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Laundering durable antibacterial cotton fabrics grafted with pomegranate-shaped polymer wrapped in silver nanoparticle aggregations

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To improve the laundering durability of the silver functionalized antibacterial cotton fabrics, a radiation-induced coincident reduction and graft polymerization is reported herein where a pomegranate-shaped silver nanoparticle aggregations up to 500 nm can be formed due to the coordination forces between amino group and silver and the wrapping procedure originated from the coincident growth of the silver nanoparticles and polymer graft chains. This pomegranate-shaped silver NPs functionalized cotton fabric exhibits outstanding antibacterial activities and also excellent laundering durability, where it can inactivate higher than 90% of both *E. coli* and *S. aureus* even after 50 accelerated laundering cycles, which is equivalent to 250 commercial or domestic laundering cycles.

Silver has been used as antibacterial material since ancient times. The study of antibacterial mechanisms have shown that free silver ions released from silver can effectively kill bacteria, and is primarily responsible for silver's antibacterial property¹⁻⁵.

Various forms of silver including zero-valent silver, silver oxide, ionic silver and silver-containing molecular complexes have been shown to be active against different bacterial strains. Recent work reveals that nanoscale forms of silver⁶⁻⁹ (and other antibacterial metals, copper¹⁰ and zinc oxide¹¹ for example) are particularly effective at deactivating bacterial growth. Although the general antibacterial mechanism of nanoscale metal-based antimicrobial agents is largely unclear, its ultra-small size and super-large specific area has been proposed as a main rationale¹²⁻¹⁶.

Silver nanoparticles (NPs) are increasingly used to fabricate antimicrobial textiles¹⁷ for their broad-spectrum antibiotic property against different bacterial strains including common gram-negative bacteria, gram-positive bacteria¹⁸⁻²¹, even drug-resistant bacteria²²⁻²⁴, fungus, viruses and parasites²⁵⁻²⁹, e.g., Monkeypox Virus²⁹ and HIV-1^{27,28}.

Laundering of such silver NPs-based textiles has already been identified as an important issue with concerns about increasing silver exposure in the environment and the general population³⁰. Silver NPs can become released from textile surfaces into wastewater during laundering. The migration of silver NPs from the textile to human sweat can also increase dermal exposure, creating concerns about effects on human physiology¹⁷.

Various methods have been adopted for coating silver NPs on a textile surface, such as by generating active groups via plasma^{31,32} and UV irradiation³³, or sol-gel processing³⁴, *in-situ* reduction of the silver ions to metallic silver onto the fabrics³⁵⁻³⁸ and so on. However, conventional surface modification of textiles by silver NPs is not persistent, especially against laundering^{39,40}. Thus, long-term antibacterial activity that resists home laundering is urgently needed⁴¹.

Some coordinating groups such as amino groups, carboxylic acid groups⁴² or hydrosulfide groups⁴³, even the σ -bond formed with long alkyl chains⁴⁴ have been further used to bind silver ions-containing silver NPs onto a textile surface. Antimicrobial Ag-loaded silk has been created through a one-step method of radiation-induced reduction, and the results showed that Ag-loaded silk possessed some washing stability because the silver NPs interact with the amino acids on the surface of silk fibers via an electrostatic and coordination interaction⁴⁵. The electrostatic and coordination interaction forces between ions, however, are insufficient against harsh laundering.



It thus remains essential to introduce more powerful forces into the antibacterial system. Additionally, in consideration of concerns about increasing silver exposure in the environment and the general population, further attention needs to be paid to developing techniques that create lossless silver NPs during laundering. The strong binding of silver NPs onto the surface of the fabric not only provide the long-term effective antibacterial clothing which can keep us healthy while avoiding the abuse of antibiotics but also reduce the leakage of silver NPs into the environment with the waste water thus lessening the potential risks.

Here, we report a novel “one-step” radiation-induced coincident reduction of silver ions and graft polymerization of monomer containing amino group onto cotton fabric, to fabricate an antibacterial cotton fabric with a pomegranate-shaped structure of wrapped silver nanoparticle aggregations (NPAs) by polymer chains grafted onto the surface. The monomer, 2-aminoethyl methacrylate (AEMA), with a primary amine group that provides a coordination group and a carbon-carbon double bond that offers a grafting group was chosen in the experiment. The pomegranate-shaped polymer wrapped silver NPAs grown on the surface of the functionalized fabric allows an excellent laundering durability together with outstanding antibacterial activity, even after 50 accelerated laundering cycles, which is equivalent to 250 instances of home laundering. Meanwhile, the total loss of silver were below 10% after 50 accelerated laundering cycles, which means the product would be an environmental-friendly at the same time of protecting people’s healthy.

Results

Wrapped silver NPAs formation. Cotton fabrics functionalized with pomegranate-shaped silver NPAs wrapped by poly(2-aminoethyl methacrylate) (PAEMA) graft chains were obtained by radiation-induced coincident reduction and graft polymerization. The cotton fabric samples were simply soaked in a solution of AgNO_3 and an AEMA monomer with different concentrations and then irradiated by γ -ray up to an absorbed dose of 34 kGy under the protection of nitrogen gas. The sample was extracted by boiling milliQ water in a Soxhlet extractor for 24 hours to remove the unreacted AgNO_3 , residual monomer and homopolymers, and then was vacuum dried for further measurements. The obtained sample was named as PAEMA-co-Ag cotton fabric in the following sections. The extraction of the product by hot water, is well accepted as the effective routine to remove not only the hydrophilic homopolymers^{46–49} but also the free silver ions^{35,36}.

The PAEMA-co-Ag fabric looks slightly whitened as compared to pristine cotton fabric (Figure 1a). Hence, the success in graft polymerization can be proven by FT-IR spectroscopy study, where the new absorption band at $1,722\text{ cm}^{-1}$ in the spectrum of the PAEMA-co-Ag fabric can be attributed to an ester group in the PAEMA graft chains (Figure 1b). Due to the radiation-induced coincident reduction and graft polymerization, the weight increment ratio, which is called degree of grafting (DG), is composited by two sources, where DG_{PAEMA} accounts for the PAEMA graft chains and DG_{Ag} accounts for the silver NPAs. DG_{Ag} is the weight ratio of silver NPAs attached on the fabric versus the pristine cotton fabric in percentage. Therefore, the silver contents can be re-written in mg/g unit by just multiply DG_{Ag} by 10, when the pristine cotton fabric is used as the substrate. Figure 1c shows the effect of the monomer concentration on the DG_{PAEMA} and DG_{Ag} of PAEMA-co-Ag fabrics, where the Ag mass percentages were determined by microwave digestion followed by inductively coupled plasma-mass spectrometry (ICP-MS) and DG_{PAEMA} were determined by subtracting DG_{Ag} from total DG. It was found that there was a tendency for the two DGs to work against each other with the PAEMA-co-Ag fabric, because of competition during the reduction reaction and graft polymerization, with both consuming the same amount of free radicals generated in the reaction system by γ -ray irradiation to the same total absorbed doses.

Therefore, higher monomer concentration results in a higher reaction ratio of the graft polymerization which consumes more radicals, and hence fewer radicals are left for the reduction reaction, resulting in a lower amount of generated silver NPAs.

Figure 1d shows the effect of the silver ion concentration on the DG_{PAEMA} and DG_{Ag} of PAEMA-co-Ag fabrics with the monomer concentration was set at 10%. It was found that there was also a tendency for the two DGs to work against each other with, although the DG_{PAEMA} seems less affected by the concentration of silver ions. DG_{Ag} is linearly increasing with the increase in $\log[\text{Ag}^+]$. The higher silver ion concentration, of cause, will raise DG_{Ag} . But over higher silver ion concentration could produce precipitation with the existence of chloride ions, and also lead to the higher cost.

The contact angle (CA) results showed that the PAEMA-co-Ag fabric ($\text{DG}_{\text{Ag}}=0.54\%$ and $\text{DG}_{\text{PAEMA}}=14.6\%$) is superhydrophilic, with the water drops spreading out within 0.2 s (Figure 1e). The superhydrophilicity should be attributed to the hydrophilicity nature of the abundance in amino groups in the graft chains attached on the surface of the cotton fabric. The superhydrophilicity of PAEMA-co-Ag cotton fabric makes it more beneficial for the Ag NPAs contact with solution such as our sweat and therefore the ionized silver could be release through the solution and kill bacteria effectively and continuously.

Additionally, XPS spectroscopy study was performed to confirm the existence and the chemical state of the elements on the surface of the fabrics. The successful introducing of silver NPAs on the surface of PAEMA-co-Ag fabric by radiation is defined by the narrow scan spectra, where the new doublet peak of Ag(0) at 368 eV (3d5/2) and 374 eV (3d3/2) in the spectra of PAEMA-co-Ag fabric (Figure 2a). As contrast, there is no peaks attributed to Ag on the surface of the pristine cotton fabric and only PAEMA grafted cotton fabric. The coordination force of the amino group in the monomer with silver should account for the interaction of the PAEMA chains with the silver NPAs, which can be defined by XPS N 1s spectroscopy study (Figure 2b), where the new peak of 407.7 eV attributed to the highly de-electronated state of N elements indicated the happening of the electron transfer between N and Ag elements, therefore, it means the strong interaction between the amino groups and the Ag NPAs on the surface of the PAEMA-co-Ag fabric.

Figure 3a–3d shows the scanning electron microscopy (SEM) images of the pristine cotton fabric and PAEMA-co-Ag fabrics prepared under different monomer concentration. The radiation-induced simultaneous reduction and graft polymerization onto cotton fabric did not change the woven structure, which indicates good preservation of air-breathing properties that are important for clothing comfort.

Zoomed-in images show many nanoparticles attached to the surface of the fibers (Figure 3e–3h). Based on our knowledge^{46–49}, there should be no particle formation on the surface of the fibers if only the methacrylate monomer was graft polymerized onto the cotton fabric under radiation. Therefore, these nanoparticles should be attributed to the silver NPAs that were due to the reduction reaction. However, the size and numbers of silver NPAs was found to not correspond with the DG_{Ag} of the PAEMA-co-Ag fabrics. At a DG_{Ag} of 0.82% and DG_{PAEMA} of 10.9%, the size of the silver NPAs were around 50 nm (Figure S1). When DG_{Ag} decreased to 0.54% and DG_{PAEMA} increased to 14.6%, the size of the silver NPAs suddenly increased to 500 nm (Figure S2). Further at a $\text{DG}_{\text{Ag}}=0.16\%$ and $\text{DG}_{\text{PAEMA}}=21.1\%$, the decrements left fewer and smaller nanoparticles to within 50 nm (Figure S3). Further, there were increasingly more wrinkled structures formed on the surface of the fibers with increasingly higher DG_{PAEMA} , which can be attributed to aggregation of the grafted PAEMA chains. The abnormal size change in the silver NPAs hinted that they are complex particles due to the interaction of the grafted polymer chains and the silver NPAs, most possibly the pomegranate structure where the silver NPAs are the seeds. This also

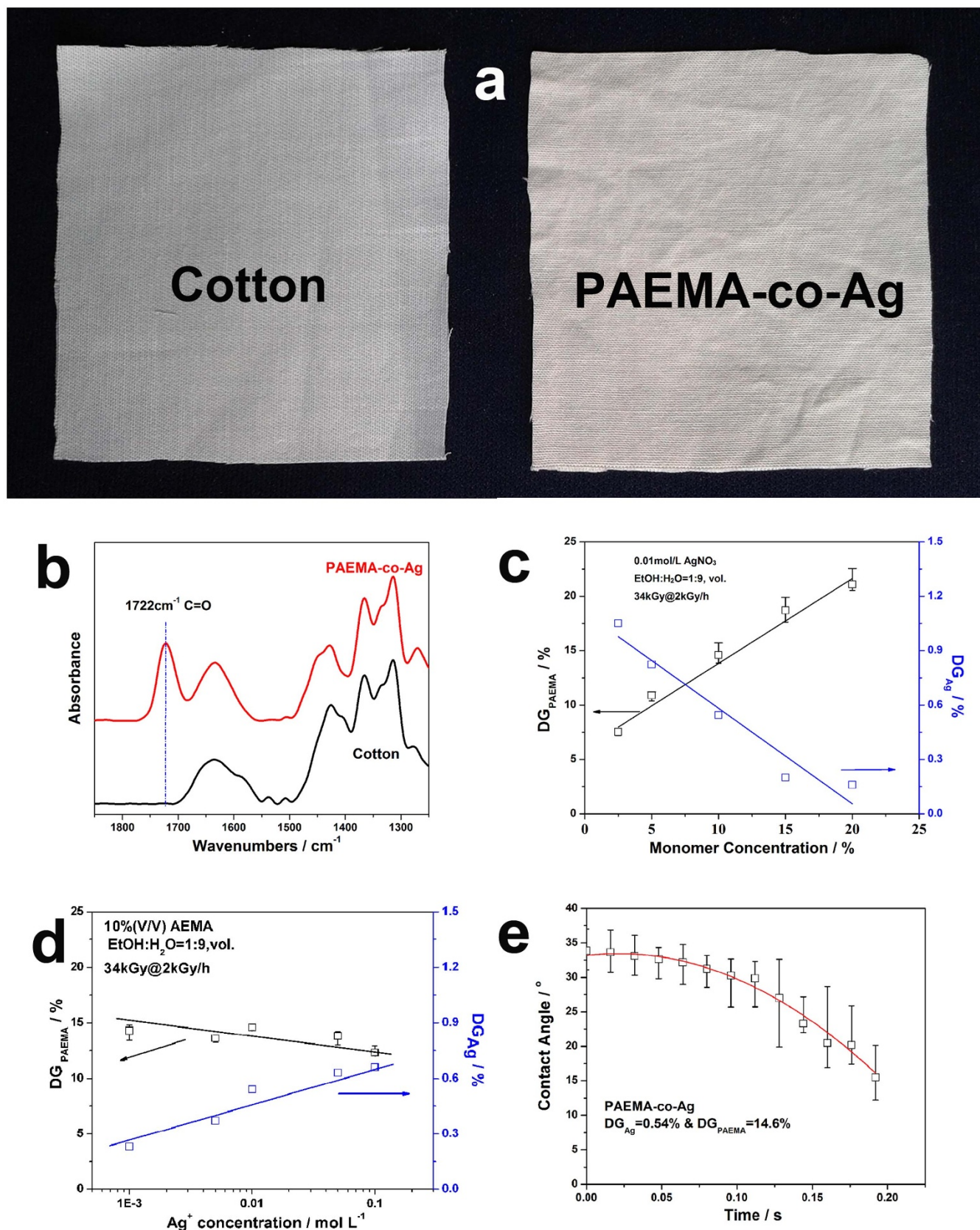


Figure 1 | (a) Photos of the cotton fabric and the PAEMA-co-Ag fabric; (b) FT-IR ATR spectra of the cotton fabric and the PAEMA-co-Ag fabric; (c) Dependence of the DG_{PAEMA} and DG_{Ag} of PAEMA-co-Ag fabrics on the AEMA monomer concentration under radiation-induced coincident reduction and graft polymerization; (d) Dependence of the DG_{PAEMA} and DG_{Ag} of PAEMA-co-Ag fabrics on the Ag⁺ ion concentration under radiation-induced coincident reduction and graft polymerization; (e) Contact angle of the PAEMA-co-Ag fabric with DG_{Ag}=0.54% and DG_{PAEMA}=14.6%.

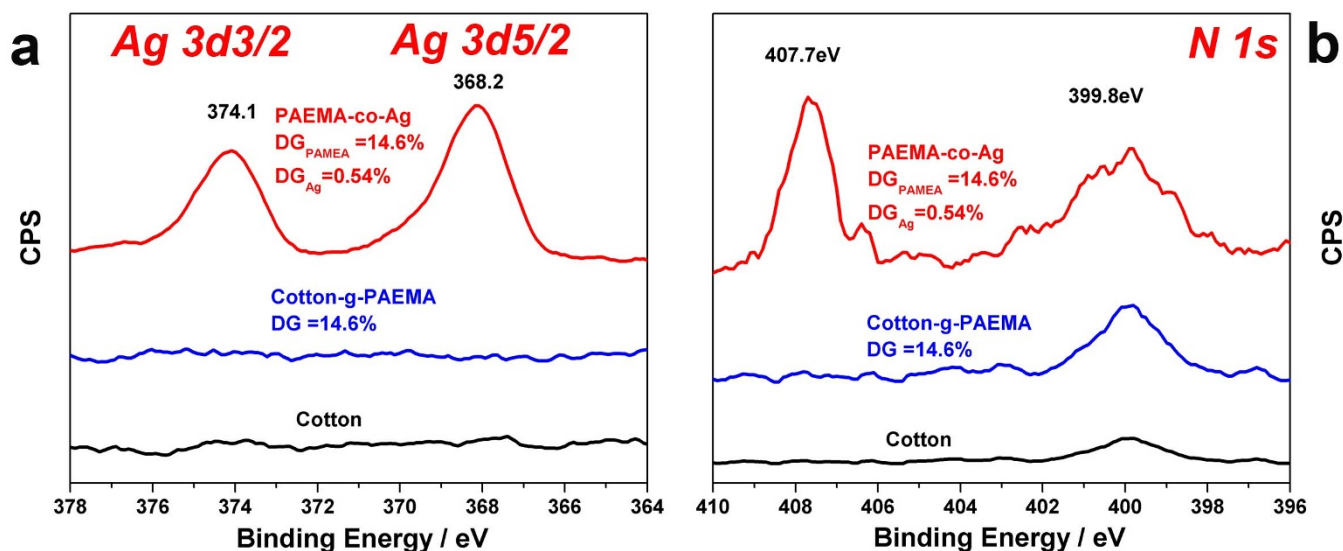


Figure 2 | XPS narrow-scan spectra of the cotton fabric, the cotton-g-PAEMA and the PAEMA-co-Ag fabric: (a) Ag 3d_{3/2} and Ag 3d_{5/2} spectra and (b) N 1s spectra.

could be partially proven by the color of the functionalized PAEMA-co-Ag fabric, which is almost unchanged when compared with pristine cotton fabric, indicating that the silver NPs were wrapped by PAEMA graft chains. If otherwise, the color of the functionalized fabric should be much darker if the silver NPs are exposed on the surface of the fibers, as demonstrated by the control samples.

To reveal the internal structure of the “pomegranates” grown on the surface of the fibers of PAEMA-co-Ag fabric ($DG_{Ag}=0.54\%$ and $DG_{PAEMA}=14.6\%$) (Figure 4a), high-resolution transmission electron microscope (TEM) images (Figure 4b and 4c) were taken, despite the difficulty in doing this due to the insulation nature of the fabric. From the images, the silver “seeds” were clearly found to be less than 10 nm in diameter with a lattice spacing of 0.25 nm and 0.236 nm belonging to the Ag 1/3{422} reflection and {111} facet^{50,51}, which are well wrapped by the thick PAEMA “membrane” and “rind” (Figure 4d). The coordination force of the amino group in the monomer with silver should account for the interaction of the PAEMA chains with the silver NPs, and the coincident growth of the

silver NPs and PAEMA chains should account for the wrapping procedure. These two interactions are both important, and resulted in the final pomegranate structure. The size of the “pomegranates” are determined by the reaction ratio of the reduction and graft polymerization. Seeding speed accompanied by a proper wrapping speed resulted in large fruits, and explains why only a 10% monomer concentration at the fixed 0.01 mol/L AgNO₃ concentration resulted in the wrapped silver NPAs pomegranate structure with sizes of up to hundreds of nanometers.

Control sample preparation. To demonstrate that the coordination force of the amino group with silver and the coincident reduction and graft polymerization are the two key processes for pomegranate-structure formation, we prepared two control samples. One control sample was prepared by radiation-induced coincident reduction and graft polymerization using a monomer, 1-allylurea, with a carbamido group instead of an amino group. The concentration of AgNO₃ and the reaction conditions were exactly the same as that used in

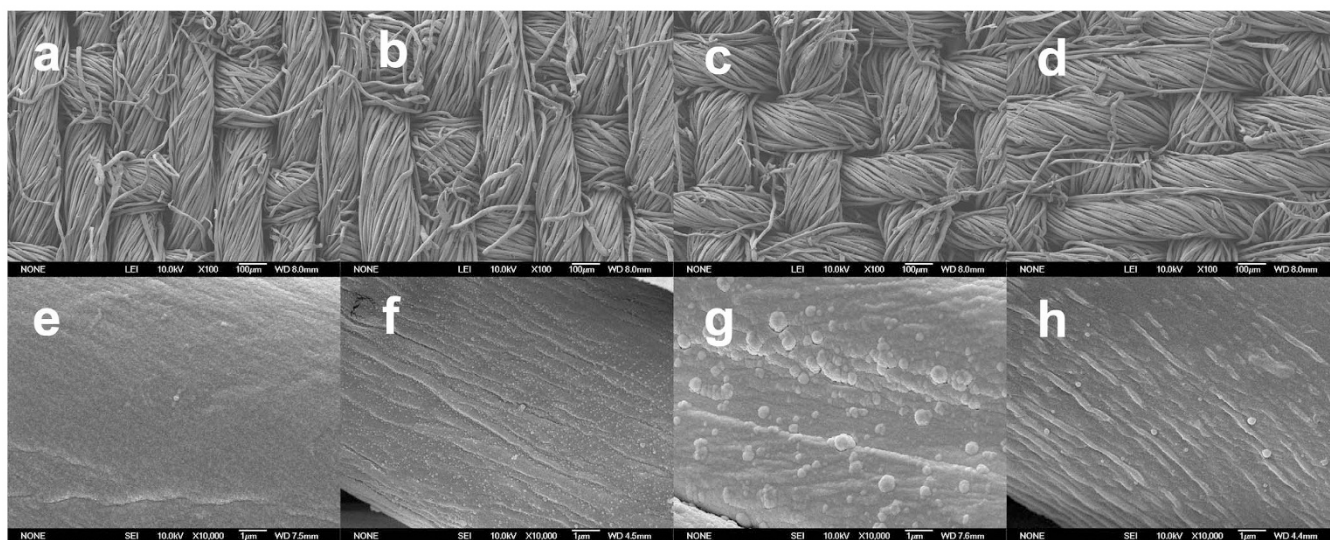


Figure 3 | SEM images of the cotton fabric (a,e) and the PAEMA-g-Ag fabric with $DG_{Ag}=0.82\%$ and $DG_{PAEMA}=10.9\%$ (b,f), the PAEMA-g-Ag fabric with $DG_{Ag}=0.54\%$ and $DG_{PAEMA}=14.6\%$ (c,g), the PAEMA-g-Ag fabric with $DG_{Ag}=0.16\%$ and $DG_{PAEMA}=21.1\%$ (d,h). The scale bars are 100 μm in (a,b,c,d) and 1 μm in (e,f,g,h).

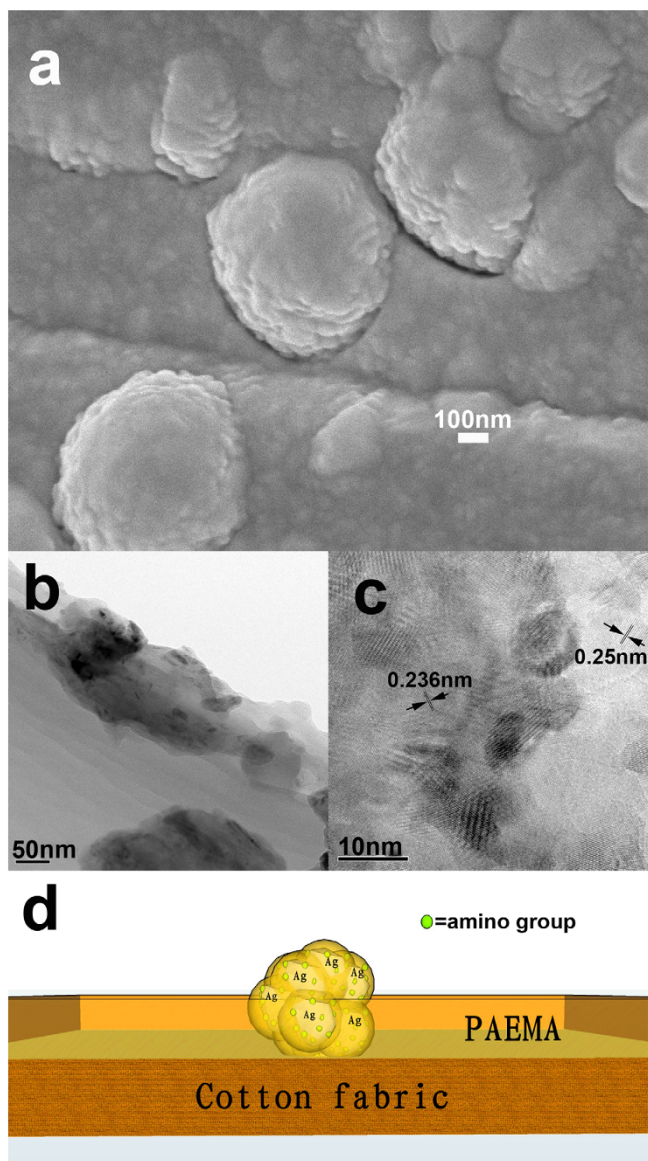


Figure 4 | (a) SEM image of the PAEMA-g-Ag fabric with $DG_{Ag}=0.54\%$ and $DG_{PAEMA}=14.6\%$ ($\times 50,000$); (b,c) TEM images of the PAEMA-g-Ag fabric with $DG_{Ag}=0.54\%$ and $DG_{PAEMA}=14.6\%$; (d) The cartoon of the pomegranate-shaped polymer wrapped in silver NPs on a fiber of the functionalized cotton fabric.

preparation of the PAEMA-co-Ag fabric. The product was named as PAU-co-Ag fabric. When the feed monomer concentrations were exactly the same as the PAEMA-co-Ag fabric with $DG_{Ag}=0.54\%$ and $DG_{PAEMA}=14.6\%$, the PAU-co-Ag fabric has a $DG_{Ag}=11.5\%$ and $DG_{PAU}=2.5\%$ which should be attributed to the poor polymerization kinetic of AU.

The second control sample was prepared by radiation-induced graft polymerization followed by radiation-induced reduction. That is, the cotton fabric was grafted by AEMA and an intermediary product was named Cotton-g-PAEMA. The Cotton-g-PAEMA with $DG_{PAEMA}=14.6\%$ was then deposited with silver NPs by soaking in an $AgNO_3$ solution and irradiated by γ -ray under nitrogen protection. The obtained sample was denoted as PAEMA-dep-Ag fabric and the corresponding DG_{Ag} is 0.12%.

After the functionalization, the color of the PAU-co-Ag and PAEMA-dep-Ag fabrics became chocolate brown and French gray, respectively (Figure S4). Due to the high amount of silver reduced onto the fabric, the SEM images of PAU-co-Ag fabric (Figure S5)

show that there were many cubic- or cuboid-shaped silver NPs up to 400 nm in length deposited on the surface of the fiber and an absence of any round or wrapped structures. And in the SEM images of PAEMA-dep-Ag fabric (Figure S6), there were fewer and transformed cuboid-shape silver NPs up to 200 nm in the field of vision because of low amount of silver loading on the fabric. These results indicated the lack of coordination forces between amino groups and silver NPs together with coincident reaction rate leading to giant silver NPs formation due to the trends to reduce surface energy. Also, the depositing of the silver NPs on the amino group's rich surface encountered with poor efficient and cannot result in a wrapped structure, which leads to fewer and free-standing silver NPs.

Anti-bacterial ability against *E. coli* and *S. aureus*. The antibacterial activity of pomegranate-shaped polymer wrapped in silver NPAs-based cotton fabrics was qualitatively evaluated and compared using gram-negative *E. coli* and gram-positive *S. aureus* bacteria cells according to American Association of Textile Chemists and Colorists (AATCC) Test method 100-2004 by TÜV SÜD PSB Product Testing (Shanghai) Co., Ltd. The detailed procedure is described in the Experiment section. After 24 hours of incubation at 37°C, a higher number of colony-forming units (CFU) appeared on pristine cotton and Cotton-g-PAEMA cotton fabrics that had no silver nanoparticles on their surfaces (Figure 5a–5d). In contrast, fewer *E. coli* and *S. aureus* cells were growing on the PAEMA-co-Ag fabric and fewer *E. coli* and *S. aureus* colonies were found (Figure 5e and 5f), implying that PAEMA-co-Ag fabric possess superior antibacterial activity. Subsequently, the loss of bacteria viability was calculated according to the inactivation efficiency test as detailed in the Experimental section. The results showed that PAEMA-co-Ag fabric possess excellent bactericidal activity, and the inactivation activity was 96.5% for *E. coli* and 99.5% for *S. aureus* (Figure 5g), while cotton fabric and Cotton-g-PAEMA fabric were 0% for *E. coli* and *S. aureus*. PAEMA-co-Ag fabric was the excellent antibacterial material because of higher inactivation efficiency and lower Ag mass. Additionally, the inactivation efficiency of the PAEMA-co-Ag fabric for gram-positive bacteria was better than gram-negative, even though the gram-positive bacteria possessed more complicated cell wall structures.

Laundering durability. Laundering durability is an important and necessary factor for reuse of any modified fabric. A laundering durability test was carried out according to AATCC (American Association of Textile Chemists and Colorists) Test method 61–2006, condition 2A⁵², tested with Without Optical Brightener (WOB) detergent (0.15%, w/w) and 50 stainless steel balls in warm water (Figure S7). One accelerated laundering cycle equals five home or commercial laundings. The inactivation efficiency of PAEMA-co-Ag fabric was measured after 5, 30 and 50 laundering cycles and the results were recorded. After 30 circles, the inactivated efficiencies of PAEMA-co-Ag fabric were still higher than 90% for both *E. coli* and *S. aureus*. After 50 wash cycles, the inactivation efficiency of PAEMA-co-Ag still remained higher than 90%, indicating that the sample possesses excellent laundering durability (Figure 6a).

As for the control samples, after 30 laundering cycles, the inactivated efficiencies of PAEMA-dep-Ag was depressed to both types of bacteria ($R=14.1\%$ to *E. coli* and $R=46.6\%$ to *S. aureus*, Figure S8), which means it is unstable against laundering. Although the inactivated efficiency was around 80% to *E. coli* for PAU-co-Ag fabric after 30 laundering cycles (Figure S9), which was achieved by with an about 20 times higher amount of silver NPs loaded on the surface as compared with PAEMA-co-Ag fabric. Figure 6b and 6c shows the SEM images of the PAEMA-co-Ag fabric before and after 30 laundering tests, and it is obvious that the pomegranates are retained well on the surface of the fibers of PAEMA-co-Ag fabric. But most of the silver NPs were washed off the surface of PAU-co-Ag (Figure S10) and PAEMA-dep-Ag fabric fibers (Figure S11). The results implied

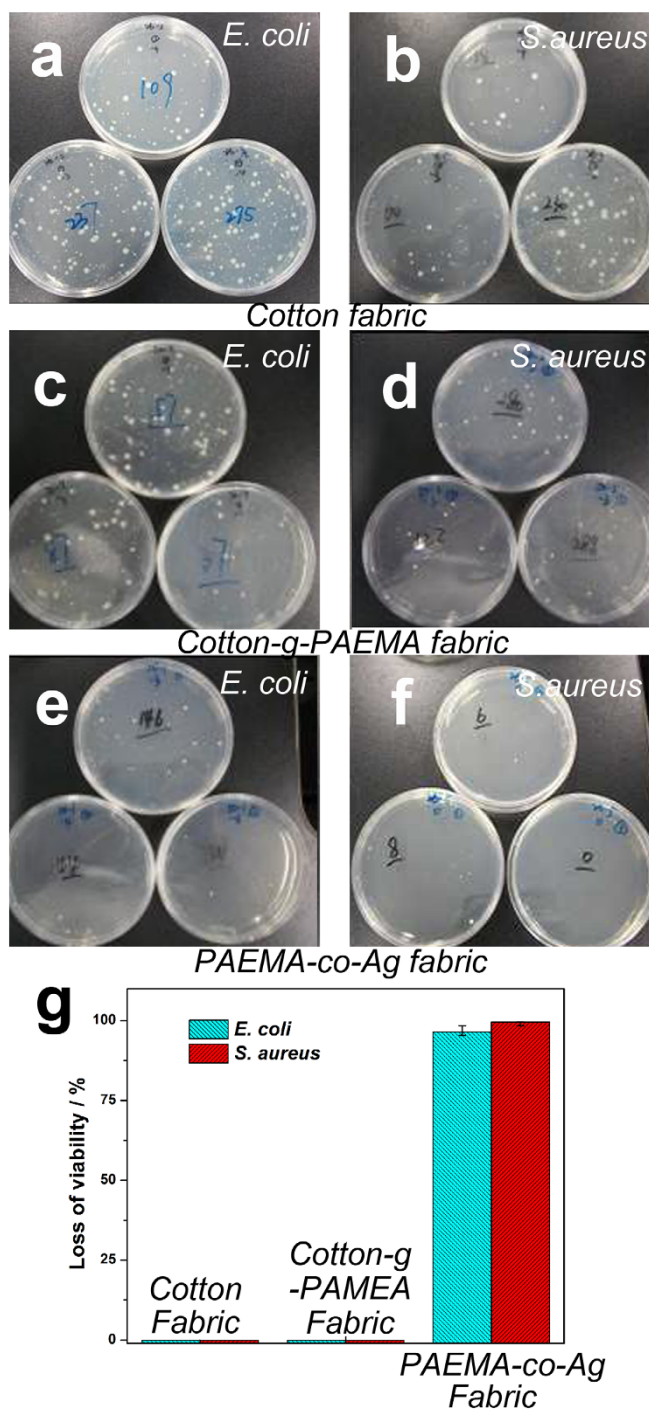


Figure 5 | (a–f) Images of the antibacterial activity test of the cotton fabric, cotton-g-PAEMA fabric and PAEMA-co-Ag fabric ($DG_{Ag}=0.54\%$ and $DG_{PAEMA}=14.6\%$) for *E. coli* and *S. aureus*; (g) The loss of viability for *E. coli* and *S. aureus* of the cotton fabric, cotton-g-PAEMA fabric and PAEMA-co-Ag fabric ($DG_{Ag}=0.54\%$ and $DG_{PAEMA}=14.6\%$).

that PAEMA-co-Ag cotton could effectively reduce the outflow of silver NPs, and therefore, showed excellent laundering durability. Based on inactivated efficiency after the laundering test, it is obvious that the PAEMA-co-Ag fabric is the most stable and efficient antibacterial functionalized cotton fabric, with the laundering stability originating from the polymer-wrapped silver NPs pomegranate structure. Further, taking color changes into account, PAEMA-co-Ag fabric was the best choice in this present work.

In consideration of concerns about increasing silver exposure in the environment, silver ion concentrations in the washing liquid were characterized by the ICP-MS of the PAEMA-co-Ag fabric (Figure 7a). It is obvious that silver element loss is severe in the first 5 cycles, where the highest silver ion concentration is about 0.9 ppm in 150 mL of washing liquid, and becomes steady after 20 cycles. Although the silver NPs in domestic effluent are concerned as expressed in the literature^{53–55}, there is no national standard which gives the limitation of the silver NPs in waste water. For the easier understanding and operation, we measured the silver ion concentration instead here. The total silver ion of wash wastewater are limited to below 0.5 ppm according to the GB18466-2005, while the consumption of washing cloth are 15 L (water)/1 kg (cloth). The 0.9 ppm of silver ions in washing liquids was form 5 accelerated laundering cycles, which is equivalent to 25 instances of home laundering. Therefore, in real laundering, the silver ion concentration should be 0.18 ppm, which is much lower than emission standards of GB18466-2005, which show that the released silver from PAEMA-co-Ag cotton fabrics during washing should be acceptable.

In consideration of concerns about potential threats to humans from silver ions, the cytotoxicity of the diluted washing solution at the 5th cycle for PAEMA-co-Ag cotton fabrics was characterized using HaCaT cells. The washing solution and WOB detergent (0.15%, w/w) were diluted by Phosphate Saline Buffer (PBS) to a certain ratio and then used as the cultivation solution. An MTT assay based on mitochondrial succinic dehydrogenase was employed to detect the metabolic activity of HaCaT cells. As shown in Figure 7b, when the ratio is low, both the washing solution and WOB solution are almost harmless to the cell. But when the ratio increased to 20%, the WOB solution has extremely intense cell toxicity. Meanwhile the washing solution containing an extra 0.18 ppm of silver ions continued to show very low cell toxicity. From the cell morphology, it is obvious that HaCaT cells were slightly different with the blank ones with exposure to the washing solution, however, noticeable cell death occurred with exposure to the WOB solution (Figure S12). It is interesting that the silver ions in the washing solution depressed the cell toxicity of the detergent. And considering that this was an accelerated laundering test and the silver ion concentration is higher than home laundering, the released silver ions from PAEMA-co-Ag cotton fabrics during washing should be safe for human health.

Discussion

Pomegranate-shaped PAEMA wrapped silver NPs functionalized cotton fabrics were prepared by radiation-induced coincident reduction and graft polymerization. The maximum size of the pomegranate-shaped silver NPs can be up to 500 nm with a DG_{Ag} of 0.54% and DG_{PAEMA} of 14.6%. The unique pomegranate-shaped formation can be attributed to the interaction of the PAEMA chains with the silver NPs that originate from coordination forces⁵⁶, and the wrapping procedure originated from the coincident growth of the silver NPs and PAEMA chains, which cannot find on the surface of PAEMA-dep-Ag and PAU-co-Ag cotton fabric. This pomegranate-shaped silver NPs functionalized cotton fabric exhibits outstanding antibacterial activities and also excellent laundering durability, where it can inactivate higher than 90% of both *E. coli* and *S. aureus* even after 50 accelerated laundering cycles (equivalent to 250 commercial or domestic laundering cycles), which are also superior to PAEMA-dep-Ag and PAU-co-Ag cotton fabric. This amazing performance and durability can be attributed to the super-hydrophilic polymer wrapped silver NPs where the silver NPs are strongly covalent-bound to the cotton fibers, but the silver ions can be steadily released³⁶. The simply radiation-induced coincident reaction is also promising for large-scale fabrication of antibacterial cotton fabrics thru pomegranate-shaped polymer wrapped silver NPs.

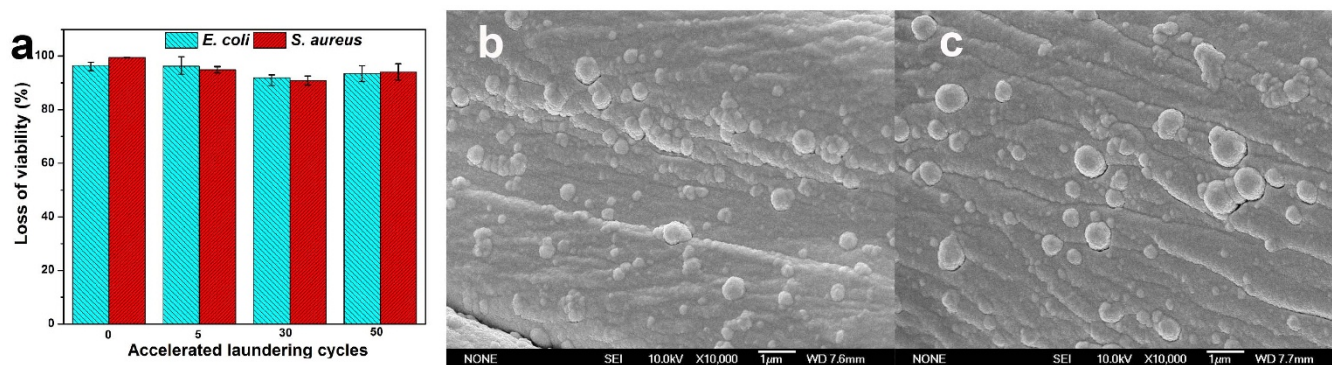


Figure 6 | (a) The loss of viability for *E. coli* and *S. aureus* of the PAEMA-co-Ag fabric ($DG_{Ag}=0.54\%$ and $DG_{PAEMA}=14.6\%$) after accelerated laundering test; SEM image of the pomegranate-shaped polymer wrapped in silver NPs on the fiber before laundering test (b) and after 30 accelerated laundering cycles (c).

Methods

PAEMA-co-Ag fabric preparation. The cotton fabric samples were put into irradiation tubes, then a 0.01 mol/L $AgNO_3$ alcohol-water solution (1 : 9, vol.) with a defined monomer concentration was added to keep the samples immersed. The tubes were purged with nitrogen for 15 min and sealed, and then irradiated with a ^{60}Co γ -ray source at a constant dose rate of 2 kGy/h at room temperature for 17 h, until reaching a total absorbed dose of 34 kGy.

After the reaction, the samples were washed with large amounts of milliQ water, and then the fabric was extracted for 24 h by hot milliQ water in a Soxhlet apparatus to remove residual monomer, homopolymers and unreacted ions. Finally, the samples were vacuum-dried to constant weight.

Determination of the degree of polymer grafting. DG_{Ag} denote the DG of silver NPs on the cotton fabric, which measured by ICP-MS.

DG_{PAEMA} was determined by gravimetric method, which was calculated by eq. (1)

$$DG_{PAEMA}(\%) = \frac{W_g - W_0}{W_0} \times 100\% - DG_{Ag} \quad (1)$$

where W_0 and W_g denote the weights of the pristine and the grafted cotton fabric, respectively.

Inactivation efficiency test. An antibacterial activity test was carried out according to AATCC Test method 100–2004 by TÜV SÜD Products Testing (Shanghai) Co., Ltd. *E. coli* (ATCC 8739) or *S. aureus* (ATCC 6538) was vaccinated in LB (Luria Bertani broth, Lennox modification) liquid media for 24 hours at 37°C. The solution with bacterial cells was diluted to $1 \sim 2 \times 10^5$ /ml in 0.9% sodium chloride solution (NS). One ml of the final bacterial cell density of $1 \sim 2 \times 10^5$ /ml NS solution was evenly put onto different cotton fabrics (diameter = 4.8 cm, circle) which were sterilized in an autoclave at 105°C for 10 min and spread on the bottom of the jar. Then 100 mL neutralizing solution was added to the jar under vigorous agitation and incubated at 37°C and 90% RH for 24 h. 1 ml solution (at '0' contact time) and 1 ml solution (after

24 h contact time) were diluted to 5×10^3 /ml (2×10^3 /ml, *S. aureus*) with 0.9% NS and then coated uniformly on three replicate LB agar plates (with 1.5% agar) per specimen solution (cotton as control group). These plates were incubated at 37°C for 24 h. The colony-forming units (CFU) spread on the agar plates were manually counted. The disk diffusion assay has antibiotic controls. The reduction of bacteria named Loss of viability (%) was calculated with the following formulas:

$$\text{Loss of viability (\%)} = (B-A)/B \times 100\% \quad (1)$$

A: The number of bacteria recovered from the inoculated treated test specimen swatches in the jar incubated for 24 h.

B: The number of bacteria recovered from the inoculated treated test specimen swatches in the jar immediately after inoculation (at "0" contact time).

Laundering durability test. Laundering durability evaluation was carried out according to AATCC Test method 61–2006, condition 2A. The samples were cut into 50 mm \times 150 mm patches and washed in a rotating closed canister containing 150 mL aqueous solution of standard WOB detergent (0.15%, w/w) and 50 stainless steel balls in a thermostatically controlled water bath at 49°C, 40 ± 2 rpm. After being washed, the samples were washed using deionized water and vacuum dried for the inactivation efficiency test.

Cytotoxicity test. Human HaCaT cell lines (Cellbank, Chinese Academy of Sciences, China) were incubated in dulbecco's minimum essential medium (DMEM) medium (Gibco, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Gibco, USA), penicillin (100 U/mL), streptomycin (100 µg/mL) in a humidified atmosphere containing 5% CO_2 at 37°C.

Human HaCaT cell lines were seeded in a 24-well plate (5×10^4 cells/well), and grown overnight prior to the test. Then the cells were maintained in fresh media containing different concentrations of washing liquid. After 48 hours, the metabolism activities of cells were measured via MTT assay. Briefly, 50 µL of 5 mg/mL 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution (MTT, sigma, USA) was added to each well of the 24-well plate, followed by incubation at 37°C for

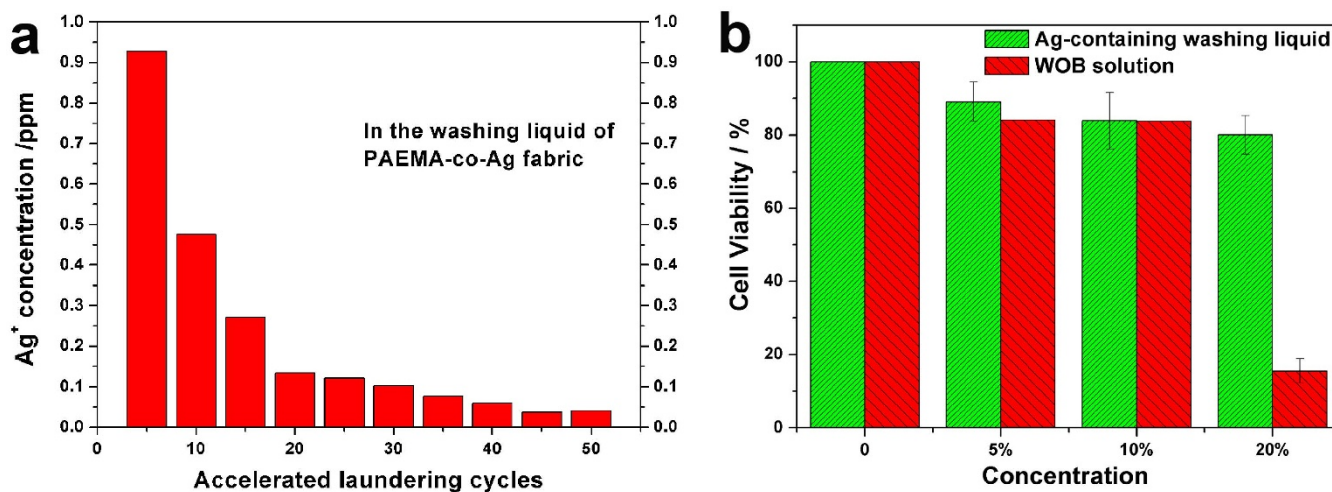


Figure 7 | (a) Ag^+ ions concentration measured by ICP-MS in washing liquids of the PAEMA-co-Ag fabric ($DG_{Ag}=0.54\%$ and $DG_{PAEMA}=14.6\%$) in accelerated laundering test; (b) The cytotoxicity of washing liquid on human HaCaT cells. The cells were exposed to different concentrations (0%, 5%, 10%, 20%, v/v) of PAEMA-co-Ag washing liquid at 5th. accelerated laundering cycle and WOB detergent for 48 h.



4 hours. Then cells were lysed with 10% acid sodium dodecyl sulfate solution (SDS, Sigma, USA). The absorbance of 200 μ L cell lysis solution was measured at 570 nm by using a microplate reader (Bio-Rad 680, USA). The MTT assay was independently performed at least three times.

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Author contributions

J.L. designed and supervised the project. H.L. and M.Y. performed the material preparation. H.L., M.L. and B.D. conducted the characterization. H.L., M.L., B.D., J.L., Q.H. and C.F. analysed the data. H.L., M.L., B.D. and J.L. wrote the manuscript. All authors reviewed the manuscript.

Additional information

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