology; Chuang Xiao: conceptualization, methodology and investigation; Wang Yin: supervision and writing (review and editing); Yong Guan: resources; Meidong Lang: supervision and writing (review and editing).

Data Availability All data that support the findings of this study are included within the article (and any supplementary files).

Declarations

Ethical Statement All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Shanghai Jiao Tong University and approved by the Animal Ethics Committee of Renji Hospital Affiliated to Tongji University.

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