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# Counterion-Activated Nanoactuator: Reversibly Switchable Killing/Releasing Bacteria on Polycation Brushes

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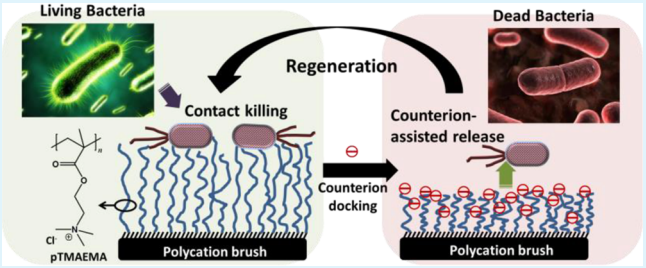
Supporting Information

**ABSTRACT:** A strategy to release attached bacteria from surface-grafted bactericidal poly((trimethylamino)ethyl methacrylate chloride) (pTMAEMA) brushes has been proposed. The pTMAEMA brushes were fabricated via the surface-initiated atom transfer radical polymerization for contact killing of bacteria, including *Escherichia coli*, *Staphylococcus epidermidis* and *Stenotrophomonas maltophilia*. The bacteria-conditioning surfaces, afterward, were washed with electrolyte solutions containing anions with different lipophilic characteristic, charge density, polarity and adsorbility to quaternary ammonium groups in polymers. Because of the special ion-pairing interactions, the interfacial properties, including wettability and $\zeta$-potential, can be manipulated in a controlled manner. Therefore, the counterion-assisted modulation of pTMAEMA brushes facilitates the bacterial release and regeneration of antimicrobial polymer films. The physicochemical properties of polymer brushes and their interactions with counterions were characterized using an ellipsometer, contact angle goniometer, X-ray photoelectron spectroscopy and an electrokinetic analyzer. The repetitive killing and releasing actions of pTMAEMA through unlocking and locking counterions were demonstrated, showing the robust effectiveness of the pTMAEMA-based nanoactuator in controlling the physical action by the chemical stimuli. The real-world implementation of the nanoactuator was demonstrated with a surgical scalpel by repelling killed bacteria and retaining reusability.




**KEYWORDS:** *polymer brushes, antimicrobial biointerfaces, Hofmeister series, polyelectrolytes, responsive polymers*

## INTRODUCTION

Bacterial adsorption is routinely observed on medical apparatus, implants, foods and housewares, leading to a high rate of contamination and pathogenic infection. This can threaten the public health, or deteriorate the function and applicability of devices. For instance, healthcare associated infections (HAIs) cause considerable deaths annually.[1] The majority of the HAIs stemmed from the bacterial adsorption at the surfaces of implants and medical devices, resulting in persistent bacterial growth and a recurrent source of contamination. Once the formation of mature biofilms, bacteria are almost indestructible against the immune system and antimicrobial agents in hostile environments.[2−4]

In the process of the biofilm formation, the initial stage is to condition the substrate by the bacteria-derived substances or by the interfacial properties, e.g., unbalanced net charge and low wettability, to be favorable for bacterial adhesion. Restraining the bacterial adhesion is regarded as the most effective approach to prevent bacterial colonization. Therefore, antifouling coatings based on hydrophilic poly(ethylene glycol) (PEG) materials,[5−7] or zwitterionic materials[8−14] have been successfully developed to passivate surfaces from bacterial adhesion. However, a little amount of bacteria was still found on these materials after a long-term cultivation, likely leading to the sequential growth into the biofilm.[9] In addition to passive resistance against bacterial adhesion, a variety of active surface coatings containing antimicrobial agents were fabricated to kill approaching bacteria or inhibit their growth on the substrates. These antimicrobial agents include cationic polymers,[15−17] hydrolytic enzymes,[18] antimicrobial peptides,[19] antibiotics[20,21] and silver ions.[22,23] Yet, they may fail when coatings were conditioned by a layer of killed bacteria to shield off agents, and to trigger immune response and inflammation.[24] Recently, synergistic approaches to incorporate passive antifouling and active bactericidal functions into a surface design have been achieved. Owing to structure diversity of zwitterionic materials, Jiang and Cheng's groups developed a series of functionally switchable polymers, which are capable of killing and releasing bacteria.[20,24−29] The bactericidal functions of these materials were given by bearing quaternary ammonium moieties in the pendants of polymers or clicking counterions of antibiotics. For the antifouling and/or bacterial releasing functions, the actions

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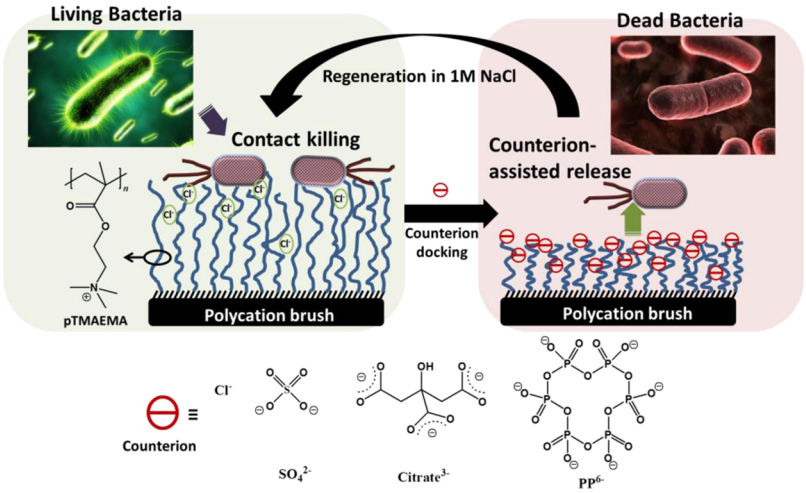
**Scheme 1. Schematic Illustration of Contact Killing and Counterion-Assisted Release of Bacteria on pTMAEMA[a]**




[a]The pTMAEMA film was prepared via SI-ATRP and its interfacial properties were modulated by counterions, including chloride, sulfate, citrate and hexametaphosphate.

were conducted by tuning the environmental pH[27] or hydrolyzing from cationic to zwitterionic groups.[20,24−26,28,29] Besides, Lopez's group carried out alternatively nanopatterned thermoresponsive and biocidal quaternary ammonium polymer brushes to modulate the attachment, killing and release of bacteria in response to changes in temperature.[30] These intelligent designs have attracted a great deal of attentions and open up a new possibility to surface chemistry. However, developed materials rely on stimuli, such as treatments with agitated basic/acidic solutions, pH or temperature changes, to repel adsorbed bacteria or to regenerate the bactericidal properties, which may not be applicable to some applications. In addition, the effectiveness of the bactericidal potency of coatings was only useful under a particular condition, for instance in an anhydrous environment,[25] a solution with a low pH value,[27] or high temperature.[30] Thus, a strategy to effectively kill and release bacteria from bactericidal surface coatings in an environmental friendly, rapid and reversible fashion is to be realized.

Recently, considerable efforts were contributed to the design of smart interfaces based on polyelectrolyte materials as their dynamically controllable properties in response to environmental stimulus. Several research groups have demonstrated the tunable wettability, friction, stiffness and conformational changes of poly((trimethylamino)ethyl methacrylate chloride) (pTMAEMA) thin films by docking proper counterions.[31−42] The films interact with ions via ion-pairing interactions, which have been shown highly stable to perform interfacial properties in a chemically selective way. Huck's group demonstrated that the surface-grafted cationic polyelectrolyte brushes can hold different anions by strong Coulombic forces and exhibit dramatic changes in hydrophobicity following the characteristics of anions in an order of the Hofmeister series.[31−33] The promising evidence was found by reversibly switching the pairing anions between chaotropic bis(trifluoromethane)-sulfonamide (TFSI) and kosmotropic polyphosphate (PP) anions, showing the switching of advancing contact angles between 90° and 15°.[33] Moreover, the ion-pair induced highly hydrated cationic polyelectrolyte brushes possess ultralow friction.[41] Therefore, the special interactions between polyelectrolytes and their counterions offer possibility to modulate surface properties and gain control over the ability to coordinate the adsorption phenomena.

In this work, we aim to develop a novel strategy to release bacteria from bactericidal surfaces via clicking kosmotropic counterions into cationic polyelectrolyte brushes for reversible switching of surface functions between killing and release of bacteria (Scheme 1). We prepared cationic pTMAEMA brushes via surface-initiated atom transfer radical polymerization (SI-ATRP) as an antimicrobial supramolecular platform. The counterions with different valences and hydrophobic characters following the Hofmeister series were used for the ion-pairing interaction. The physicochemical properties of pTMAEMA brushes and their interactions with anions will be characterized using an ellipsometer, contact angle goniometer, electrokinetic analyzer and X-ray photoelectron spectroscopy (XPS) for identification of the thicknesses, surface wettability, $\zeta$-potentials and elemental compositions, respectively. Common pathogens, including *Escherichia coli* (*E. coli*, Gram-negative), *Staphylococcus epidermidis* (*S. epidermidis*, Gram-positive) and *Stenotrophomonas maltophilia* (*S. maltophilia*, Gram-negative), are introduced in contact with pTMAEMA brushes, followed by washing with electrolyte solutions containing Hofmeister anions with attempt to release killed bacteria from the surfaces. The active killing-release modulation will be demonstrated by the pTMAEMA-based nanoactuator fueled by kosmotropic ions for potential applications in reusable medical devices and industrial facilities.

## EXPERIMENTAL SECTION

**Materials.** Methanol, $\omega$-mercaptoundecyl bromoisobutyrate (Br-thiol), copper(I) bromide, magnesium sulfate anhydrous ($MgSO_4$), sodium citrate dehydrate, sodium hexametaphosphate (PP), phosphate buffered saline (PBS) and albumin from human serum (HAS) were purchased from Sigma-Aldrich (St. Louis, MO). Ethanol, tetrahydrofuran (THF), toluene, 2,2′-bipyridiyl (BPY), 3-aminoprpyltrimethylsilane ($NH_2$-silane) and *n*-octyltriethoxysilane ($CH_3$-silane) were obtained from Acros Organics (Geel, Belgium). 2-(Methacryloyloxy) ethyltrimethylammonium chloride solution (TMAEMA) was purchased from Alfa Aesar (Ward Hill, MA). Sodium chloride (NaCl) was acquired from Showa Chemical Industry (Tokyo, Japan). Beef extract Baco Peptone were purchased from Scharlau and Becton, respectively. LIVE/DEAD BacLight was bought from Invitrogen (Carlsbad, CA).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**Preparation of Polyelectrolyte Brushes.** The substrate was prepared via the thermal deposition of a 50 nm gold thin film on a glass slide. The UV-ozone cleaned Au substrate was immersed in ethanol containing 1 mM Br-thiol at room temperature for 24 h in order to form a self-assembled monolayer (SAM) of ATRP initiators, followed by intensive rinsing with ethanol, THF and drying in a stream of filtered air. The Br-thiol modified chip was transferred to a reaction tube containing 1.5 M TMAEMA monomers, BPY and copper(I) bromide with a mole ratio of [TMAEMA]:[BPY]:[CuBr] = 45:4:2. The reaction tube was placed under nitrogen protection in a glovebox and reacted for 24 h. After the polymerization, the substrates were removed and rinsed with ethanol and deionized (DI) water to remove loosely bound polymers, followed by drying in a stream of nitrogen. The modified substrates were stored in DI water at 4 °C.

**Thickness Measurements by Ellipsometry.** The dry thicknesses of pTMAEMA brushes were measured using an ellipsometer (Auto SE, Horiba Jobin Yvon) with a light source with wavelengths ranging from 440 to 1000 nm at a fixed incident angle of 70°. The thickness measurements were taken on four random spots on each sample. The refractive index of polymer films was around 1.45.

**Contact Angle Measurement.** Before the measurement, the substrates were washed with ethanol and dried in a stream of nitrogen. The substrates were then immersed into the 0.1 M electrolyte solutions of NaCl, sodium citrate, $MgSO_4$ and PP, followed by washing with ethanol and drying in a stream of nitrogen. The substrates were transferred to an oven at 80 °C for 2 h. The water contact angles were measured via the sessile drop method using a contact angle goniometry (FTA1000, First Ten Ångstroms). The droplets used were 4 $\mu$L with a microsyringe, and the measurements were performed at least three times at random positions on samples.

**XPS for Elemental Analysis.** The elemental compositions of the pTMAEMA brushes incorporating with couterions were determined using XPS (K-Alpha, Thermo Fisher Scientific). The measurements were performed with a spectrometer equipped with an Al KR X-ray source (1486.6 eV photons) in a vacuum of $2 \times 10^{-8}$ Pa. The angle of incidence was set at 45°. The photoelectron spectra were acquired with pass energy ranging from 50 to 150 eV. The binding energy was estimated to be accurate within 0.2 eV. The high-resolution C 1s, N 1s, Cl 2p, P 2p and S 2p spectrum were recorded for further analysis.

**Zeta Potential Measurements.** The potential at the interface between the immobile and the mobile layer is studied as the electrokinetic or $\zeta$-potential that is analyzed by an electrokinetic analyzer (SurPass, Anton Paar GmbH, Graz, Austria) with a height-adjustable channel. Two planar samples (20 × 10 mm) covered with pTMAEMA brushes facing each other were aligned in parallel with a ~100 $\mu$m spacer to form a microchannel. Solutions containing 0.1 M counterions of NaCl, $MgSO_4$, sodium citrate,and PP dissolved in DI water were prepared. The solution was pumped through the microchannel at 0−300 mbar with a syringe pump. The samples were rinsed thoroughly with the 1 M NaCl solution between measurements so that the counterionis in the surface-confined brushes can be replaced by the ion-exchanging reaction,[32] followed by washing with DI water. The results of the streaming current measurement were converted to $\zeta$ using the Helmholtz−Smoluchowski equation and the Fairbrother−Mastin approach. Each $\zeta$ in different electrolyte solutions represents an average of at least four measurements.

**Bacterial Killing and Release.** Three bacterial species, including *Escherichia coli* (*E. coli*), *Staphylococcus epidermidis* (*S. epidermidis*) and *Stenotrophomonas maltophilia* (*S. maltophilia*), were used in this work. Several colonies from a LB broth agar plate were used to inoculate in 25 mL of LB broth (25 g/mL). After a 16 h culture in the incubator at 37 °C, 5% $CO_2$, the bacteria solutions were diluted to the optical density at 600 nm (OD600) of 0.15 with PBS, corresponding to the concentration of ~$1.2 \times 10^8$ cells/mL. The bacteria were collected by centrifugation at 3000 rpm for 5 min. Cell pellets were washed with sterile PBS and resuspended in PBS, repeating three times. The substrates were first immersed in a 6-well plate containing 4 mL of bacteria solution in each well. Afterward, the plates were incubated at 37 °C, 5% $CO_2$ at 100 rpm for 1 h for bacteria adsorption. The substrates were subsequently washed with DI water and then 5 mL of counterion solutions at a shaking speed of 100 rpm for 2 h for detachment of bacteria. After release, the surface was regenerated by ion-exchanging reaction with 1 M NaCl for 30 min, and then washed with DI water. The substrates were treated with LIVE/DEAD BacLight at its working concentration for 25 min at room temperature. The coverage of adherent cells was expressed as the ratio between the bacteria-covered area and total imaged area using fluorescence upright microscope (Eclipse 80i, Nikon) equipped with a fluorescein isothiocyanate (FITC) filter. For the release rate, it was determined as the ratio between the original and reduced bacteria coverages before and after the treatment of electrolyte solutions.

## RESULTS

**Preparation of pTMAEMA Brushes.** A compact and well-defined polymer brush film was prepared via the bottom-up approach of the SI-ATRP. In this work, we fabricated the polymer films on 50 nm-thick gold layers that were deposited by the physical vapor deposition on glass substrates. The thiolated ATRP initiator (Bi-thiol) was dissolved in EtOH solution and spontaneously formed a SAM onto UV-ozone cleaned gold substrate. Afterward, the pTMAEMA brushes were grown as reported previously.[31−33,43] After thoroughly washed with ethanol and DI water to remove trace residues, the substrates were dried in an oven at 80 °C for 2 h. The contact angle of the films was determined as 59.8 ± 2.6°. The thickness measured by the ellipsometer was 56.4 ± 6.7 nm, which is in agreement with previous work.[43]

**Elemental Composition Measurement by XPS.** The counterion-dependent interfacial properties of pTMAEMA films were investigated for development of an intelligent nanoactuator. The pTMAEMA brush films were brought to contact with electrolyte solutions individually containing 0.1 M NaCl, $MgSO_4$, sodium citrate and PP. The samples treated with corresponding electrolyte solutions were referred to pTMAEMA-$Cl^-$, pTMAEMA-$SO_4^{2-}$, pTMAEMA-$citrate^{3-}$ and pTMAEMA-$PP^{6-}$. After substrate washing, the elemental compositions and docked anions in polymers were determined by XPS (Figure 1). The full-range spectra of samples are shown in Figure 1S (Supporting Information). The N 1s spectra for all samples appeared at a binding energy (BE) of 402.3 eV, indicating the presence of $-N(CH_3)^{3+}$ of pTMAEMA.[44,45] For detecting of couterions after ion docking, spectra of Cl 2p, S 2p, C 1s and P 2p were measured for the corresponding samples of

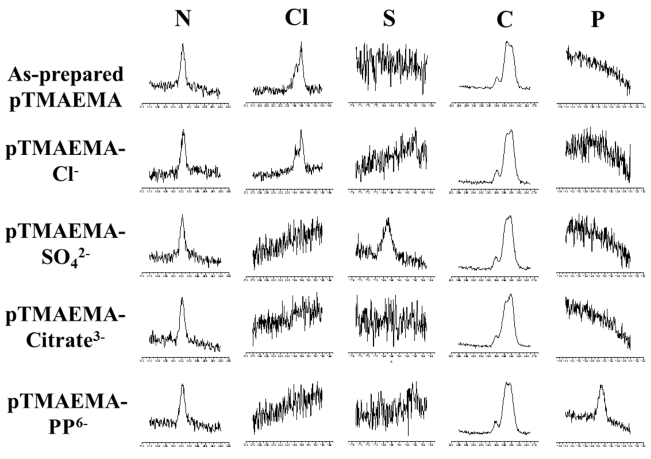


**Figure 1.** XPS spectra of N 1s, Cl 2p, S 2p, C 1s and P 2p for the samples of as-prepared pTMAEMA, pTMAEMA-$Cl^-$, pTMAEMA-$SO_4^{2-}$, pTMAEMA-$citrate^{3-}$ and pTMAEMA-$PP^{6-}$.


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pTMAEMA-$Cl^-$, pTMAEMA-$SO_4^{2-}$, pTMAEMA-citrate$^{3-}$ and pTMAEMA-$PP^{6-}$, respectively. The Cl 2p spin−orbit doublets spectra located at BE = 201.0 and 199.0 eV and S 2p at BE = 168.3 and 168.6 eV, indicating the presence of $Cl^-$ and $SO_4^{2-}$ ions in polymers.[46,47] The C 1s and P 2p core level spectra at BE = 289.1 and 134.6 eV were associated with $COO^-$ and $PO_3^-$ in citrate and PP, respectively.[48] It should be noted that the spectrum signals for $Cl^-$ were not found in the samples of pTMAEMA-$SO_4^{2-}$, pTMAEMA-citrate$^{3-}$ and pTMAEMA-$PP^{6-}$, indicating the ion-exchanging reaction to occur. Therefore, the XPS results reveal the coordination of desirable counterions with pTMAEMA by replacing the $Cl^-$ anions from the as-synthesized films.

**Tunable Surface Wettability and ζ-Potential by Docking Counterions.** Because of the lipophilic characteristics of anions, the wettability of pTMAEMA films can be tunable in a controlled manner by docking counterions.[32,33] The hydrophilicity of as-synthesized pTMAEMA and those with treatment of counterions were determined by contact angle ($\theta$) measurements (Figure 2). The result shows that the

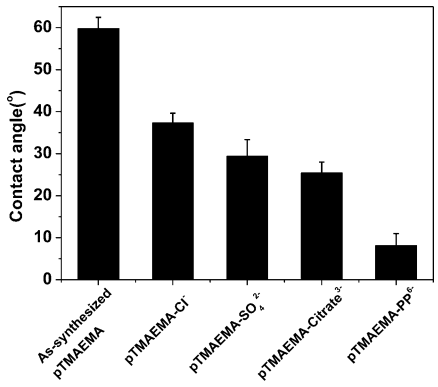


**Figure 2.** Contact angle measurements for the samples of as-prepared pTMAEMA, pTMAEMA-$Cl^-$, pTMAEMA-$SO_4^{2-}$, pTMAEMA-citrate$^{3-}$ and pTMAEMA-$PP^{6-}$. The data were collected from at least three measurements.

change in the wetting characteristics was in accordance with the lipophilic scale, following the order of $Cl^-$ > $SO_4^{2-}$ > citrate$^{3-}$ > $PP^{6-}$. The contact angle can be substantially tuned from hydrophobicity of $\theta$ = 60° to hydrophilicity of $\theta$ = 8°. It is worthwhile to mention that the surface wettability of pTMAEMA can be repetitively changed by unlocking anions in a 1 M NaCl solution and then relocking the anion of interest as a consequence of reversible manipulation of interfacial properties in a chemically selective way.

Because the anions used in the study have different valences and pairing strengths to cations in polymers, we applied the electrokinetic analyzer for measuring the ζ-potentials of pTMAEMA films in response to the interaction of anions (Figure 3). In this work, the ζ-potential of the as-synthesized pTMAEMA brush exhibited a positive net charge in DI water, reflecting the exposure of quaternary ammonium groups at the interface. Moreover, the ζ-potentials of the pTMAEMA changed upon the coordination with anions in electrolyte solutions. The potentials shifted substantially from +166 mV in DI water to −142 mV in the PP solution. The conversion of net charge signs from positive to negative can be ascribed to the valence and propensity of the anions to dock in polymers. Evidently, the strong ion-pairing interactions and unequal

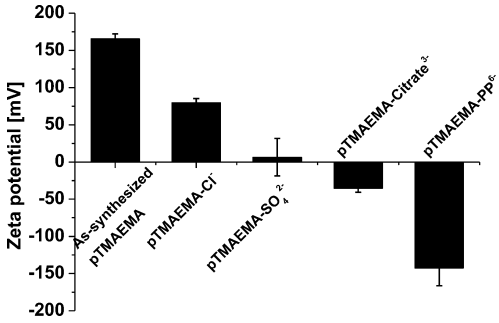


**Figure 3.** ζ-Potential measurements by electrokinetic analyzer for samples of pTMAEMA in solutions of DI water, NaCl, $MgSO_4$, sodium citrate and PP.

adsorption of multivalent anions, such as citrate$^{3-}$ and $PP^{6-}$, at the interface of pTMAEMA films were attributable to the negative ζ-potential.

**Antimicrobial Properties of pTMAEMA and Bacterial Detachment by Docking Counterion.** The pTMAEMA has been employed to actively kill the bacterial cells.[49,50] However, the accumulation of killed cells can condition the surfaces, facilitating the adhesion of following bacteria and likely leading to the formation of biofilms. In this study, we prepared bacterial solutions of *E. coli*, *S. epidermidis* and *S. maltophilia* to challenge the pTMAEMA and to test the capability of anions to detach cells from surfaces for regeneration of pTMAEMA. As shown in Figure 4, after coculture of bacteria and pTMAEMA films, the presence of cells and effectiveness of bactericidal capability of pTMAEMA were determined by using the Live/Dead cell staining and observation under a fluorescence microscope. The results reveal that most bacteria were killed while contacting with pTMAEMA surfaces, manifesting as a large number of bacteria in red. The substrates after bacterial treatments were subsequently immersed into solutions containing NaCl, $MgSO_4$, sodium citrate and PP at a concentration of 0.1 M for 2 h. Interestingly, the numbers of bacteria on surface decreased following the order of pTMAEMA-$Cl^-$ > pTMAEMA-$SO_4^{2-}$ > pTMAEMA-citrate$^{3-}$ > pTMAEMA-$PP^{6-}$ for all bacterial species. The quantitative results in Figure 5 for *E. coli* and in Figure 2S for *S. epidermidis* and *S. maltophilia* in the Supporting Information revealed that the bactericidal rates of pTMAEMA reached at least 94.3%. More importantly, 98.3, 96.2 and 93.7% of the attached *E. coli*, *S. epidermidis* and *S. maltophilia*, respectively, were released from pTMAEMA after the treatment of the PP solution. It has to be noted that the substrates were immersed in the electrolyte solutions and gently horizontally shook at 100 rpm without applying the other drag forces from fluids on bacteria. Therefore, the peeling of bacteria should not be attributable to the mechanical stress.

The releasing action of the pTMAEMA platform by adding counterions may be suspected to be the result of the interaction only between bacteria and anions, rather than that between pTMAEMA and anions. To clarify this argument, we prepared two substrates modified separately with hydrophobic $CH_3$- and $NH_2$-terminated silane coatings for the deposition of *E. coli* and subsequently washing with the PP solution, following the same the protocol as experiments with pTMAEMA. As shown in Figure 6a, the bacteria before and after PP washing on $CH_3$−silane and $NH_2$−silane coatings were imaged using the fluorescence microscope. The surface coverage area ratios in Figure 6b indicate that the cells adhered on both substrates with high survival rates to be 95.9 and 88.0% on $CH_3$−silane


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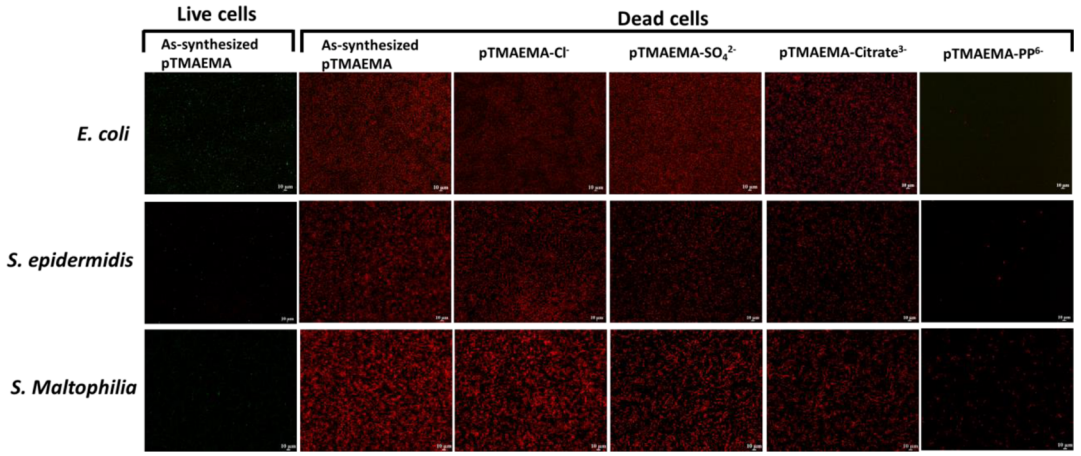


**Figure 4.** Fluorescent images of bacteria on pTMAEMA brushes before and after washing with electrolyte solutions. The bacteria used were *E. coli*, *S. epidermidis* and *S. maltophilia*. The color in green means live cells, whereas that in red means dead cells.

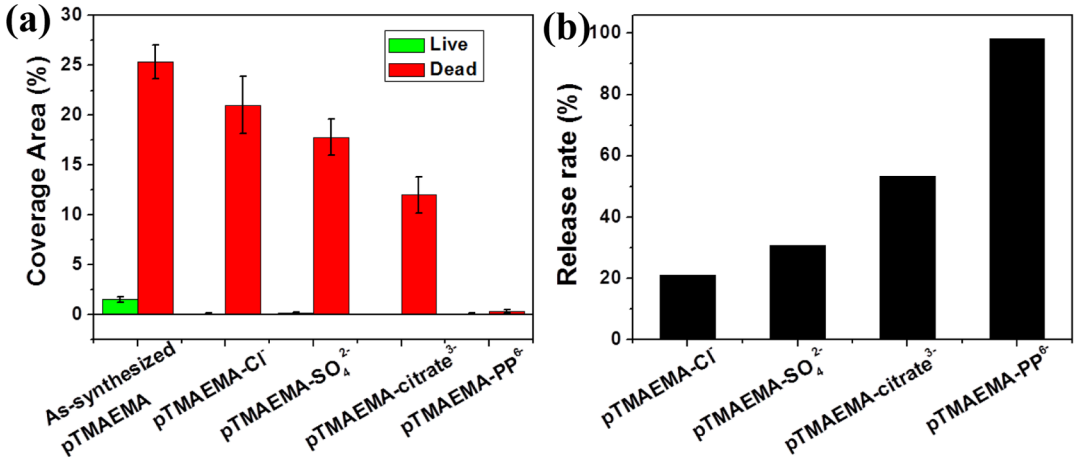


**Figure 5.** Quantification of the coverage area ratio (a) and release rate (b) for *E. coli* on pTMAEMA before and after the treatments of electrolyte solutions.

and $NH_2$−silane coatings, respectively. After treatment with PP, only 2.1 and 9.3% reduction in the total bacteria coverage on $CH_3$−silane and $NH_2$−silane were observed with respect to those before PP washing. The losses of bacteria are negligible compared with the case on pTMAEMA. Interestingly, even under the adverse condition, e.g., the nutritional deficiency or the changes in the osmotic pressure, for bacteria during the PP washing, we did not observe a significant decrease in the bacteria survival rates. Moreover, the control results for the release of bacteria from pTMAEMA with water and PBS washing are shown in the Supporting Information (Figure 3S), indicating no comparable effectiveness as that with the PP treatment in releasing bacteria. Nevertheless, from the control experiments, we can conclude that PP is incapable of killing bacteria and directly interacting with bacteria in concerning of the release action.

To demonstrate the capabilities of the repetitive release of attached bacteria in the PP solution and the regeneration of the bactericidal function of pTMAEMA in the 1 M NaCl solution, we successively deposited and removed *E. coli* on the same pTMAEMA substrate for five cycles. The coverages for live and dead bacteria on pTMAEMA are presented in Figure 7. The result indicates that the cationic polymer films can repel bacteria in the presence of $PP^{6-}$ counterions. However, the release rate gradually decayed from 98.1 to 74.3% after five killing/release circles. The deteriorated capability to release cells may be due to irreversible adsorption of small debris, such as polysaccharides and proteins, which served as conditioning layers to shield off pTMAEMA. Similarly, we conducted the bacteria release with the PP solution after 5 h and 24 h bacterial incubation with pTMAEMA substrates (Figure 4S, Supporting Information). The release rate for the 24-h incubation slightly dropped to 92.4%. Nevertheless, the bactericidal function of pTMAEMA can be effectively recovered for the use in the following circle. The efficiency of bactericidal activity reached 99.3% after five circles.

Herein, the real-world application of the counterion-activated bacteria release approach was demonstrated on a surgical scalpel coated with pTMAEMA brush. The bare scalpel without bacterial deposition was presented in Figure 8a. The pTMAEMA-coated and bare scalpels were incubated in the *E. coli* solution, followed by washing with the PP solution. The samples were imaged by using scanning electron microscopy (SEM), as shown in Figure 8b,c. Clearly, a large amount of rod-shaped bacteria still remained on the bare surface, whereas almost all bacteria were removed from the pTMAEMA surface through the special ion-pairing interactions.


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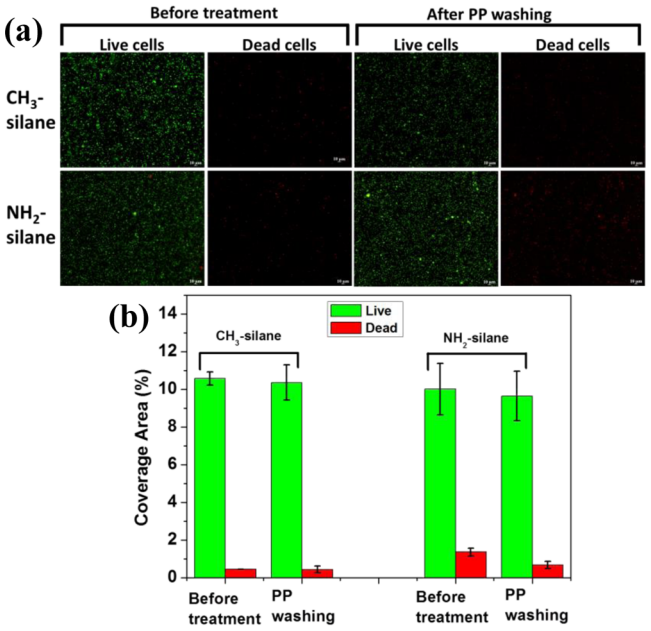


**Figure 6.** Control experiments with $CH_3$−silane and $NH_2$−silane coatings for examining the interactions between *E. coli* and PP ions. The fluorescent images of bacteria on $CH_3$−silane and $NH_2$−silane coatings were taken before and after the PP treatment (a). The coverage area ratios for bacteria on $CH_3$−silane and $NH_2$−silane coatings were estimated before and after the PP treatment (b).

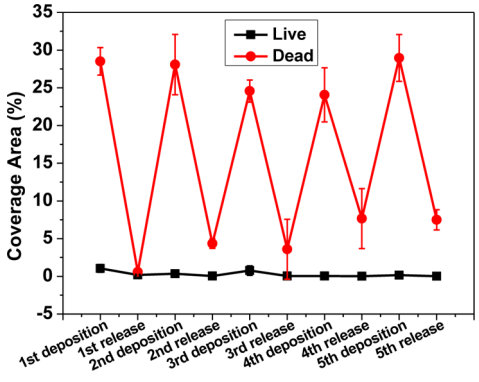


**Figure 7.** Repetitive release of bacteria in the PP solution and the regeneration of bactericidal function of pTMAEMA in the 1 M NaCl. The surface coverage area ratio of *E. coli* was estimated by using a fluorescence microscope, and the live and dead bacteria were distinguished by fluorescent dye.

## DISCUSSION

The surface-based strategies to resist bacteria have been developed for decades.[51] These designs namely relied on (1) positively charged bactericidal polymers; (2) surface-confined antimicrobials; (3) antifouling materials. Although the effectiveness of the works was successfully proved, the contact-killed bacteria on surfaces usually serve as a conditioning layer for the bacterial colonization and finally biofilm formation. The combinative approaches have been achieved to kill and release bacteria on intelligent surface platforms.[20,24−30] However, these platforms either are workable under a limited operation environment or acquired sophisticated chemistry or fabrication. In this work, we perform a novel approach to repetitively kill and remove bacteria from surfaces. pTMAEMA brushes on substrates were prepared via SI-ATRP, which have been

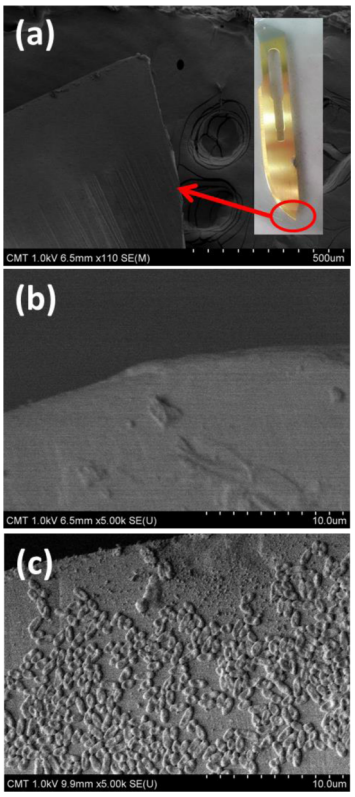


**Figure 8.** SEM images of bare (a, c), pTMAEMA-coated (b) scalpels. The tip of the scalpel as the inset picture before bacteria adsorption was imaged (a). *E. coli* were brought to contact with scalpels and then washed in the PP solution (b, c). The rod-shaped bacteria were observed on the bare scalpel (c).

realized to potently kill the bacteria once contacting.[49,50] A common feature of quaternary ammonium is its ability to cause rearrangement of the membrane and subsequent leakage of intracellular constituents.[52] Herein, after exposure to bacteria, the substrates were then immersed in electrolyte solutions with four types of anions. The interactions between pTMAEMA and anions enable to change the interfacial properties of films, and turn out to affect the association between polymers and adhered bacteria.

Specific ion effects ubiquitously occur in nature, such as on solution properties of density, viscosity, heat capacity, refractive index, freezing point depression and osmotic pressure. A simple ion pair can be elucidated by complex interactions involving ionic radii, hydration, polarization and charge density. In the 1870s, Hofmeister carried out the relative effectiveness of different on the precipitation of protein and colloids, showing a reoccurring trend called the Hofmeister series.[53] Generally, the ions are grouped into kosmotrope and chaotrope, referring to the ion's ability to alter the hydrogen boding network of water. The difference gives rise to salting-out and salting-in behaviors. The finding highlights the specific ion effects on biomolecules. Recently, considerable attention has been paid to understanding the effects of the Hofmeister phenomena on macromolecules.[54−56] In this work, we investigated the ion effects on changes of the wettability, $\zeta$-potential and the interactions with adsorbed biological objectives with surface-confined polycation brushes.

From the XPS experiments (Figure 1), we confirmed docked ions and quaternary ammonium groups in pTMAEMA brushes. The surface wetting measurements were carried out, showing


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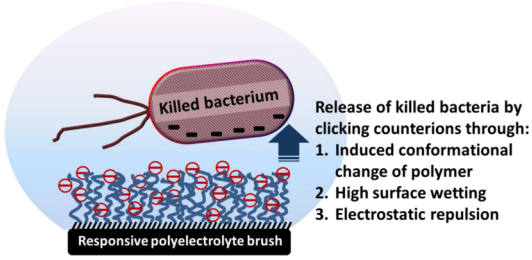
that the order of wettability correlates to the Hofmeister series (Figure 2). Cremer's group proposed a model from time-resolved and thermodynamic studies, showing that the adsorbability of anions onto the interface determines its influence on macromolecular properties, instead of being central to the bulk water structure.[55] The statement was supported by Tahara's recent study on the counterion effect on the interfacial water at the charged surfactant interfaces by using advanced heterodyne-detected vibrational sum frequency generation spectroscopy.[56] The results clearly demonstrated that the anions with a strong hydration shell gets difficult to approach to the surface, resulting in a relatively thick Helmholtz layer and structured water layer, which is opposite to chaotropic anions. Therefore, the propensity of the ion to adsorption determined the order of the halide anion effects at the quaternary ammonium interface. Huck's group reported a series of works on tuning interfacial properties of polyelectrolyte brushes by clicking counterions.[31−33,38,42] They found that while in contact with counterions, the conformational changes of the surface-grafted polymers can be due to "hydrophobic collapse" or "electrostatic-driven collapse".[31,42] The ion-pairing interactions also attributed to the changes in surface wetting and polymer stiffness.[32,33,38] Herein, our results from the contact angle measurements are well in accordance with that from Huck's works. Additionally, the $\zeta$-potentials can be varied from positive to negative in an order of $Cl^- > SO_4^{2-} >$ citrate$^{3-}$ > $PP^{6-}$ (Figure 3). Evidently, in the PP solution, excess anions appear at the panel between the dispersion medium and the stationary layer of fluid.

Bacteria generally possess negative charge at the outmost membranes under a physiological condition. The pTMAEMA platform provides an electrostatic-driven attraction to draw the bacteria to contact the locally abundant ammonium moieties in polymers to kill bacteria. Besides to active bactericidal function, we applied the specific ion-pairing interaction between quaternary ammonium and its counterions to repel killed bacteria from polymer films. We suggest that PP associates with ammonium to induce the collapse of polymer chains due to electrostatic screening,[32,42] and to increase the surface wettability (Figure 2), leading to weakening of the bonding to bacteria. In addition, the repulsive force from the excess negative net charges (Figure 3) to bacteria enables to repel the cells from surfaces. Together with induced conformational change, strong hydration and negative net charge, $PP^{6-}$ is capable of removing the adhered bacteria from the pTMAEMA brushes and to regenerate the bactericidal properties of the platform (Figures 5 and 7). Moreover, the reusability of the pTMAEMA platform was achieved as shown in Figure 7. $PP^{6-}$ can repetitively unlock and lock to pTMAEMA to modulate the surface activities between bactericidal and release. Therefore, the pTMAEMA platform acts as a nanoactuator that is activated by proper anions to attach/detach bacteria through the specific ion pair interaction. The plausible mechanism of counterion-driven repulsion to bacteria is proposed in Scheme 2. The system possesses several advantages, including (1) pTMAEMA is commercially available and commonly used as antimicrobial agent for contact killing; (2) the antimicrobial function of pTMAEMA can be active under various conditions (different temperatures, pH values, and so on); (3) the switching of interfacial properties of pTMAEMA does not need to use strong basic or acidic solutions and heat; (4) electrolyte solutions do not cause significant adverse effect to the ecological environment. Therefore, this work offers a step toward creating a smart dynamic biointerface, regulated via the specific ion pair interaction. In addition to the scalpel (Figure 8), we can envision its wide spectrum of implementation in most reusable surgical devices, healthcare-used clothes, filtration apparatus, coatings for pipes or reactors, and so on.

**Scheme 2. Mechanism of the Counterion-Assisted Bacterial Release from the pTMAEMA-based Nanoactuator**




## CONCLUSIONS

In summary, a novel strategy for the repetitive regeneration of the surface-grafted antimicrobial pTMAEMA films has been developed. The anions in electrolyte solutions possess distinct lipophilic characteristic, charge density, polarity and adsorbility to ammonium groups in polymers, which leads to different degrees of changes in the polymer conformation, wettability and $\zeta$-potential at interfaces. Three kinds of bacteria, including *E. coli*, *S. epidermidis* and *S. maltophilia*, in solutions were introduced to contact with pTMAEMA films. The results displayed the excellent effectiveness of the antimicrobial function of cationic polymers in accordance with previous works. For the counterion-assisted bacterial release from the pTMAEMA films, electrolyte solutions, containing NaCl, $MgSO_4$, sodium citrate and PP individually, were used to interact with bacteria-conditioned polymer films. After gentle washing, the release rates of bacteria from pTMAEMA surfaces follow the orders of wetting characteristics and $\zeta$-potentials, attributing to the ion-pairing interactions of counterions with quaternary ammonium in polymers. Herein, the kosmotropic $PP^{6-}$ anion enables to effectively repel adsorbed bacteria to a release rate of more than 93%. According to the results from the control experiments with alkyne- and amine-terminated silane adlayers, we exclude the effect of the direct anion-bacteria interaction on bacteria release and surface regeneration. Thus, the nanoactuator based on the polycation brush offers a great versatility for introducing chemical functionalities by interacting with a wide range of counterions to trigger physical modulation in a control manner.

## ASSOCIATED CONTENT

### Supporting Information

Full-range XPS spectra; quantification of the coverage area ratio and release rate for *S. epidermidis* and *S. maltophilia*; *E. coli* release from pTMAEMA by treating with water and PBS; long-term cultivated *E. coli* release from pTMAEMA. This material is available free of charge via the Internet at http://pubs.acs.org

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### Notes

The authors declare no competing financial interest.


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