Non-Leaching, Rapid Bactericidal and Biocompatible Polyester Fabrics Finished with Benzophenone Terminated N-halamine

Shu Wang1 · JianNa Li2 · Yihong Cao1 · JingWei Gu1 · YuanFeng Wang1 · ShiGuo Chen1

Received: 9 June 2021 / Accepted: 20 August 2021 / Published online: 20 September 2021
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Abstract
Pathogenic bacteria can proliferate rapidly on porous fabrics to form bacterial plaques/biofilms, resulting in potential sources of cross-transmissions of diseases and increasing cross-infection in public environments. Many works on antibacterial modification of cotton fabrics have been reported, while very few works were reported to endow poly(ethylene terephthalate) (PET) fabrics with non-leaching antibacterial function without compromising their innate physicochemical properties though PET is the most widely used fabric. Therefore, it is urgent to impart the PET fabrics with non-leaching antibacterial activity. Herein, a novel N-halamine compound, 1-chloro-3-benzophenone-5,5-dimethylhydantoin (Cl-BPDMH), was developed to be covalently bonded onto PET fabrics, rendering non-leaching antibacterial activity while negligible cytotoxicity based on contact-killing principle. Bacterial was easily adhered to Cl-BPDMH finished PET fabrics, and then it was inactivated quickly within 10 s. Furthermore, the breaking strength, breaking elongation, tearing strength, water vapor permeability, air permeability and whiteness of Cl-BPDMH finished PET fabrics were improved obviously compared to raw PET fabrics. Hence, this work developed a facile approach to fabricate multifunctional synthetic textiles to render outstanding and rapid bactericidal activity without compromising their physicochemical properties and biocompatibility.

Keywords Poly(ethylene terephthalate) · Fabric · Bactericidal · N-halamine · Photochemical reaction

Introduction
Poly(ethylene terephthalate) (PET) fabrics have been widely used in our daily clothes due to their excellent strength, easy processability, quick drying, dimensional stability, and wrinkle resistance [1]. However, pathogenic bacteria can proliferate rapidly on porous PET fabrics to form bacterial plaques/biofilms, resulting in irreversibly damaging their appearance, adversely affecting human health, and increasing cross-infection in public environments [2], since bacterial infection is one of the most serious threat to global safety especially drug-resistant bacteria [3–6]. To endow fabrics with antibacterial activity, ciprofloxacin [7], metal salts and nanoparticles [8–10], metallic oxide [11], rose Bengal [12], chitosan [13–15], betaine [16, 17], surface stereochemistry [18], quaternary ammonium salt (QAS) [19–21], dopamine-menthol [22], guanidine [23, 24], and N-halamine [25–27] have been developed, and impressive achievements have been gained. Among them, N-halamine has become a popular antimicrobial due to its remarkable antibacterial activity against wide-spectrum pathogens even drug-resistant pathogenic bacteria [25, 28], rapidly inactivating bacteria [29], and reproducible biocidal activity [30, 31]. Moreover, strenuous efforts have been made to develop N-halamine as disinfectants [32, 33] for water treatment [30], food packaging [34, 35], hydrogel [25], and antibacterial fabrics [36, 37]. Siloxane [38–40] or epoxide terminated N-halamines have been developed to fabricate antibacterial cotton fabrics through a powerful covalent-bonding method.
The aforementioned strategies were suitable for natural fibers containing reactive NH₂ or OH group, while an effective finishing method to fabricate antibacterial PET fabrics without compromising their intrinsic physicochemical properties is still not satisfactory owing to no reactive group in the PET molecular skeleton [2]. To endow PET fabrics with permanent antibacterial, reactive NH₂, COOH, and OH groups were introduced by local destruction of polymer chains, and then the antibacterial constituents were covalently bonded to the locally damaged PET molecular chain through surface coupling reaction [42]. However, those strategies would lead to significant decrease in the mechanical properties and surface roughness of PET fabrics [43]. Therefore, it is urgent to develop a convenient strategy to fabricate permanent antibacterial PET fabrics with well-preserved physicochemical properties. Recently, we developed a benzophenone terminated quaternary ammonium salt (BPQAS) for PET fabric by a photochemical finishing method, rendering perdurable antibacterial, and anti-mite activities without compromising their physicochemical properties [2]. Therefore, the benzophenone terminated N-halamine may be an optimal antibacterial agent for durable antibacterial PET fabrics. However, hydrophilic surface is not suitable for protective textiles such as medical surgical masks and protective clothing with waterproof requirements.

Herein, 1-chloro-3-benzophenone-5,5-dimethylhydantoin (Cl-BPDMH) was developed to finish PET fabrics by covalent bond via a photochemical hydrogen abstraction reaction. The Cl-BPDMH finished PET fabrics exhibited (i) excellent bactericidal activities against Gram-negative Escherichia coli (E. coli) and Gram-positive Staphylococcus aureus (S. aureus); (ii) greatly improved mechanical properties, softness, and air permeability; (iii) negligible cytotoxicity; (iv) rapidly attaching and killing bacteria; (v) well-preserved water vapor permeability and whiteness. This work proposed a potential avenue to develop non-leaching bactericidal synthetic fabrics with great potential applications including but not limited to water purification, masks, home textiles and protective clothing.

**Experimental**

**Materials**

5,5-dimethylhydantoin (DMH, 99%), 4-(Bromomethyl) benzophenone (BP-Br, 99%), trichloroisocyanuric acid (TCCA, 99%), sodium hydroxide (NaOH, 99%), sodium hypochlorite (available chlorine ≥ 8.25%), sodium thiosulfate (Na₂S₂O₃, 99%), and potassium iodide (KI, 99%) were purchased from Energy Chemical Co. Ltd., China. Ethyl acetate (EA, AR), hexane (AR), and acetone (AR) were purchased from Guangzhou Chemical Reagent Factory, China. Gram-negative bacteria *E. coli*, (ATCC25922), Gram-positive bacteria *S. aureus* (ATCC6538) were purchased from Guangdong Institute of Microbiology, China. Cell Counting Kit-8 (CCK-8) was purchased from Beyotime Biotechnology Inc. China. NIH 3T3 mouse embryonic fibroblast cells were sent by Zhejiang University.

**Synthesis of Cl-BPDMH**

The typical synthetic reaction of Cl-BPDMH was following. The intermediate product 3-benzophenone-5,5-dimethylhydantoin (BPDMH, a pale yellow powder) was obtained by a nucleophilic substitution reaction of 5,5-dimethylhydantoin and 4-(bromomethyl) benzophenone (BP-Br) under constant stirring at 80 °C for 24 h (yield, 65%). Then, the crude product CI-BPDMH was prepared by the chlorination reaction of the BPDMH and trichloroisocyanuric acid under continuous stirring for 12 h at room temperature. And the final product Cl-BPDMH was obtained after being purified by silica gel column chromatography using petroleum ether as the eluent (yield, 84%).

**Antibacterial Finishing**

The PET fabrics were finished with Cl-BPDMH as following. Typically, the commercial PET fabrics were sequentially immersed in acetone solution for 2 min and purified water for 5 min under ultrasonic vibration, after being dried in blast drying oven at 50 °C, the clean PET fabrics (defined as raw PET) were obtained. The raw PET fabrics were completely immersed in finishing solution containing Cl-BPDMH (liquor ratio 6:1) at room temperature, then the PET fabrics were irradiated under mild light (1.5 mW, λ = 365 nm) for 1 min. Finally, the Cl-BPDMH finished PET fabrics were obtained after being washed with purified water and air-dried. The PET fabrics finished with different Cl-BPDMH content were defined PET-2 (20 mg/ml), PET-3 (30 mg/ml), and PET-4(40 mg/ml), respectively.

**Structural Characterization**

Fourier transform infrared (FTIR) spectra of BP-Br, MDH, BPDMH, and Cl-BPDMH were recorded on a Nicolet 6700 FTIR spectrometer with a scan range of 400–4000 cm⁻¹ at a resolution of 4 cm⁻¹ and 32 scans. The proton magnetic resonance (¹H-NMR) spectra of BPDMH and Cl-BPDMH were collected on a Bruker Avance-400 spectrometer at 400 MHz by using CDCl₃ and DMSO-d₆ as solvents, respectively, and tetramethylsilane (TMS) was used as the internal standard. The morphologies of the raw PET and Cl-BPDMH finished PET fabrics were observed by field emission scanning electron microscope (FE-SEM, Hitachi SU-70). The chemical element compositions of the raw PET and Cl-BPDMH...
finished PET fabrics were determined by energy dispersive X-ray spectroscopy (EDS) elemental mapping. The oxidative chlorine content of Cl-BPDMH finished PET fabrics were determined by the iodimetric/thiosulfate titration method as compared with raw PET fabrics[44, 45].

**Comprehensive Performance Evaluation**

The tearing strength, bursting strength, breaking strength, breaking elongation, water contact angle (WCA), moisture permeability and air permeability of raw PET and PET-3 were characterized according to our previous work[16, 46], and the detailed test processes were provided in supporting information. The whiteness of raw PET and PET-3 was evaluated by the AATCC 110–2015 test method on a WSB-3A Intelligent Whiteness tester.

**Antibacterial and Biocompatibility**

The quantitative antibacterial activity of the Cl-BPDMH finished PET fabrics against *E. coli* and *S. aureus* was evaluated by flask oscillation method and the plate count method according to FZ/T 73,023–2006 standard [20]. The antibacterial kinetics were carried out using the above experimental operation method while the shaking time was replaced by other predetermined time. The diameters of the inhibition zone (DIZs) were measured by the agar plate diffusion method to evaluate release behavior of the antibacterial constituent from the Cl-BPDMH finished PET fabrics [17]. To investigate the washing durability of antibacterial activity, the antibacterial rates of PET-3 compared with raw PET (control) against *E. coli* and *S. aureus* after being washed for 5, 10 and 20 laundering times, respectively, were performed according to FZ/T 73,023–2006 standard method. Each experiment was carried out in triplicate and the mean results were reported for analysis [2].

To further visualize the antibacterial process of the Cl-BPDMH finished PET-3 fabrics, *E. coli* was stained using the live/dead BacLight Bacterial Viability Kit [47, 48]. Afterward, the dynamic process of stained *E.coli* contacting with PET-3 fabrics compared with raw PET fabrics was observed by rotating confocal fluorescence microscope (Olympus IX explore SpinSR10 instrument). Living *E.coli* was green and dead *E.coli* was red.

The cytotoxicity of Cl-BPDMH finished PET fabrics was evaluated by a CCK-8 assay using NIH 3T3 mouse embryonic fibroblast cells, and rabbit skin irritation was carried out to evaluate their skin irritation according to our previous work[24].

**Results and Discussion**

**Preparation and Structural Characterization**

The Cl-BPDMH was synthesized by two step typical chemical reactions as showed in Fig. 1a. The FTIR spectra of DMH, BP-Br, BPDMH and Cl-BPDMH were shown in Fig. 1b. A peak at about 1650 cm$^{-1}$ assigned to C=O characteristic peaks of diaryl ketone appeared in the FTIR spectra of BP-Br, BPDMH and Cl-BPDMH. While a peak at 3240 cm$^{-1}$ assigned to the N–H characteristic peaks of Hein ring appeared in the FTIR spectra of DMI and BPDMH. Compared with BDMMH, a new peak at 3300 cm$^{-1}$ appears assigned to N–H stretching vibration.

**Fig. 1 a Synthetic route of 1-chloro-3-benzophenone-5,5-dimethylhydantoin (Cl-BPDMH); b FTIR spectra of BP-Br, DMH, BPDMH, Cl-BPDMH; 1H-NMR spectra of c BPDMH and d Cl-BPDMH**
peak at 752 cm\(^{-1}\) assigned to N–Cl group appeared and the characteristic peak of N–H group at 3240 cm\(^{-1}\) disappeared in the FTIR spectrum of the Cl-BPDMH\[49\]. The molecular structures of BPDMH and Cl-BPDMH were further confirmed by \(^1\)H-NMR spectra (Fig. 1c, d). \(^1\)H-NMR of BPDMH (400 MHz, CDCl\(_3\), TMS, Fig. 1c): \(\delta6.38–6.53\) (s, 1H), \(4.58–4.86\) (s, 2H), \(1.40–1.54\) (s, 6H), \(7.40–7.89\) (m, 9H). \(^1\)H-NMR of Cl-BPDMH (400 MHz, DMSO, TMS, Fig. 1d): \(\delta4.58–4.69\) (s, 2H), \(1.27–1.41\) (s, 6H), \(7.34–7.77\) (m, 9H). Therefore, we confirmed that the Cl-BPDMH was synthesized successfully.

The Cl-BPDMH finished PET fabrics were fabricated by covalently binding Cl-BPDMH to raw PET fabrics though a facilely photochemical hydrogen abstraction reaction as showed in Fig. 2a. Compared to raw PET, the surface and cross-sectional morphology of PET-3 exhibited no obvious damage (Fig. 2b), indicating that PET-3 fabrics could maintain their original structures well during the finishing process. Compared to raw PET fabric, a weak signal of Cl (0.12 wt%) ascribed to N–Cl bond appeared in PET-3 (Fig. 2b, insert figure), and the oxidative chlorine content of the Cl-BPDMH finished PET fabrics increased from 0.067 to 0.18 wt% as the concentration of the finishing agent increased (Fig. 2c). The distribution mapping of N and Cl elements (Fig. 2d) could be further confirmed that Cl-BPDMH was successfully bonded to the PET fabrics.

**Antibacterial Activities**

As shown in Fig. 3a, no live *S. aureus* could be observed when it was incubated with Cl-BPDMH finished PET fabrics for 24 h, while about 65% of live *E. coli* could be observed when it incubated with PET-2 for 24 h. Obviously, no live bacteria could be observed for both test bacteria when they incubated with PET-3 and PET-4 for 24 h.

The DIZs were all zero, and no live bacteria was existed under antibacterial PET fabrics (Fig. 3b), indicating that no Cl-BPDMH was leached from the Cl-BPDMH finished PET fabrics. Therefore, the antibacterial mechanism of the Cl-BPDMH finished PET fabrics was proven to contact killing mechanism [28] by directly transferring the oxidative chlorine from N-halamine to bacterial receptors [31, 50]. Non-leaching characteristics of antibacterial fabrics usually exhibited long-lasting antibacterial activity [46]. The antimicrobial rates of PET-3 were higher than 99% against both *S. aureus* and *E. coli* after being washed for 5, 10, and 20 laundering times, respectively (Fig. 3c), which were much than the reference values of AA class (FZ/T 73,023–2006 standard). The bactericidal kinetics of PET-3 were carried out compared with raw PET fabrics as shown in Fig. 3d. PET-3 could inactivate all of *S. aureus* and *E. coli* within 1 h and 3 h, respectively. Gram-positive *S. aureus* were more susceptible to *N*-halamine than Gram-negative *E. coli* may be the cell walls of Gram-negative *E. coli* were more complex.

![Fig. 2](image-url)  
**Fig. 2** a Photochemical reaction principle of PET finished with Cl-BPDMH under UV irradiation; b FE-SEM images and EDS (insert figure); c Oxidative chlorine content; d The chemical element mapping images of the raw PET fabric and antibacterial PET-3
than that of Gram positive *S. aureus* [51]. Thus, the non-leaching Cl-BPDMH finished fabrics exhibited excellent antibacterial activity and PET-3 was selected as the following experimental sample.

To further visualize the process of bacteria contacting with PET-3, and its real bactericidal speed, the in-situ dynamic process of *E. coli* contacting with PET-3 compared with raw PET fabrics was observed by rotating confocal fluorescence microscope as shown in Fig. 3e. The ultra-high resolution fluorescence microscopy image of *E. coli* treated with PET-3 was full of live bacterial (green). Interestingly, *E. coli* was inactivated (red) after contacting with PET-3 within 10 s. Therefore, bacteria can be easily adhered to Cl-BPDMH finished PET fabrics, thereby being inactivated quickly within a few seconds, further indicating the main antibacterial mechanism of the Cl-BPDMH finished PET...
fabrics was contact killing. Though antiviral activity was not provided here, and it would be carried out in the future, N-haloamines have been proved to have excellent antiviral activity [52]. Therefore, Cl-BPDMH finished PET fabrics have potential applications in quick sterilization, including but not limited to water purification, biochemical protective clothing, mask, food packaging, antibacterial textiles.

**Comprehensive Performance Evaluation**

Mechanical property is one of the most critical requirements of the fabrics. Compared with the raw PET fabric, the breaking strength and breaking elongation, tearing strength and bursting strength of PET-3 all showed a general increase (Fig. 4a–d). Specifically, the breaking strength increased by 8.3% in the warp direction from 1450.6 to 1570.3 N, and it increased by 38.5% in the weft direction from 511.7 to 708.4 N (Fig. 4a). Additionally, the breaking elongation increased by 8.0% in the warp direction from 31.7 to 34.2%, and it increased by 69.22% in the weft direction from 11.3 to 19.2% (Fig. 4b) because the increase of the interaction between Cl-BPDMH and PET chains improved the portion of amorphous region [53]. The tearing strength of PET-3 increased by 10.1% in the warp direction from 15.4 to 17.0 N, and it increased by 20.5% in the weft direction from 7.0 to 8.5 N (Fig. 4c). Therefore, the breaking strength, breaking elongation and tearing strength of PET-3 increased obviously compared with raw PET fabrics because the intermolecular forces could increase by introducing polar chlorine group [54] after binding Cl-BPDMH, and extracting an active hydrogen to generate free radicals by mild light irradiating on the surface of PET fabric for a short time would not damage to PET structure [53]. The bursting strength remained basically unchanged as compared with raw PET (Fig. 4d), while the bending stiffness decreased obviously.

![Fig. 4 Comprehensive performance of raw PET and Cl-BPDMH finished PET fabrics.](image)
(Fig. 4e), indicating improved wear comfort property due to its enhanced softness.

The whiteness of Cl-BPDMH finished PET fabrics was well remained without obvious changes as compared with raw PET (Fig. 4f). The water vapor transmission (Fig. 4g) and air permeability (Fig. 4h) of PET-3 both increased slightly may be the pore size of the Cl-BPDMH finished PET fabric were slightly increased during the finishing process, resulting in improving the ability of water vapor and air flow to penetrate the PET fabrics [2]. Compared to raw PET, the water contact angle (WCA) of PET-2, PET-3 and PET-4 all increased, meaning that Cl-BPDMH finished PET fabrics can improve the hydrophobicity of PET fabrics due to the mainly influence of the diaryl group (hydrophobic group) on the chain of Cl-BPDMH (Fig. 4i), which was conducive to adhering bacteria, and thereby inactivated them quickly (Fig. 3e). This strategy has obvious advantages over hydrophilic antibacterial surfaces for medical surgical clothing and masks with waterproof requirements.

Cytotoxicity and Skin Irritation

Cytotoxicity of PET-2, PET-3 and PET-4 compared with raw PET were shown in Fig. 5a. After being co-cultured with PET fabrics for 24 h, the viability of NIH 3T3 cell treated with Cl-BPDMH finished PET fabrics showed a slight decrease. Interestingly, compared with raw PET fabrics, the cell viability of PET-2, PET-3 and PET-4 were all more than 95%. Almost no cell was inactivated (red) after incubated with both raw PET and PET-3 for 24 h compared with NIH 3T3 cell only (Fig. 5b). Therefore, the Cl-BPDMH finished PET fabrics exhibited negligible cytotoxicity.

Raw PET and PET-3 were further exposed to the back of rabbit’s skin to test their irritation responses. After being contacted for 12, 24, 48, and 72 h, respectively, no erythema and edema of rabbit’s skin treated with both raw PET and PET-3 was found on (Fig. 5c). Besides, no distinct histopathological abnormal was found in hematoxylin & eosin (H&E) stained images at any evaluated areas masked by raw PET and PET-3 (Fig. 5d).
Conclusion

Cl-CBPDMH was synthesized to fabricate non-leaching bactericidal PET fabrics by photocatalytic hydrogen abstraction reaction. The Cl-CBPDMH finished PET fabrics can inactivate bacteria within 10 s, indicating their outstanding bactericidal activity against both Gram-positive S. aureus and Gram-negative E. coli. Moreover, PET fabrics can maintain even improve their mechanical properties, comfort properties and biocompatibility after being finished with Cl-BPDMH. Therefore, the Cl-BPDMH was a preferred antimicrobial to fabricate excellent bactericidal PET fabrics while negligible toxicity. Selecting C–H bond as the modified active site, this strategy can be widely applied to finish synthetic fibers, including but not limited to polyester, polyamide, polypropylene, and polyacrylonitrile. This work can promote the development of permanent antibacterial finishing strategy of synthetic fibers. And Cl-BPDMH finished synthetic fibers promises applications including but not limited to water purification, masks, and medical textiles, socks, curtains, carpet and so on.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42765-021-00100-z.

Acknowledgements This work was financially supported by the National Natural Science Foundation of China (No.51773117), the Science and Technology Project of Shenzhen city (No. KC2014ZDZJ0001A).

Declarations

Conflict of interest The authors declare that there is no financial and non-financial competing interest.

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Shu Wang received the bachelor degree of Food Science and Engineering from Beijing Forest University, the master degree of Material Engineering from Shenzhen University. Her research focuses on antibacterial materials.
**Jianna Li** is an associate professor at School of Basic Medical Sciences at Shenzhen University Health Sciences Center. She graduated from School of Life Sciences at Sun Yat-sen University with a PhD, and College of engineering at Hainan University with a master’s degree. She was a visiting scholar at Johns Hopkins University School of Medicine in 2019. Her main research interests are multifunctional wound dressings and skin regeneration, and biosafety evaluation of antibacterial materials.

**Yihong Cao** received the bachelor degree of textile engineering from Wuyi University, and she is presently completing his Master study in the College of Materials Science and Engineering at Shenzhen University, China. Her current research interests are antibacterial materials.

**Yuanfeng Wang** is currently an assistant professor at the College of Materials Science and Engineering at Shenzhen University. He graduated from The Hong Kong Polytechnic University with a PhD, and Donghua University with a master’s degree. His main research interests include smart and functional fibers/textiles, biomimetic materials/interfaces, super-wettable materials, and bio-/medical-materials.

**Jingwei Gu** received the bachelor degree of Nonwoven Material and Engineering from Soochow University, the master degree of Material Science and Engineering from Shenzhen University. His research focuses on antibacterial materials.

**Shiguo Chen** received his BSc degree in chemistry from Jishou University in 2000, a PhD degree in polymer chemistry and physics from Sun Yat-sen University in 2005. He is now a professor of the College of Materials Science and Engineering at Shenzhen University. His research interests include structure–property–processing relationships, characterization techniques and applications of multifunctional fibers/textiles/coatings, and bio-/medical-materials.