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Integrated Antimicrobial and Nonfouling Zwitterionic Polymers

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antimicrobial · bactericidal · nonfouling · polymers · zwitterions

Zwitterionic polymers are generally viewed as a new class of nonfouling materials. Unlike their poly(ethylene glycol) (PEG) counterparts, zwitterionic polymers have a broader chemical diversity and greater freedom for molecular design. In this Minireview, we highlight recent microbiological applications of zwitterionic polymers and their derivatives, with an emphasis on several unique molecular strategies to integrate antimicrobial and nonfouling properties. We will also discuss our insights into the bacterial nonfouling performance of zwitterionic polymers and one example of engineering zwitterionic polymer derivatives for antimicrobial wound-dressing applications.

1. Introduction

By definition, zwitterionic polymers bear an equimolar number of homogenously distributed anionic and cationic groups along their polymer chains.[1–3] This combination of oppositely charged moieties (German: zwitter) grants the polymers their ultra-hydrophilicity, while, at the same time, maintaining an overall charge neutrality. From an engineering perspective, zwitterionic polymers are generally considered to be an alternative to the widely used poly(ethylene glycol) (PEG) polymers for nonfouling applications to prevent nonspecific protein adsorption as well as to minimize bacterial or mammalian cell adhesion.[4,5] Chemically, however, this empirical juxtaposition between PEG and zwitterionic polymers can be misleading, as all PEG polymers essentially share the same repeating unit, while zwitterionic materials encompass a broad spectrum of polymers with distinctive monomeric chemical structures. Some major aspects of this aforementioned chemical diversity include: 1) the choice of ionic groups to be incorporated into the polymer structure (e.g., carboxylate,[6] sulfonate,[7] or phosphate[8] as anionic groups, and quaternary ammonium,[9] phosphonium,[10] pyridinium,[11] or imidazolium[12] as cationic groups); 2) the spatial arrangement of charged groups, that is, the proximity between positive and negative charges within the same monomeric unit[13] or the total separation of oppositely charged ionic groups onto different polymer side chains (the latter case is also known as "mixed charge" polymers);[14] 3) various derivatives of conventional zwitterionic polymers that can either switch between zwitterionic and non-zwitterionic forms,[15–17] or carry a charged biologically active molecule as a part of the zwitterionic constituent.[18] This structural diversity brings functional versatility to zwitterionic materials beyond nonfouling. This functional aspect is further accentuated by the ionic nature of zwitterionic materials, which enables the adjustment of polymer charge density, pH sensitivity, counterion association, etc., thus distinguishing this type of polymers from other non-ionic nonfouling materials, including polyoxazolines[19] and polysarcosine.[20] Because of their practical importance, microbiological applications of zwitterionic polymers and their derivatives have been studied in depth in recent years, resulting in new mechanistic understandings and novel molecular designs. Surveying these studies should serve as the focus of this Minireview, while readers interested in the synthesis, applications, and nonfouling mechanisms of zwitterionic polymers are referred to previous reviews.[1,21,22] To clarify some key nomenclatures: "nonfouling" means the repelling of bacterial adhesion through polymer hydration, "bactericidal" indicates the active killing of bacterial cells, and "antimicrobial" is a broader term, covering both bactericidal and bacteriostatic activities. Methacrylate (MA) and acrylamide (AA) are two common backbones for zwitterionic polymers, and their influence on nonfouling properties is quite similar.

Though surface resistance to nonspecific protein adsorption and bacterial adhesion still remains central to the studies of zwitterionic polymers, the chemical diversity and malleability of zwitterionic polymers make it possible to impart functionality into otherwise biologically inert materials

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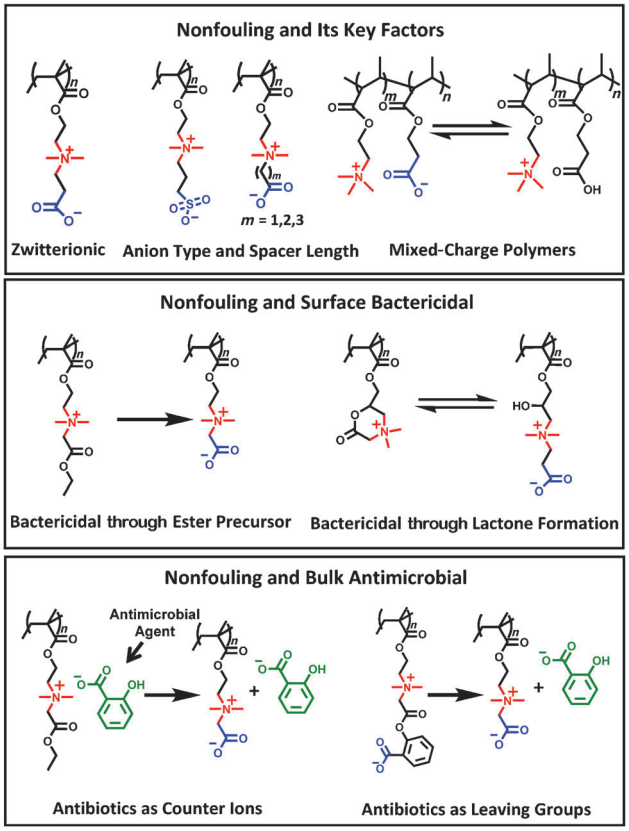


Figure 1. Chemical structures and microbiological applications of several zwitterionic polymers and their derivatives.

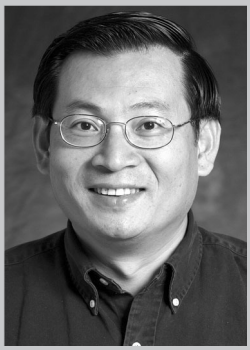
through rational molecular design. In this Minireview, we aim to demonstrate the strengths of zwitterionic polymers and their derivatives in microbiological applications by showing several selected examples, starting with our recent understanding of structural features that govern the bacterial nonfouling properties of zwitterionic polymers (Figure 1 a), then shifting toward integrating surface bactericidal (Figure 1 b) or bulk antimicrobial (Figure 1 c) functionalities into the nonfouling framework. Within the realm of nonfouling studies, polymer density/stability (Figure 2),[23,24] choice of anionic species/spacer length (Figure 3),[25] and charge separation[26] (Figure 4) were all shown to have a pronounced impact on material performances, which in turn sheds light on the underlying mechanism of bacteria–surface interactions. Beyond the pursuit of nonfouling, several cases in which the integration of nonfouling and antimicrobial properties into a single zwitterionic-based polymer—through side-chain ester hydrolysis (Figure 5),[15] reversible lactonization (Figure 6),[16,17] or controlled release of antimicrobial agents as counterions[27] or leaving groups[18] (Figure 7)—have been reported in the past years and are highlighted herein. One practical example of applying zwitterionic polymer-derived antimicrobial hydrogels for wound dressing is also presented (Figure 8).[28] Lastly, several unique aspects for future microbiology-related zwitterionic polymer research are discussed.

2. Nonfouling and Its Key Factors

2.1. From SAM to Polymer Brush: Polymer Density and Stability

Early studies by Whitesides and co-workers showed that a single layer of nonfouling groups formed on a surface through self-assembled monolayers (SAMs) can effectively reduce nonspecific protein adsorption.[29–31] However, fending off bacterial adhesion turned out to be a more challenging task, as many protein-resisting SAM surfaces have been proven to be insufficient to prevent bacterial fouling.[4] This aspect can be partially attributed to the limited stability of SAM surfaces in complex biological environments and also the sheer mass of bacterial cells compared to individual protein molecules.[32,33]

In one of the first studies, in which zwitterionic polymers were used to prevent bacterial surface fouling, a head-to-head comparison was made between nonfouling SAM surfaces and surfaces modified with surface-initiated atom-transfer radical polymerization (ATRP).[23] For the SAM surfaces, a single layer of nonfouling groups was formed on the gold substrates, giving a total film thickness of 1–2 nm, while in the case of ATRP, the high grafting density and molecular weight propelled the surface-tethered polymers to adopt a brush-like conformation, typically leading to a 20 nm dry thickness. In this study, two polymer surfaces (zwitterionic poly(2-(*N*-3-sulfopropyl-*N*,*N*-dimethylammonium)ethyl methacrylate) (pSBMA) and PEG-based poly(oligo(ethylene glycol)methylether methacrylate) (pOEGMA)) and three SAM surfaces (mixed sulfonate and trimethylamine (SA/TMA SAM), oligo(ethylene glycol) (OEG SAM), and 1-dodecanethiol



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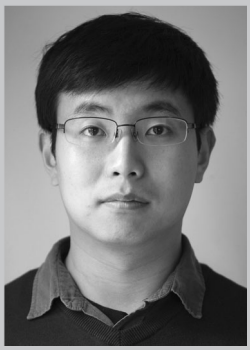

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(CH_3 SAM)) were challenged with protein fibrinogen and bacteria *P. aeruginosa*. Nonfouling SAM and polymer brush surfaces are indistinguishable in reducing nonspecific protein surface adsorption, determined using surface plasmon resonance (SPR) sensors. However, the 24 hour bacterial fouling test demonstrated that the ATPR surfaces are far superior to their SAM counterparts, thus indicating that a more chemically stable and densely-packed thicker layer of nonfouling moieties is necessary to thwart bacterial fouling (Figure 2a and b).

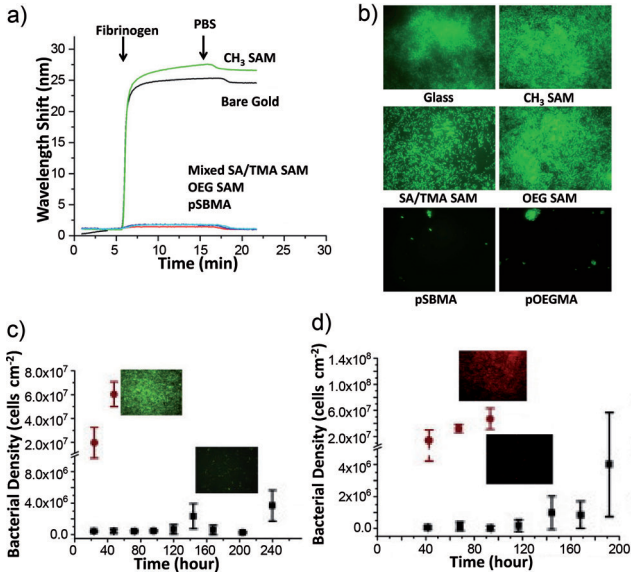

Figure 2. a) SPR sensorgram to measure the adsorption of 1 mg mL^{-1} fibrinogen in phosphate-buffered saline (PBS) on various surfaces. b) Representative fluorescence micrographs of *P. aeruginosa* attachment on various surfaces after 24 h. c) *P. aeruginosa* accumulation on pCBMA-2 (black ■) and glass (red ●) surfaces as a function of time at 25 °C. d) *P. putida* accumulation on pCBMA-2 (black ■) and glass (red ●) surfaces as a function of time at 30 °C.[23,24]

In a follow-up study to further test zwitterionic polymer nonfouling properties against bacterial adhesion and biofilm formation, zwitterionic poly(2-carboxy-*N*,*N*-dimethyl-*N*-(2′-(methacryloyloxy)ethyl)ethanaminium) (pCBMA-2) was optimized and tested for its resistance to long-term biofilm formation.[24] It was reported that pCBMA-2-coated surfaces can reduce *P. aeruginosa* and *P. putida* biofilm formation by 95% for up to 10 days at 25 °C and 8 days at 30 °C in comparison to the bare glass (Figure 2c and d). However, despite their excellent nonfouling properties, traditional zwitterionic polymers do not possess bioactivities against surface and bulk bacterial growth and proliferation. Several strategies that aim to remedy this functional constraint will be discussed in detail in Sections 3 and 4.

2.2. From Protein to Polysaccharide: Divalent Cations and Bacterial Phenotypes

Nonspecific protein adsorption has been commonly postulated as the molecular basis for initial bacterial adhesion and the subsequent biofilm formation.[30] Despite its general acceptance, this assumption is tainted by its oversimplification. Extracellular polysaccharides (EPS), strikingly disproportionate to their abundance on bacterial cell surfaces, have been scarcely studied for their roles in the biomaterial fouling process.[34,35] In a recent study seeking to expand our current understanding on the issue, a system was set up to measure divalent cation-mediated polysaccharide interactions with several zwitterionic surfaces using SPR (Figure 3a).[25] Alginate, an anionic polysaccharide rich in carboxylate groups,

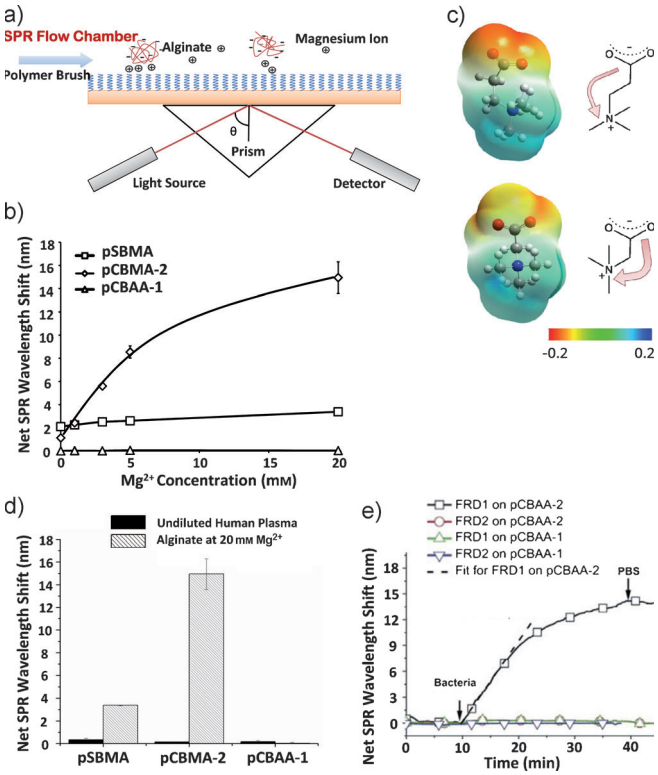

Figure 3. a) Mg^{2+}-mediated alginate surface adsorption measured by SPR. b) Alginate surface fouling under varying Mg^{2+} concentrations on three zwitterionic surfaces: pSBMA (sulfonate anion), pCBMA-2 (carboxylate anion at β position of quaternary ammonium group), and pCBAA-1 (carboxylate anion at α position of quaternary ammonium group). c) Electron density surfaces of carboxybetaine with two (upper) and one (lower) carbon atoms between oppositely charged ionic groups. d) Comparison between protein (undiluted human plasma) and polysaccharide (alginate) adsorption on three zwitterionic surfaces. e) In situ long-range SPR measurements of alginate-rich (FRD1) and alginate-deficient (FRD2) *P. aeruginosa* adhesion on pCBAA-1 (one-carbon spacer) and pCBAA-2 (two-carbon spacer) surfaces.[25,43]

was chosen for its natural abundance in many bacterial species.[36] It was observed that both the type of anionic groups and the charge separation impacts the zwitterionic material polysaccharide resistance. High Mg^{2+} concentration, though uncommon in natural environments, was found to trigger alginate fouling on carboxybetaine polymers with two carbon atoms between the carboxylate and quaternary ammonium groups (pCBMA-2), but not on pSBMA surfaces. Furthermore, the cation effect on poly(carboxybetaine) was

suppressed when the charge distance was reduced from two carbon atoms to one, as is the case in poly(1-carboxy-*N*,*N*-dimethyl-*N*-(3′-acrylamidepropyl)methanaminium) (pCBAA-1; Figure 3b). This finding was explained on the basis of specific ion interactions:[37–39] the higher surface charge density of carboxylate compared to sulfonate favors Mg^{2+} binding through Coulombic interactions. Reducing the proximity of strongly electron-withdrawing quaternary ammonium group decreased the partial charge on carboxylate, consequently lowering Mg^{2+} affinity. In comparison, monovalent cations generally do not interfere with the nonfouling properties of zwitterionic polymers. Consistent with this theory, as the spacer is reduced by one carbon atom, a shift in the carboxybetaine pKa value from 3.2 to 1.8 occurs.[40] A quantum chemical calculation that compared the electron density of the two carboxybetaine molecules further confirmed the trend observed (Figure 3c).[25] While non-ionic nonfouling materials are generally not sensitive to multivalent cations, zwitterionic nonfouling materials clearly respond to cations differently, depending on their specific chemical structures. As both the divalent cation concentrations and biomacromolecular anionic charge densities in human body fluids are considerably lower than the experimental conditions used in this study, pCBMA-2 polymers are expected to maintain their overall material performance in vivo, which was confirmed in a recent hydrogel implantation study.[41] It is also worth noting that all zwitterionic materials used in this work were shown to resist nonspecific protein adsorption from undiluted human plasma (< 0.3 nm SPR wavelength shift; Figure 3d), regardless of their difference in polysaccharide fouling. This contrast between protein and polysaccharide fouling results underlined the necessity to go beyond the protein nonfouling criteria in the design and evaluation of bacteria-resistant materials.

As the amount of anionic EPS varies significantly among different bacterial strains, the above-mentioned finding suggests that the performance of nonfouling materials can also change in accordance with bacterial phenotypes. In a related study, one alginate-rich *P. aeruginosa* strain (FRD1) was compared with its alginate-deficient isogenic mutant strain (FRD2) for their adhesion on two carboxybetaine polymers. Measured in situ using long-range SPR, both poly(carboxybetaine) coatings, pCBAA-1 and pCBAA-2 (poly(1-carboxy-*N*,*N*-dimethyl-*N*-(3′-acrylamidopropyl) ethanaminium)) were shown to resist alginate-deficient mutant bacterial adhesion,[42,43] but only the polymer with one carbon spacer was effective against alginate-rich wild-type bacteria (Figure 3e). Similar to the previous study, both pCBAA-1 and pCBAA-2 surfaces were verified to withstand protein fouling conditions as challenging as undiluted human plasma. These results further emphasized that the surface resistance to bacterial adhesion and biofilm formation is not a simple extension of its protein nonfouling property.

2.3. From Betaine to Mixed Charge: pH Response

In comparison to the permanently charged sulfonate group, the carboxylate group is a weak acid with a typical pK_a between 3 and 5, making it prone to protonation under low pH conditions. As most bacteria in nature are negatively charged, it is then feasible to design a pH-sensitive carboxybetaine material that attracts bacteria under slightly acidic conditions, and then release them, for example, for detection, at a higher pH value. Understandably, the tendency for carboxylate protonation is strongly affected by the neighboring quaternary ammonium group: the further apart the cationic group, the stronger the carboxylate pronation tendency as the inductive effect diminishes.[40] A mixed-charge zwitterionic polymer that separates the carboxylate and quaternary ammonium groups in different polymer side chains (Figure 4a) should therefore be more pH-responsive

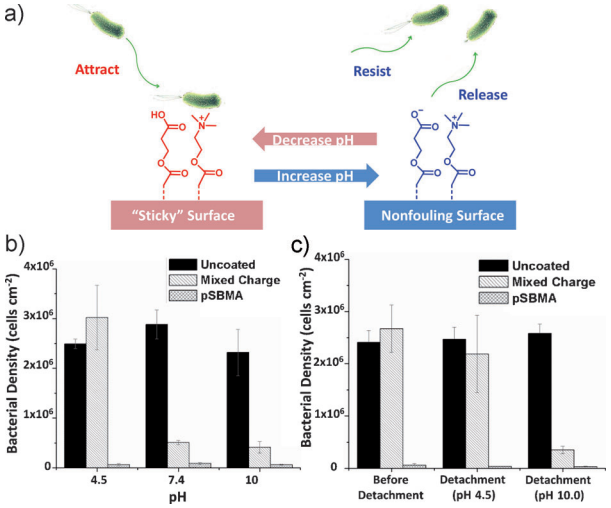

Figure 4. a) A zwitterionic surface that attracts and releases bacterial cells in response to the environmental pH value. b) *S. epidermidis* accumulation on pH-responsive mixed charge surfaces, pH-insensitive pSBMA surfaces and uncoated bare gold surfaces under acidic, neutral, and basic conditions. c) *S. epidermidis* accumulation on mixed-charge, pSBMA-coated, and uncoated surfaces before and after a 30 min hydrodynamic detachment assay under pH 4.5 and pH 10. The initial attachment was carried out at pH 4.5.[26]

than regular carboxybetaine polymers.[26] A study showed that this random copolymer of positive and negative (1:1) units largely retained the protein and bacteria nonfouling properties of zwitterionic materials under neutral conditions while promoting bacteria surface binding under mildly acidic environments (Figure 4b). Furthermore, bacterial cells attracted to the mixed charge surface can later be hydrodynamically detached at a higher pH value (Figure 4c).

3. Combining Nonfouling and Surface Bactericidal Properties

Infections caused by surface-bound bacterial cells and biofilms are a major problem in clinical surgeries and implantation operations. Antibiotics aside, two coating strategies generally exist to counter this threat. The first is to use nonfouling materials, exemplified by zwitterionic polymers

and PEG polymers, to prevent bacteria from adhering onto the surface.[44–47] The second is to use cationic surfaces with high charge density to actively kill the bacterial cells attached. All polymer coatings based on quaternary or primary ammonium polymers fall into this category.[48–50] Despite their great popularity and general effectiveness, there are fundamental limitations to both approaches. Nonfouling materials are powerless against bacterial cells once they adhere onto the surface, while bactericidal surfaces suffer from very poor biocompatibility and quick surface accumulation of dead bacterial cells shielding surface functional groups. An ideal material should combine the strength of these two complementary strategies, and possess both nonfouling and bactericidal capabilities at the same time. While it is possible to achieve dual functionality using multilayered/multicomponent systems,[51] we here focus on strategies that realize this functional unification through single-component monomer design.

3.1. "Kill and Release"

In one report, a hydrolyzable carboxybetaine ester precursor, poly(*N*,*N*-dimethyl-*N*-(ethoxycarbonylmethyl)-*N*-[2-(methacryloyloxy)ethyl]ammonium bromide) (pCBMA-1 C2), was utilized to accomplish this functional integration (Figure 5a).[15] In this design, the material started at a cationic state, inactivating bacterial cells that come into direct contact (Figure 5b). As with any bactericidal surfaces, the killed microbes remained on the surface. But unlike permanently charged cationic polymers, the ethyl ester groups in this zwitterionic polymer derivative can be readily hydrolyzed into anionic carboxylates. This ester hydrolysis process converted the cationic polymers into their zwitterionic and nonfouling forms, releasing the dead bacterial cells at the same time (Figure 5c). This separation of functionality into distinctive stages ensured the minimum interference between two distinct functionalities.[52] It was experimentally shown in this work that before hydrolysis, this switchable material possessed a bactericidal potency similar to a commonly used quaternary ammonium polymer, poly(methacryloyloxy ethyldimethyloctyl ammonium bromide) (pC8NMA), while, after the chemical transition, a nonfouling property comparable to conventional zwitterionic surfaces. Its initial bactericidal functionality, self-cleaning property and long-term biocompatibility made this zwitterionic polymer derivative particularly suitable for coatings of implantable biomedical devices. In these applications, the switchable polymers should kill the bacteria attached onto the surface at the initial in vitro stage and then be hydrolyzed into their nonfouling forms right before the implantation operation to prevent any further surface fouling and to provide in vivo biocompatibility.

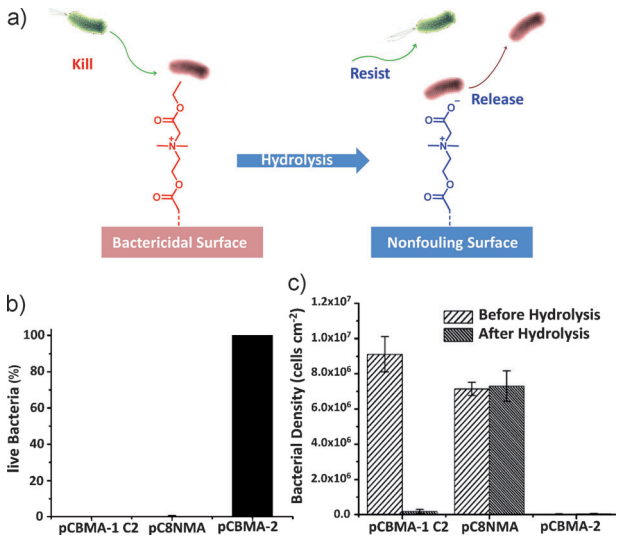

Figure 5. a) A bactericidal surface based on a zwitterionic ester precursor kills bacteria at the cationic state and then undergoes hydrolysis to release dead bacterial cells and to provide long-term bacterial resistance. b) The bactericidal activity of pCBMA-1 C2 (hydrolyzable cationic zwitterionic precursor), pC8NMA, (cationic), and pCBMA-2 (zwitterionic) as measured using *E. coli* K12 colony formation assay. c) The bacterial surface accumulation on pCBMA-1 C2, pC8NMA, and pCBMA-2 surfaces after one hour exposure to a suspension of 10^{10} cells mL^{-1} *E. coli* K12, before and after hydrolysis.[15]

3.2. "Kill, Release, and Regenerate"

Although a single transition between cationic and zwitterionic states is adequate for certain clinical scenarios, in many applications, such as most reusable surgical devices, renewable bactericidal surfaces are often needed. To this end, a reversible lactonization reaction was used to achieve multiple cycles of bactericidal–nonfouling transitions (Figure 6a).[16] In the ring/lactone form, polymers are strongly positively charged and can prevent the surface proliferation of bacterial cells that fall onto the surface, as demonstrated by a colony formation assay after directly spraying bacterial aerosols onto the material (Figure 6b). Upon exposure to water, the ring form is very quickly hydrolyzed to a linear zwitterionic form, releasing inactivated bacteria and preventing further bacterial fouling (Figure 6c). Finally, the cationic lactone form can be restored from the zwitterionic state through dehydration under acidic conditions, thus completing the cycle. It was shown that this switchable material underwent seven cycles of regeneration without significant loss of polymer film thickness, thus indicating its overall chemical stability.

4. Integrating Nonfouling with Bulk Antimicrobial Properties

Although switchable materials that have distinctive cationic and zwitterionic states can effectively inactivate surface-bound bacteria and, after the functional transition, provide long-term protection against further bacterial incursions, their antimicrobial functionality does not extend beyond the immediate interface and attack planktonic bacterial cells. As a result, releasable antimicrobial agents need to be incorporated into the zwitterionic materials for those applications that require both surface nonfouling and bulk

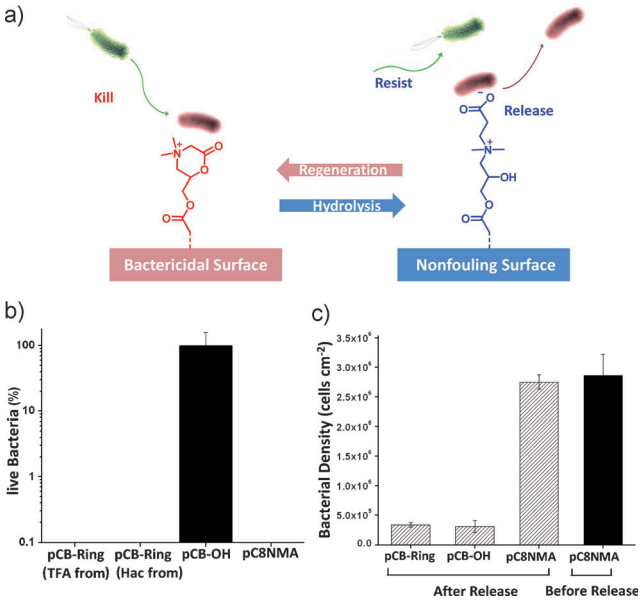

Figure 6. a) A switchable material undergoing reversible lactonization that prevents bacterial surface proliferation at its cationic state, releases inactivated bacterial cells at its zwitterionic state, and regenerates through a dehydration process. b) Bactericidal activity of the switchable polymer at cationic *N*,*N*-dimethyl-2-morpholinone state (CB-ring) and at zwitterionic carboxybetaine state (CB-OH) against *E. coli* K12. pC8NMA is the cationic bactericidal surface included as the positive control. The notes in parentheses indicate the dehydration agent used to generate pCB-ring. c) *E. coli* K12 density on the surfaces before and after the releasing procedure (shaking in PBS for 1 h). The labelled pCB-ring, pCB-OH, and pC8NMA correspond to the initial dry-state chemistry. Before release, pCB-ring, pCB-OH, and pC8NMA have the same density of bacteria sprayed onto their surfaces.[16]

antimicrobial properties. Two chemical approaches have so far been reported in order to meet this requirement.

4.1. Antibiotics as Counterions

One straightforward way to accomplish this goal is to dock an antimicrobial anionic molecule to a positively charged zwitterionic ester precursor as its counterion.[27] Over time, the antimicrobial molecule will be released and can inhibit the growth of bacterial cells in the surrounding environment, while the cationic polymer matrix should gradually be hydrolyzed into its final zwitterionic form in a similar fashion as described in the previous section. This approach has the advantage of being chemically simple and easily implementable for most negatively charged antimicrobial agents. However, one potential downside is a limited control over the release of small hydrophilic drugs under high salt conditions, in which case, exchange with environmental ions can deplete the loaded bioactive agents in a short time frame, leading to an unfavorable burst release.[53] This lack of control over release profile may also be a problem in the physical embedment of antimicrobial silver nanoparticles within zwitterionic polymer brushes.[54]

4.2. Antibiotics as Leaving Groups

In addition to carrying a bioactive counterion to a zwitterionic ester precursor, another approach is to covalently conjugate antimicrobial molecules to the polymer matrix through hydrolyzable linkers. In a recent study, a mild antimicrobial agent salicylate (SA) was attached to the carboxylate group of a carboxybetaine molecule through an easily hydrolyzable ester bond to form a functional zwitterionic unit, poly(2-{2-[2-(methacryloyloxy)ethyl]dimethylammonio}acetoxybenzoate) (pCBSA; Figure 7a).[18] The release

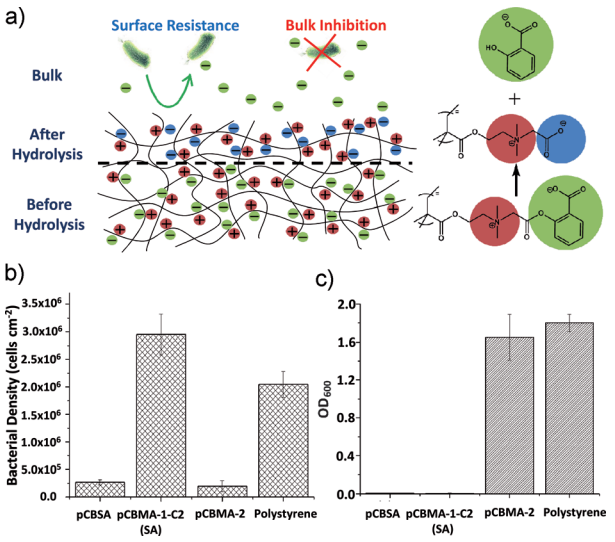

Figure 7. a) A zwitterionic hydrogel with built-in antimicrobial functionality. For every antimicrobial salicylate anion released upon hydrolysis, one carboxylate anion is formed at the hydrolysis site to maintain its zwitterionic nonfouling state. b) Bacterial surface adhesion on pCBSA (antimicrobial agent as the hydrolyzable leaving group), pCBMA-1 C2 (SA; antimicrobial agent as the counterion), pCBMA-2 (zwitterionic with no antimicrobial activity) hydrogels as well as on the polystyrene control surface. The four surfaces were subjected to bacteria *S. epidermidis* suspension in PBS (OD600 ≈ 0.1) at room temperature and mild shaking condition for 2 hours before visualization. c) 16-hour growth-inhibition results of pCBSA, pCBMA-1 C2 (SA), and pCBMA-2 hydrogels against *S. epidermidis*. The medium optical densities (OD_{600}) were indications for bacteria bulk density.[18]

of the drug conjugated through a covalent linkage was no longer sensitive to the environmental ionic strength in comparison to the counterion strategy, and can be further fine-tuned by adjusting the linkage type and the neighboring chemical groups. Compared to the previous reports of drug release using hydrolyzable linkages, this particular zwitterionic-polymer release system had its own unique property. The anionic salicylate leaving group was designed to be a constituent of the zwitterionic moiety, which enabled a high drug-loading capacity unattainable through post-polymerization modification and a good nonfouling property characteristic of a zwitterionic polymer. The cationic quaternary ammonium group on the polymer side chain was initially balanced by the negatively charged salicylate moiety. Over time, for every salicylate molecule released, a new carboxylate group was formed at the site of hydrolysis to maintain the

overall neutrality. After the drug release was completed, the material naturally turned into a chemically stable and biologically inert zwitterionic material, further providing long-term protection against bacterial adhesion. In this study, it was shown that this particular zwitterionic material can resist nonspecific protein adsorption and bacterial fouling during the entire drug release process while exerting antimicrobial function to the bulk bacteria (Figure 7b and c). The general concept of "zwitterionic materials with built-in functions" may also be adapted for a wide array of biological applications.

5. An Antimicrobial Wound-Dressing Hydrogel Based on Zwitterionic Polymer Derivatives: A Case Study

Many biological processes are intrinsically complex and have multiple stages, thus posing great challenges to material design that aims at such biomedical applications. For example, an ideal wound-dressing material should have a) the antimicrobial activity to reduce the risk of wound-site infections; b) the in situ formation property for ease of application and better conformation to the damaged tissue; c) the property to recruit blood platelets to help blood coagulation; and d) the biocompatible property to facilitate wound healing and tissue regeneration.[55,56] In a study of engineering zwitterionic polymer derivatives for wound-dressing applications, an ABA triblock copolymer was prepared using the reversible addition/fragmentation chain-transfer (RAFT) polymerization method (Figure 8a).[28] The inner B block was made up of cationic carboxybetaine ethyl esters with antimicrobial salicylate as the counterions. Upon application, the antimicrobial salicylate ion could be released to sterilize the wound site while the cationic polymer matrix was designed to help blood coagulation through the attraction of negatively charged blood platelets. By releasing antimicrobial agents as the counterions, a complete inhibition of bacteria growth for 16 hours was reported (Figure 8b and c). Over time, the positively charged ethyl ester block can be hydrolyzed into its biocompatible and nonsticky zwitterionic form in the later stages of the wound-healing process. The two flanking A blocks consisted of thermoresponsive poly(*N*-isopropylacrylamide) (PNIPAM), whose lower critical solution temperature is slightly below the human physiological temperature.[57] As a result, once the polymer solution was applied to the wound site, the heightening of temperature quickly led to the phase transition of PNIPAM, which served as a hydrophobic crosslinking site, turning the liquid polymer solution into a mechanically useful physical gel (Figure 8d). It was underscored through this work that, through molecular design and chemical manipulation, zwitterionic polymers and their derivatives can orchestrate nonfouling, antimicrobial, and other biological functionalities together to cater to complex engineering and biomedical applications.

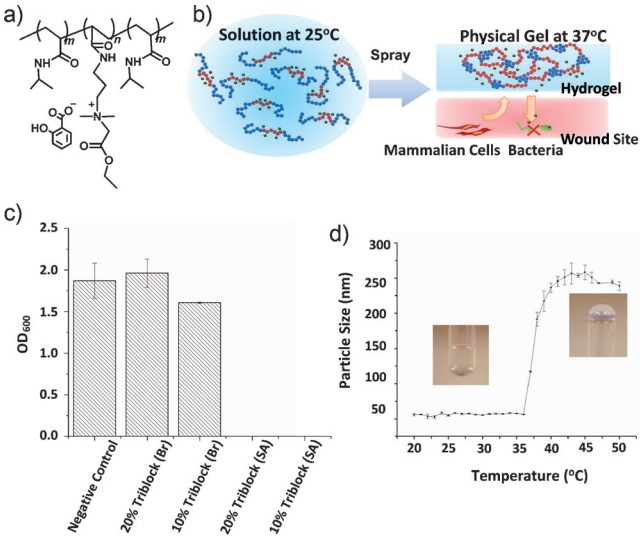

Figure 8. a) Chemical structure of an antimicrobial wound-dressing ABA triblock copolymer with zwitterionic ester precursors in the middle, carrying antimicrobial counterions, while the flanking B blocks consist of pNIPAM thermo-responsive polymers. b) Schematic illustration of the in situ formation of an antimicrobial wound-dressing hydrogel at the wound site. c) Bacterial growth inhibition property of hydrogels at different mass concentrations and counterions (SA or Br). The media OD600 readings of *E. coli* K12 were indications for bacteria bulk density. d) The temperature-dependent phase transition behavior of a triblock copolymer solution as measured using dynamic light scattering (DLS). Representative pictures of the polymer solution before and after gelation were also included.[28]

6. Summary and Outlook

In this Minireview, microbiological applications of zwitterionic polymers and derivatives were presented through several selected examples. The development of zwitterionic materials has come a long way from a simple nonfouling coating to a functionally switchable polymer, a unique platform to release antimicrobial drugs, and a multicomponent system designed to address various aspects of a complex biological scenario. This functional versatility of zwitterionic polymers and their derivatives is inseparable from their structural diversity, which makes them a promising complement and improvement to conventional PEG-based nonfouling polymers.

Several unique aspects for future microbiology-related zwitterionic polymer research are worth mentioning in particular. First, zwitterionic polymers are among the most osmotically active macromolecules thanks to their high density of ionic groups.[58] For this reason, zwitterionic polymer-based materials naturally create a region of high osmotic pressure at the interface. By studying PEG polymers as a model system, osmotic pressure exerted by a polymeric scaffold was recently unveiled to have a profound impact on bacterial physiology and gene expression profile, including the production of virulent factors.[59] It will thus be of great interest to investigate the bacterial physiological response when interacting with zwitterionic polymer materials as well as its implication in the nonfouling material design. Second, as demonstrated in Section 4.2, it is possible to integrate

a charged biologically active molecule as the zwitterionic constituent, achieving high drug loading without sacrificing the polymer property. This design strategy can be readily adopted for many in vivo antimicrobial drug-delivery systems that enjoy the additional advantage of maintaining bulk material nonfouling. Third, several recent reports suggest a higher bactericidal activity[60] and a lower toxicity for mammalian cells[61] of polyphosphonium polymers compared to their commonly used quaternary ammonium counterparts, thus making phosphonium polymers an interesting alternative in various biological applications. Last, though polymeric materials, including zwitterionic polymers, have been extensively tested for their ability to fend off bacterial surface adhesion, their effectiveness against virus particles and bacterial endospores have not yet been reported. Bacterial spores and viruses have their own distinctive surface structures and bear great biomedical relevance. Research efforts in this direction will undoubtedly help further broaden the microbiological applications of zwitterionic polymers and their derivatives.

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