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Novel *N*-chloramine precursors for antimicrobial application: synthesis and facile covalent immobilization on polyurethane surface based on Perfluorophenyl azide (PFPA) chemistry

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Abstract

To control the pathogen cross contaminations on medical material surface, there is a pressing need to develop antimicrobial materials with highly efficacious surface biocidal activity. In this work, *N*-Chloramine precursors containing quaternary ammonium unit and perfluorophenyl azide unit were synthesized and covalently immobilized on inert polyurethane (PU) film upon UV light irradiation. The surface modification was confirmed by contact angle, Fourier transform infrared (ATR FT-IR) spectroscopy, X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM) analyses. After bleaching treatment, satisfactory biocidal activity was achieved for the surface modified PU films. It was found that the introduced surface QA center contributed an even faster surface contact killing behavior, and that precursors with longer structural linker caused higher surface chlorine content and higher antimicrobial efficacy. This approach provides a novel and facile method which enables the covalent immobilization of *N*-chloramine precursors on inert polymeric surface to produce durable antimicrobial materials.

Keyword: *N*-chloramine antibacterial agents; Organic synthesis; Perfluorophenyl azide chemistry; Polyurethane surface modification

Introduction

In past decades, there has aroused an increasing concern regarding healthcare associated infections and water-related diseases caused by pathogens.¹ Particularly, microorganism contamination on substrate surface greatly added to the risk of bacterial infections and cross infections, as it has been reported that some pathogenic bacteria could survive on surface for several months.² Therefore, development of highly efficacious antimicrobial materials is imperative to control the bacteria spread and cross infections. Considering prevalent bacterial resistance caused by extensive use of biocides such as quaternary ammonium (QA) salts³ and Ag based agents,⁴ much attention has been directed to immobilize antimicrobial agents onto polymers or fibers to develop non-leaching antimicrobial materials,⁵ especially those based on *N*-chloramines (*N*-Cl)s.⁶

Organic *N*-Cl is usually regarded as a stable storage of oxidizing chlorines (Cl^+) in terms of organic compounds. By transfer of Cl^+ to biological receptors, *N*-Cl displayed high antibacterial efficacy with wide spectrum of microorganisms and facile recharge ability upon ordinary bleaching treatment. Few bacteria resistance of *N*-Cl agents has been reported due to the contact-killing manner based on series of oxidizing biochemical reactions,⁷ thus making it one of the most extensively applied antimicrobial agents.⁶ Recently, by covalent binding a QA unit and an *N*-Cl unit together, Li et al⁸ developed a "composite" *N*-Cl biocide **1** (Fig. 1) started from 5, 5-dimethyl hydantoin (DMH). The QA *N*-Cl precursor **3** (Fig. 1) was covalently immobilized on pre-treated poly(ethylene terephthalate) (PET) and cotton fabrics by "click" reaction method, and antibacterial tests demonstrated that the introduced QA unit greatly boosted the biocidal efficacy of *N*-Cl moieties.⁸ More recently, much efforts has been directed to develop *N*-Cl antibacterial materials based on conferring cationic *N*-Cl precursors on various surface.⁹ However, to the best of our knowledge, there still lacks a universal and efficient surface modification strategy that could realize facile immobilization of quaternized *N*-Cl precursors, such as **2** in Fig. 1, on inert polymeric surface.

Generally, integration of *N*-Cl precursors on polymer surface via covalent bonds produces non-leaching antibacterial materials. Surface nucleophilic-electrophilic chemical reactions or surface grafting polymerization¹⁰ has been considered. To produce covalent bonds between *N*-Cl precursors and substrate surface, some tethering groups, such as the hydroxyl group (**4-5** in Fig. 1),¹¹ the epoxide group (**6** in Fig. 1)¹² and the alkoxy silane group (**7** in Fig. 1),¹³ were needed to realize surface

immobilization on cotton fabrics and silica gels. However, surface modification on inert substrates still remains a challenge due to the lack of surface bonding groups. For instance, harsh chemical hydrolysis¹⁴ was entailed for biofunctionalization of PET substrate, but as a result, the physical and mechanical properties may be compromised. By Friedel-Crafts reaction and Bucherer-Bergs reaction on polystyrene (PS) resin surface, Sun et al introduced handantoinyl *N*-Cl precursors based on the phenyl group of PS backbone, however, a large amount of AlCl₃ and toxic KCN were technically involved.¹⁵ Another strategy involves radical induced polymerization¹⁶ of *N*-Cl precursor monomers. For instance, allyl monomer **8** (Fig. 1) was used for immobilization on inert polymers such as polypropylene (PP) and PET.^{16(b)} However, surface radical induced polymerization is quite complicated and other monomers were usually required to be copolymerized with **8** due to its self-inhibition phenomenon.

Upon photolysis at 254 nm, perfluorophenyl azides (PFPA) are capable of generating nitrene intermediate, which readily proceeds -CH/-NH insertion and C=C addition reaction to practically produce new covalent bonds.¹⁷ In this respect, molecules that tethered with PFPA units could be efficiently photo-immobilized on surface.¹⁷ For instance, Pastine¹⁸ and Liu¹⁹ introduced hydrophilic hydroxypropyl and QA functionalities onto carbon nano tubes and graphene materials respectively. Li and colleges²⁰ developed a versatile polyurethane (PU) platform by imparting surface PFPA groups, and as a result, native heparin was directly photo-coupled within minutes, yielding a film substrate with favorable antithrombogenic properties.

Fig. 1

We herein synthesized QA *N*-Cl precursors 9-11 (Fig. 1) that contain a PFPA unit as the coupling handle. Upon UV light irradiation, the synthetic precursors were conferred onto commercial PU film, one of the most extensively used biomedical materials.²¹ The surface modification was characterized by contact angle, Fourier transform infrared (ATR FT-IR) spectroscopy, X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM) analysis. After bleaching treatment, the surface antibacterial activity was confirmed against *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923), and the relationship between the precursor structure and the final surface antimicrobial efficacy was discussed.

Scheme 1 Synthesis protocol of precursors **9-11** and **24**. Reagents and conditions: a) $\text{BrCH}_2\text{CH}_2\text{OH}$, DCC/DMAP, CH_2Cl_2 (for **14**), $\text{Cl}(\text{CH}_2)_n\text{OH}$ ($n=4$ or $n=6$), concentrated H_2SO_4 , CCl_4 (for **15-16**) reflux; b) NaI , acetone, reflux; c) **12**, CH_3CN , reflux, and then Ion-exchange (Amberlite R IRA-900, Cl^-); d) NaN_3 , acetone/ H_2O , reflux; e) propargyl alcohol, DCC/DMAP, CH_2Cl_2 ; f) $\text{CuSO}_4\text{-Na ascorbate}$, $\text{CHCl}_3/\text{H}_2\text{O}/\text{MeOH}$ (1:1:1), rt; g) NaN_3 , acetone/ H_2O , reflux.

Experimental

Materials and agents

All chemicals and solvents were from commercial suppliers (such as Matrix Scientific and Aladdin) and used as received without further purification unless otherwise stated. Purification of synthesized compounds was conducted by flash column chromatography on silica gel received from Haiyang Chemical Co., Ltd China. PU film was purchased from Wanhua Chemical Group Co., Ltd China. *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were obtained as a gift from the Dalian Medical University, and used as model microorganism to challenge the antimicrobial activity on surface modified PU films.

FTIR spectra were taken on a Nicolet iN10 MX & iS10 spectrometer (Thermo Electron Corporation). XPS spectra were taken on an ESCALAB™ 250Xi spectrometer (Thermo Electron Corporation). ^1H NMR and ^{13}C NMR spectra were recorded on a VARIAN INOVA-300 spectrometer and a VARIAN INOVA-500 spectrometer with chemical shifts reported as ppm. AFM observations were carried out in air at atmosphere pressure with a microscope Dimension ICON (Bruker Company). Mass spectrometric data were obtained on a Q-ToF MS spectrometer (Micromass, Manchester, England). Contact angle was measured by contact angle measurement (OCAH200, Dataphysics Instruments).

Compound **12** and compound **26** were prepared respectively as described previously.^{8, 22} All the obtained NMR data were identical with those found in literatures.

Chemistry synthesis

Synthesis of precursor **9**

Compound **14**

To a solution of pentafluorobenzoic acid (2.12 g, 10 mmol) and 2-bromoethanol (1.87 g, 0.74 mL, 15 mmol) in CH_2Cl_2 (100 mL) was added dicyclohexyl carbodiimide (DCC)

(2.06 g, 10 mmol) and 4-dimethylaminopyridine (DMAP) (0.05 g, 0.41 mol). The suspension was stirred at room temperature overnight, and then the reaction mixture was filtered and evaporated under vacuum, giving the crude product which was further purified by column chromatography eluting with EtOAc/petroleum ether (1:9, v/v). Compound **14** was obtained as a colorless liquid (2.65 g, 83.1%); ^1H NMR (500 MHz, CDCl_3) δ 4.70-4.66 (m, 2H), 3.62 (td, J = 6.1, 1.9 Hz, 2H); ^{13}C NMR (126 MHz, CDCl_3) δ 158.6, 146.6 (m), 144.6 (m), 142.4 (m), 138.6 (m), 136.4 (m), 107.5 (m), 65.7, 27.5; HRMS calcd. for $\text{C}_9\text{H}_4\text{BrF}_5\text{O}_2$ $[\text{M}+\text{Na}]^+$ 340.9207, found: 340.9211.

Compound **19**

Compound **14** (1.75 g, 5.5 mmol) and compound **12** (1.07 g, 5 mmol) was dissolved in 30 mL acetonitrile, and the mixture was then heated to reflux for 20 h before the solvent was removed under vacuum. The crude product was purified by column chromatography eluting with $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$ (1:9, v/v) to give **19** (Br^- form) as white solid. Then the purified product (Br^- form) was dissolved in minimum volum water and passed through anion-exchange rein (Cl^-) slowly to afford target **19** (Cl^- form) as white solid (2.34 g, 88.0%); ^1H NMR (500 MHz, D_2O) δ 4.76-4.77 (m, 2H), 3.83-3.76 (m, 2 H), 3.55-3.44 (m, 2 H), 3.44-3.32 (m, 2 H), 3.24-3.26 (m, 2 H), 3.13 (s, 6 H), 2.07-2.11 (m, 2 H), 1.31 (s, 6H); ^{13}C NMR (126 MHz, D_2O) δ 180.6, 158.6, 157.0, 146.6, 144.5, 142.5, 138.7, 136.7, 107.6, 62.3, 59.9, 59.1, 51.5, 23.3, 21.3; HRMS m/z : $[\text{M}-\text{Cl}]^+$ calcd. for $\text{C}_{19}\text{H}_{23}\text{F}_4\text{N}_3\text{O}_4^+$, 452.1603; found: 452.1598.

Compound **9**

Compound **19** (2.34 g, 4.47 mmol) and NaN_3 (0.31 g, 4.71 mmol) was added into a mixed solvent of water (5 mL) and acetone (30 mL). The mixture was heated to gentle reflux for 20 h before the solvent was removed under vacuum condition. The crude product was purified by column chromatography eluting with $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$ (1:9, v/v), affording product **9** as white solid (2.42 g, 91.4%); ^1H NMR (500 MHz, D_2O) δ 4.76 (t, J = 2.5 Hz, 2H), 3.83-3.76 (m, 2 H), 3.55-3.44 (m, 2 H), 3.44-3.32 (m, 2 H), 3.24-3.26 (m, 2 H), 3.13 (s, 6 H), 2.07-2.11 (m, 2 H), 1.31 (s, 6H); ^{13}C NMR (126 MHz, D_2O) δ 180.6, 158.6, 157.0, 146.6, 144.5, 138.7, 136.7, 114.1, 107.6, 62.3, 59.9, 59.1, 51.5, 23.3, 21.3; HRMS m/z : $[\text{M}-\text{Cl}]^+$ calcd. for $\text{C}_{19}\text{H}_{23}\text{F}_4\text{N}_6\text{O}_4^+$, 475.1711; found: 475.1712.

Synthesis of precursor **10-11**

Compound **15**

To a solution of pentafluorobenzoic acid (4.24 g, 20 mmol) and 4-chloro-1-butanol (3.26 g, 0.03 mol) in CCl_4 (20 mL) was added concentrated sulfuric acid (0.35 mL). The

mixture was heated to reflux overnight and the organic phase was washed with aqueous NaHCO₃ (5% wt, 30 mL) and water (20 mL). After being dried over MgSO₄, the reaction mixture was concentrated under reduced pressure to give the crude product which was further purified by column chromatography eluting with EtOAc/petroleum ether (1:9, v/v). Compound **15** was obtained as a colorless liquid (4.31 g, 71.2%); ¹H NMR (500 MHz, Chloroform-*d*) δ 4.43 (t, *J* = 5.0 Hz, 2H), 3.60 (t, *J* = 5.0 Hz, 2H), 1.94-1.98 (m, 4H); ¹³C NMR (126 MHz, Chloroform-*d*) δ 159.0, 146.4, 144.3, 136.7, 66.0, 44.2, 28.9, 27.1, 25.9; HRMS *m/z*: [M+Na]⁺ calcd. for C₁₁H₈ClF₅O₂Na⁺, 309.0082; found: 309.0088.

Compound **17**

Compound **15** (4.31 g, 14 mmol) and NaI (6.30 g, 42 mmol) was dissolved in 60 mL of acetone. The mixture was heated to flux overnight and inorganic salt was removed by passing the reaction mixture through a thin layer of celite. After concentration, the crude product was purified on flash chromatography column (EtOAc/petroleum ether = 1:9, v/v) to give **17** as colorless liquid (5.14 g, 93.2%) and used directly for the followed reaction; ¹H NMR (500 MHz, Chloroform-*d*) δ 4.42 (t, *J* = 6.7 Hz, 2H), 3.24 (t, *J* = 6.7 Hz, 2H), 1.99-1.88 (m, 4H).

Compound **20**

Compound **17** (1.28 g, 3.25 mmol) and compound **12** (0.46 g, 2.16 mmol) was dissolved in 30 mL of acetonitrile. The mixture was heated to flux overnight before acetonitrile was removed under vacuum condition. The crude product was purified by column chromatography eluting with CH₃OH/CH₂Cl₂ (1:9, v/v) to give **20** as slight yellow solid (I⁻ form). The solid was dissolved in minimum volume water and passed through anion exchange resin (Cl⁻ form) slowly. The corresponding fractions were then concentrated in vacuum to afford **20** (Cl⁻ form) as white solid (0.99 g, 95.3%); ¹H NMR (500 MHz, D₂O) δ 4.35 (t, *J* = 5.6 Hz, 2H), 3.51 (t, *J* = 6.6 Hz, 2H), 3.27-3.21 (m, 4H), 2.99 (s, 6H), 2.02-2.00 (m, 2H), 1.73-1.66 (m, 4H), 1.32 (s, 6H); ¹³C NMR (126 MHz, Chloroform-*d*) δ 180.6, 158.6, 157.0, 146.7, 144.5, 142.5, 138.7, 136.7, 107.6, 67.4, 60.7, 59.1, 50.9, 24.5, 23.4, 21.7, 21.2; HRMS *m/z*: [M-Cl]⁺ calcd. for C₂₁H₂₇F₅N₃O₄⁺, 480.1916; found: 480.1922.

Compound **10**

Compound **20** (0.99 g, 1.76 mmol) and NaN₃ (0.12 g, 1.87 mmol) was added into a mixed solvent of water (5 mL) and acetone (30 mL). The mixture was heated to flux overnight before the solvent was removed under vacuum condition. The crude

product was purified by column chromatography eluting with CH₃OH/CH₂Cl₂ (1:9, v/v) to give **10** as white solid (1.93 g, 95.2%); ¹H NMR (500 MHz, D₂O) δ 4.38 (t, *J* = 5.6 Hz, 2H), 3.50 (t, *J* = 6.6 Hz, 2H), 3.34-3.26 (m, 4H), 3.03 (s, 6H), 2.04-2.01 (m, 2H), 1.84-1.79 (m, 4H), 1.32 (s, 6H); ¹³C NMR (126 MHz, Chloroform-*d*) δ 180.6, 158.6, 157.0, 146.7, 144.5, 138.7, 136.7, 110.2, 108.4, 67.4, 60.7, 59.1, 50.9, 24.5, 23.4, 21.7, 21.2; HRMS *m/z*: [M-Cl]⁺ calcd. for C₂₁H₂₇F₄N₆O₄⁺, 503.2024; found: 503.2031.

Compound **16**

To a solution of pentafluorobenzoic acid (4.24 g, 20 mmol) and 6-chloro-1-hexanol (4.10 g, 30 mmol) in CCl₄ (20 mL) was added 0.35 mL concentrated sulfuric acid. The mixture was heated to reflux overnight and the organic phase was washed with aqueous NaHCO₃ (5% wt, 30 mL) and water (20 mL). After being dried over MgSO₄, the reaction mixture was concentrated under reduced pressure to give the crude product that was further purified by column chromatography eluting with EtOAc/petroleum ether (1:9, v/v); Compound **16** was obtained as a colorless liquid (5.97 g, 90.3%); ¹H NMR (500 MHz, CDCl₃) δ 4.40 (t, *J* = 6.5 Hz, 2H), 3.55 (t, *J* = 6.6 Hz, 2H), 1.84-1.75 (m, 4H), 1.58-1.39 (m, 4H); ¹³C NMR (126 MHz, CDCl₃) δ 159.1, 146.3 (d, *J* = 5.7 Hz), 144.2 (m), 142.1 (d, *J* = 13.2 Hz), 139.4-138.1 (m), 136.7 (d, *J* = 12.2 Hz), 108.4 (m), 66.7, 44.8, 32.4, 28.3, 26.4, 25.1; HRMS calcd. for C₁₃H₁₂ClF₅O₂ [M+Na]⁺ 353.0344, found: 353.0352.

Compound **18**

Compound **16** (4.31 g, 14 mmol) and NaI (6.30 g, 42 mmol) was dissolved in 60 mL of acetone. The mixture was heated to reflux overnight and inorganic salt was removed by passing the reaction mixture through a thin layer of celite. After concentration, the crude product was purified on flash chromatography column (EtOAc/petroleum ether = 1:9, v/v) to give **18** as colorless liquid (5.14 g, 93.2%) and used directly for next reaction; ¹H NMR (500 MHz, Chloroform-*d*) δ 4.39 (t, *J* = 6.5 Hz, 2H), 3.20 (t, *J* = 6.6 Hz, 2H), 1.87-1.76 (m, 4H), 1.48-1.44 (m, 4H); ¹³C NMR (126 MHz, CDCl₃) δ 159.1, 146.3 (m), 144.2 (m), 142.1 (m), 138.7 (m), 136.8 (m), 108.4 (m), 66.7, 33.2, 30.0, 28.7, 24.8, 6.1.

Compound **21**

Compound **18** (2.25 g, 5.33 mmol) and compound **12** (0.701 g, 3.29 mmol) was dissolved in 30 mL of acetonitrile. The mixture was heated to reflux overnight before acetonitrile was removed under vacuum condition. The crude product was purified by column chromatography eluting with CH₃OH/CH₂Cl₂ (1:9, v/v) to give **21** as slight yellow solid (I⁻ form). The solid was dissolved in minimum volume water and passed

through anion exchange resin (Cl^- form) slowly. The corresponding fractions were then concentrated in vacuum to afford **21** (Cl^- form) as white solid (2.01 g, 96.1%); ^1H NMR (500 MHz, Deuterium Oxide) δ 4.36 (t, J = 6.7 Hz, 2 H), 3.52 (t, J = 6.8, 2 H), 3.28-3.23 (m, 4 H), 3.01 (s, 6 H), 2.02-2.05 (m, 2 H), 1.75 - 1.66 (m, 2 H), 1.46-1.41 (m, 2 H), 1.36-1.34 (m, 2 H), 1.34 (s, 6 H); ^{13}C NMR (126 MHz, Deuterium Oxide) δ 180.6, 158.6, 157.0, 146.7, 144.5, 142.5, 138.7, 136.7, 107.6, 66.1, 63.4, 61.1, 59.1, 50.8, 35.4, 24.5, 23.4, 21.2, 18.9. HRMS m/z : $[\text{M}-\text{Cl}]^+$ calcd. for $\text{C}_{23}\text{H}_{31}\text{F}_5\text{N}_3\text{O}_4^+$, 508.2229; found: 508.2246.

Compound **11**

Compound **21** (1.84 g, 3.6 mmol) and NaN_3 (0.26 g, 4.0 mmol) was added into a mixed solvent of water (5 mL) and acetone (30 mL). The mixture was heated to reflux overnight before the solvent was removed under vacuum condition. The crude product was purified by column chromatography eluting with $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$ (1:9, v/v) to give **11** as white solid (1.80 g, 94.2%); ^1H NMR (500 MHz, Deuterium Oxide) δ 4.36 (t, J = 6.3 Hz, 2 H), 3.52 (t, J = 6.8, 2 H), 3.28-3.24 (m, 4 H), 3.01 (s, 6 H), 2.05-2.02 (m, 2 H), 1.75- 1.67 (m, 4 H), 1.46-1.41 (m, 6.7 Hz, 2 H), 1.35-1.37 (m, 2 H), 1.32 (s, 6 H); ^{13}C NMR (126 MHz, Deuterium Oxide) δ 180.6, 158.6, 157.0, 146.7, 144.5, 138.7, 136.7, 111.1, 108.4, 66.1, 63.4, 61.1, 59.1, 50.8, 35.4, 24.5, 23.4, 21.2, 18.9. HRMS m/z : $[\text{M}-\text{Cl}]^+$ calcd. for $\text{C}_{23}\text{H}_{31}\text{F}_4\text{N}_6\text{O}_4^+$, 531.2337; found: 531.2343.

Synthesis of precursor **24**

Compound **25**

To a solution of pentafluorobenzoic acid (2.12 g, 10 mmol) and propargyl alcohol (0.62 g, 11 mmol) in CH_2Cl_2 (100 mL) was added DCC (2.06 g, 10 mmol) and DMAP (0.04 g, 0.31 mmol). The suspension was stirred at room temperature overnight, and then the reaction mixture was filtered and evaporated under vacuum, giving the crude product which was further purified by column chromatography eluting with EtOAc /petroleum ether (1:9, v/v). Compound **25** was obtained as a colorless liquid (2.27 g, 91.1%); ^1H NMR (500 MHz, CDCl_3) δ 4.97 (d, J = 3.0 Hz, 2H), 2.58 (t, J = 3.0 Hz, 1H); ^{13}C NMR (126 MHz, Chloroform- d) δ 158.6, 146.7(m), 144.5(m), 142.5(m), 138.7(m), 136.7(m), 107.6, 76.2, 53.9 (corresponded with published data²³).

Compound **27**

Compound **26** (0.45 g, 2.1 mmol) and compound **25** (0.50 g, 2 mmol) was first dissolved in 30 mL mixed solvent ($\text{CHCl}_3/\text{H}_2\text{O}/\text{MeOH}$ = 1:1:1, v/v/v). Then Na ascorbate (0.16 g, 40% mol) and $\text{CuSO}_4 \cdot \text{H}_2\text{O}$ (0.05 g, 10% mol) were added to initiate the "click" reaction. The mixture was then stirred at room temperature for 24 h before the solvent was removed under vacuum. The crude product was purified by

column chromatography eluting with EtOAc/petroleum ether (1:1, v/v) to give **27** as white solid (1.73 g, 85.0%); ^1H NMR (500 MHz, Chloroform-*d*) δ 7.90 (s, 1H), 5.53 (s, 2H), 4.41 (t, J = 6.9 Hz, 2H), 3.57 (t, J = 6.5 Hz, 2H), 2.29 (p, J = 6.8 Hz, 6H), 1.46 (s, 6H); ^{13}C NMR (126 MHz, Chloroform-*d*) δ 177.3, 166.7, 155.8, 146.7, 144.5, 142.5, 134.7, 124.7, 110.6, 59.7, 58.9, 47.8, 35.6, 29.0, 25.1. HRMS m/z : $[\text{M}+\text{H}]^+$ calcd. For $\text{C}_{18}\text{H}_{17}\text{F}_5\text{N}_5\text{O}_4^+$, 462.1195; found: 461.1204.

Compound **24**

Compound **27** (0.78 g, 1.7 mmol) and NaN_3 (0.15 g, 1.8 mmol) was added into a mixed solvent of water (5 mL), acetone (30 mL), and the mixture heated to reflux for 20 h. Acetone was then evaporated and the mixture was extracted with CH_2Cl_2 (10 mL $\times$ 2). The combined organic phase was dried over anhydrous Na_2SO_4 and concentrated in vacuum to give product **24** as white solid (0.79 g, 95.1%); ^1H NMR (500 MHz, Chloroform-*d*) δ 7.90 (s, 1H), 5.53 (s, 2H), 4.41 (t, J = 6.9 Hz, 2H), 3.57 (t, J = 6.5 Hz, 2H), 2.29 (p, J = 6.8 Hz, 6H), 1.46 (s, 6H); ^{13}C NMR (126 MHz, Chloroform-*d*) δ 177.3, 166.7, 146.7, 144.5, 142.5, 138.7, 126.7, 114.3, 110.2, 59.7, 58.9, 47.8, 35.6, 29.0, 25.1. HRMS m/z : $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{18}\text{H}_{16}\text{F}_4\text{N}_8\text{O}_4^+$, 485.1303; found: 485.1309.

PU surface functionalization via photolysis reaction

Methanol solution of **9-11** and **24** (7.0 mg/mL, Fig. 2) was cast on the PU film and the resulting film was air dried before baked for 30 min in an oven preheated to 50 °C. Then the film was exposed to UV irradiation (254 nm lamps, 1.01 J/cm²) at a distance of 5 cm for 10 min. After a thorough rinse with ethanol, the film was air dried and stored before use (denoted as PU-**9**, PU-**10**, PU-**11** and PU-**24** respectively). The same process was repeated to produce PU-**9** sample for dye staining test: a drop of **9** methanol solution was spotted on the PU film, and the film sample was rinsed with ethanol after photolysis to give sample PU-**9**-s (s = spotting). PU-**9**-s was then immersed in 0.1 mg/mL⁻¹ acid orange II solution for 8 h, excess dye was washed off with water, and an image was then taken.

Surface chlorination

To transform the amino groups into *N*-chloramines, the modified PU films were immersed in a diluted commercial bleach solution (active chlorine content is about 0.5%) and stirred at 37 °C for 120 min with constant shaking at 150 rpm. After chlorination, film specimens (denoted as PU-**9**-Cl, PU-**10**-Cl, PU-**11**-Cl and PU-**24**-Cl respectively) were rinsed with copious amount of deionized and distilled water to remove unreacted free chlorine. Finally the film samples were then air-dried and ready for use.

Titration analysis

The active chlorine contents on surface modified PU film was determined using an iodometric titration method. Briefly, about 0.3 g modified PU film was cut into small pieces, and then treated with 10 mL 2% KI aqueous solution at room temperature with constant stirring for 5 min. The formed I_2 was titrated with sodium thiosulfate solution. The available active chlorine content of the bleached films was then determined from the equation below:

$$Cl^+ = \frac{35.45 \times C \times V}{2 \times S}$$

where Cl^+ is the mass on film (g/cm^2); C and V are the normality (mol/L) and volumes (L), respectively, of the $Na_2S_2O_3$ consumed in the titration; S is the area (cm^2) of the PU film samples.

Antibacterial test

The antibacterial properties of chlorinated PU films were examined against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) using a modified "Sandwich Test" Method. Luria-Bertani Agar was used for bacterial culture. After being sub-cultured from stocks, bacteria were allowed to grow at $37^\circ C$ for 18-20 hours to obtain logarithmic-phase cultures. The modified films PU-9-Cl, PU-10-Cl, PU-11-Cl and PU-24-Cl were first cut into $3 \times 3 \text{ cm}^2$ square swatches, one of which were put on a piece of glass (about 13.0 g). Then 10 μL bacterial suspension (10^6 - 10^7 CFU/mL) was placed onto the swatches, and sandwiched by another identical film swatch. Afterword, another piece of glass was put onto the sandwich to ensure good contact of bacteria with surface. After 15 min of contact, the entire "sandwich" was transferred into 10 mL of 1.0 % sodium thiosulfate aqueous solution to neutralize any active chlorine. The mixture was then vortexed for 1 min to detach the attached bacteria. An aliquot of the solution was removed from the mixture and then serially diluted and 100 μL of each dilution was placed onto a nutrient agar plate (in triplicate). The same procedure was also applied to the bleached untreated PU film to serve as control. The viable bacterial colonies on the agar plates were counted after incubation at $37^\circ C$ for 24 h. Bacterial reduction was reported according to:

$$\text{Percentage reduction of bacteria (\%)} = (A - B) / A \times 100$$

$$\text{Log reduction} = \text{Log } (A/B) \text{ if } B > 0; = \text{Log } (A) \text{ if } B = 0$$

where A is the number of bacteria counted from bleached untreated cotton, and B is the number of bacteria counted from modified cotton fabrics.

Result and discussion

Precursor synthesis

The general structure of the proposed precursors includes a DMH unit, a QA unit and a PFPA bait (Fig. 1). Initial synthesis of **9** involved quaternization of DMH-*tert* amine **12** and PFPA-bromide **13**, trying to covalently connect the DMH unit and PFPA unit via the newly formed QA unit. To this end, *p*-azidotetrafluorobenzoic acid and **12** were firstly prepared according to known procedures.²⁴ Esterification of *p*-azidotetrafluorobenzoic acid with bromoethanol produced **13** that was subsequently reacted with **12** in CH₃CN under reflux. However, no target compound **9** was obtained probably due to thermo-induced decomposition reactions of PFPA moiety. As such, an alternative synthesis strategy (Scheme 1) was proposed, which entailed the PFPA unit formation in the last step as reported.²⁵ The improved synthesis route started from esters **14-16** that were obtained via esterification of pentafluorobenzoic acid. Next, the quaternization reaction with **12** was conducted either directly (**14**) or after halogen atom exchange (**17-18**) in acetone,²⁶ affording the formation of the QA moiety as linker unit. Finally, precursors **19-21** were treated with NaN₃ in acetone under reflux,²⁵ successfully producing target compounds **9-11**. In addition, the non-ionic counterpart **22** was also proposed (Scheme 1) to manifest the role of QA moiety. However, the reaction yield for **23** was somewhat too low (less than 5%), so that we switched to an alternative synthesis protocol to give **24** which relies on the combination between the PFPA-alkyne **25** (Scheme S1) and DMH-azide **26**⁸ by means of “click” chemistry method.²⁷

Fig. 2

Validation of Surface modification

We then turned to PFPA photo grafting strategy^{17, 20} to realize the covalent immobilization on PU surface (Fig. 2). ATR was initially adopted to characterize the surface modification, but no noticeable difference was observed between pristine PU and surface modified films PU-**9**, PU-**10**, PU-**11** and PU-**12** (Fig. S1). It may be because PU and precursors share the same typical functionalities such as ester and amide groups. To this regard, PFPA-alkyl-azide **30** (see synthesis protocol in Scheme S1) was prepared and photo grafted on PU film. As depicted in Fig. 3, a characteristic peak of azide group (2097 cm⁻¹) was observed in ATR spectra, suggesting the successful

surface immobilization. Although decomposition of alkyl azides under UV light has been well documented,²⁸ our data indicated that considerable amount of alkyl azide groups survived at this point. In addition, a droplet of **9** solution was cast on a PU film followed by photolysis, and the resultant film was subjected to Orange II dye staining test. A deeper orange color appeared in the casting area (Fig. S2) probably due to electrostatic interaction between surface QA center and the ionic dye molecule, which clearly indicated that **9** was successfully conferred on PU film by taking advantage of PFPA photo-coupling strategy.

Fig. 3

Fig. 4

Characterization of Surface modification

XPS was then applied to further validate the surface modification. The F 1s signal at a binding energy of 685.7 eV (Fig. 4) indicated the existence of surface PFPA groups,²⁰ while the appearance of Cl 2p peak at 197.4 eV (Fig. S3) demonstrated the presence of surface QA moieties. Since there is no F element for pristine film, the F content calculated by XPS analysis could be used to roughly evaluate the photo-grafting efficacy. Much lower surface F concentration (Fig. 4) was detected on PU-**24**, which suggested poor photo grafting efficacy in contrast to **9-11**. The surface morphology after casting but before irradiation was then scanned under microscope to preliminarily explore the difference. The PU@**9** apparently presented a comparatively smoother surface after casting (Fig. S4), implying that natural surface defects on pristine film was covered with layers of coatings. On the contrary, a number of crystal particles appeared on PU@**24** probably due to the crystallization occurrence of **24** during natural evaporation on surface. As a result, **9** was distributed on surface more evenly as compared to **24**, leading to higher photolysis efficacy and higher surface F content. Furthermore, F content decreased (Fig. 4) with the linker length increased from $-(CH_2)_2-$ (**9**) to $-(CH_2)_6-$ (**11**), indicating that longer linker might reduce surface photo grating efficacy. It could be interpreted that smaller molecules size of **9** probably enabled higher stacking density (Fig. 5A), resulting in more precursors immobilized on surface.

By a bleaching treatment, surface *N*-Cl functionalities were produced to give PU-**9**-Cl, PU-**10**-Cl, PU-**11**-Cl and PU-**24**-Cl. Thus, a droplet of KI solution (2%) was cast on PU-**9**-Cl and a deep brown color appeared (Fig. S2), which exactly indicated the

presence of surface *N*-Cl moieties. The contact angle of the films decreased (Fig. 5C) after photolysis, meaning the successful introduction of ionic precursors on surface. After chlorination, the surface became a little more hydrophobic, also suggesting the successful transformation from *N*-H to *N*-Cl. Because there was little precursor **24** grafted on surface, the contact angle difference between PU and PU-**24** was minimal (Fig. S5). Interestingly, contact angle decreased from PU-**9** to PU-**11** with surface F content declined, which was consistent with previous reports²⁹ that higher surface F content led to more hydrophobic surface. Meanwhile, Roger et al³⁰ grafted phenyl azide-derivative carbohydrates onto PET fibers and found that polymeric carbohydrate multilayers were formed via nitrene insertion during the photolysis process. In our case, multilayers of precursors were also likely grafted on surface. As such, a preliminary conclusion could be made that higher stacking density of **9** may lead to higher photolysis efficacy and higher F content, which might shadow the surface hydrophilicity scheduled by the introduced QA units.

Fig. 5

To validate this idea, AFM was applied to investigate the surface morphology. Pristine PU presented an irregular morphology (Fig. 5B) with roughness of 4.77 nm while surface modified films were relatively smooth, suggesting that covalent coatings remedied original surface defects at different levels. Particularly, PU-**9** film provided the smoothest surface among all the films (Fig. 5B), revealing the fact that much more layers of **9** were grafted as compared to **10** and **11** (Fig. 5A). Such observations agreed well with the XPS and contact angel results. As less **24** was immobilized, PU-**24** surface had similar surface to pristine PU film (Fig. S6).

Surface chlorination

The total surface active chlorine content ($[Cl^+]$) was determined to be 1.35-1.76 $\mu\text{g}/\text{cm}^2$ (Table 1) by titration. According to the previous report,³¹ much higher content (3.43 $\mu\text{g}/\text{cm}^2$) could be achieved after 2, 2, 5, 5-tetramethyl-imidozalidin-4-one was grafted on microporous PU surface, probably due to its higher specific surface area. In case of PU-**11**-Cl, the relative higher chlorine content might also be attributed to the relatively rougher surface (Fig. 5B). However, the overall surface chlorine content level did not varied that much although different amounts of precursors were grafted. It means that probably only the outmost grafted layers were accessible for the bleaching treatment while the inner precursors were buried inside.

Antibacterial activity

Next, antibacterial activity of PU-9-Cl and PU-24-Cl were investigated to manifest the function of the surface QA moiety. As shown in Fig. 5, the slight increase of contact angle for the untreated PU film seemed to imply that a small amount of *N*-H units of PU backbone structure were transformed into *N*-Cl structure. However, the followed titration analysis and Orange II dye staining attempt failed in vain probably due to the fact that the chlorinated *N*-H was really limited. Yet, we herein adopted the bleached untreated PU film as the control for the antibacterial tests. We initially adopted 0.5×10^6 CFU/mL (*S.aureus*) as the bacterial inoculum concentration, but antibacterial tests showed that total kill was achieved for both PU-9-Cl and PU-10-Cl and thus no biocidal difference was observed. We then used higher bacterial inoculum concentration for our next antibacterial tests. Table 1 showed that a relatively higher bacteria reduction for PU-9-Cl was achieved, either against *E.coli*. (35.6% vs 24.9%) or *S. aureus* (52.9% vs 36.2%) within 15 min. Of course, slightly higher chlorine content ($1.47 \mu\text{g}/\text{cm}^2$) on PU-9-Cl may cause higher boosted killing activity, but the presence of surface QA centers may also probably facilitate the bacteria arrest (Fig. 6) and the contact killing towards microbial.⁸ However, the observed antibacterial efficacy difference between PU-9-Cl and PU-24-Cl was not that evident as expected, and we herein proposed possible reasons. Besides active chlorine content and surface hydrophilicity, surface roughness has been reported to be indispensable for surface antibacterial activity in that satisfactory surface roughness usually means higher specific area and may be in favor of bacterial adhesion³². As PU-24 displayed the most uneven surface morphology (Fig. S6), it may consequently promote bacteria adhesion although in absence of surface QA centers, which probably reduced the biocidal efficacy gap between PU-9 and PU-24. It should be stated that the cationic QA centers on PU-9, PU-10 and PU-11 might also cause bacterial reduction. Nevertheless, no noticeable bacterial reduction was observed in further antibacterial tests probably due to the minimal contact duration (15 min) and the sluggish biocidal behavior of QA centers. Such observations were consistent with our previous report in which QA moieties was immobilized on cotton fabric surface via “click” chemistry. For another example, it has been reported that QA thin layers immobilized on cellulose surface exhibited evident biocidal activity only with hours of contact time³³.

Fig. 6

Table 1

We then exploited the possible relationship between the alkyl lengths in precursors and the final surface antibacterial activity. As shown in Table 1, with the linker increased from $-(CH_2)_2-$ (**9**) to $-(CH_2)_6-$ (**11**), the bacterial log reduction increased from $23.6 \pm 2.1\%$ to $60.4 \pm 3.6\%$ (against *E.coli*) and from $60.2 \pm 0.7\%$ to $72.1 \pm 3.6\%$ (against *S.aureus*) respectively. It means that PU-**11**-Cl achieved the best antimicrobial activity probably due to its higher chlorine content, more irregular surface and better surface hydrophilicity as discussed above. The irregular surface of PU-**11**-Cl probably provided more contact killing sites³⁴ and thus basically facilitated the contact killing with assistance of electrostatic affinity between bacteria and surface QA centers (Fig. 6). However, surface contact-killing behavior is complicated, and solid conclusions regarding precise regulations of precursor structure on antimicrobial activity may not be made solely based on precursors **9-11**. We at least could preliminarily conclude that: 1) synthetic precursors were photo-immobilized on PU surface and the surface QA center well boosted the antibacterial efficacy; 2) longer alkyl length in precursors resulted in more irregular surface, higher chlorine content and higher antimicrobial efficacy. Of course, more precursor analogues are needed to pinpoint the relationship between the precursor structure and surface modification together with the final antibacterial activity, and synthesis of new *N*-Cl precursors is being undertaken in our laboratory.

In addition, the regenerability of our surface functionalized PU film was also preliminarily confirmed. After antibacterial tests, sterilization of the used samples was conducted for recycling use by rinse with diluted household bleach instead of the conventional autoclave treatments (see details in supporting information, page 3). By this approach, residual surface bacteria may be contact-killed and removed thoroughly as possible, resulting in recharging surface *N*-Cl precursors for another round of antibacterial test. As expected, the same level surface antibacterial activity was obtained for the recycled samples (see table S1). It means that the antibacterial PU film devised here could be recycled conveniently by diluted household bleach treatment, and we may deliver the detailed regeneration data toward specific medical applications later on.

Conclusion

In summary, QA *N*-Cl precursors were synthesized and photo grafted on PU film by using the introduced PFPA unit as the coupling handle. After UV light irradiation, the change on contact angle and on surface morphology together with presence of surface active chlorine (after bleaching treatment) confirmed the successful surface functionalization. The surface grafted ionic precursors endowed the resultant films significant antimicrobial activity. Precursors that contain longer linker presented better surface hydrophilicity, higher active chlorine content and more irregular surface, which contributed to an even higher contact-killing efficacy. Finally, the surface functionalized PU film could be conveniently recycled upon dilute household bleach rinse. Our work may inspire further studies on the facile covalent immobilization of *N*-Cl precursors onto a wide range of chemically inert polymers, such as PP, PS and PET, opening up a myriad of opportunities in preparation of *N*-Cl based antimicrobial materials.

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Figure captions list

Fig. 1 Structure of representative *N*-Cl_s (precursors) reported previously and proposed in this work

Fig. 2 Schematic illustration of photo-grafting of synthetic *N*-Cl precursors on PU film

Fig. 3 Validation the photo-grating feasibility: ATR spectra of (a) pristine PU film; (b) PU-**30**

Fig. 4 XPS spectra of surface modified PU films: (a) PU-**9**, (b) PU-**10**, (c) PU-**11**, (d) PU-**24**, (e) Pristine PU

Fig. 5 Characterization of surface modification: A) Schematic illustration of surface grafting; B) AFM images before and after surface modification; C) Contact angle (with contacting duration of 90 seconds)

Fig. 6 Proposed schematic illustration of boosted biocidal function of resultant modified PU films

Scheme 1 Synthesis protocol of precursors **9-11** and **24**. Reagents and conditions: a) BrCH₂CH₂OH, DCC/DMAP, CH₂Cl₂ (for **14**), Cl(CH₂)_nOH (n=4 or n=6), concentrated H₂SO₄, CCl₄ (for **15-16**) reflux; b) NaI, acetone, reflux; c) **12**, CH₃CN, reflux, and then Ion-exchange (Amberlite R IRA-900, Cl⁻); d) NaN₃, acetone/H₂O, reflux; e) propargyl alcohol, DCC/DMAP, CH₂Cl₂; f) CuSO₄-Na ascorbate, CHCl₃/H₂O/MeOH (1:1:1), rt; g) NaN₃, acetone/H₂O, reflux.

Table 1 Antibacterial activity of surface modified PU films

a) The bleached untreated PU film was herein adopted as control; b) Inoculum concentration: *E.coli* (2.17×10⁶ Colony-Forming Units (CFU)/mL), *S.aureus* (2.45×10⁷ CFU/mL); c) Inoculum concentration: *E.coli* (2.50×10⁶ CFU/mL), *S.aureus* (1.45×10⁷ CFU/mL);

Structure of representative N-Cl precursors (precursors) reported previously and proposed in this work

380x213mm (96 x 96 DPI)

Schematic illustration of photo-grafting of synthetic N-Cl precursors on PU film

470x206mm (96 x 96 DPI)

Draft

Validation the photo-grating feasibility: ATR spectra of (a) pristine PU film; (b) PU-30

372x236mm (96 x 96 DPI)

XPS spectra of surface modified PU films: (a) PU-9, (b) PU-10, (c) PU-11, (d) PU-24, (e) Pristine PU

356x243mm (96 x 96 DPI)

Characterization of surface modification: A) Schematic illustration of surface grafting; B) AFM images before and after surface modification; C) Contact angle (with contacting duration of 90 seconds)

369x267mm (96 x 96 DPI)

Proposed schematic illustration of boosted biocidal function of resultant modified PU films

345x201mm (96 x 96 DPI)

Initial synthesis protocol

Improved synthesis protocol

Synthesis protocol of precursors 9-11 and 24.

122x58mm (600 x 600 DPI)

Samples ^a	[Cl ⁺] (ug/cm ²)	Contact angle	<i>E.coli</i>		<i>S.aureus</i>	
			Percent	Log	Percent	Log
			reduction / %	reduction / log	reduction / %	reduction / log
The effect of QA moiety on surface antibacterial activity ^b						
PU-9-Cl	1.47±0.01	85.2±0.6	35.6±0.0	0.19±0.00	52.9±5.1	0.33±0.05
PU-24-Cl	1.35±0.01	92.8±1.1	24.9±6.4	0.13±0.04	36.2±2.0	0.20±0.01
The effect of alkyl linker length on surface antibacterial activity ^c						
PU-9-Cl	1.47±0.01	85.2±0.6	23.6±2.1	0.12±0.01	60.2±0.7	0.40±0.01
PU-10-Cl	1.55±0.02	83.7±1.2	32.4±7.5	0.17±0.05	63.7±0.4	0.44±0.01
PU-11-Cl	1.77±0.05	76.8±1.2	60.4±3.6	0.40±0.04	72.1±3.6	0.55±0.07

Draft

380x112mm (96 x 96 DPI)

Draft