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Antimicrobial activities of phosphonium containing polynorbornenes†

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In this study, amphiphilic polyoxanorbornene with different alkyl and aromatic phosphonium side chains was synthesized. The biological activities of these polymers were determined by the minimal inhibitory concentration (MIC) against *E. coli*, *S. aureus*, *M. tuberculosis* and the yeast *C. albicans*, and cytotoxicity studies on red blood cells were performed. A series of polymers with different alkyl and aromatic substituents (methyl, ethyl, tripropyl, *tert*-butyl, phenyl, and tris 4-methoxyphenyl) and two types different molecular weight, 3000 g mol⁻¹ and 10 000 g mol⁻¹, were prepared. It was observed that the biological activity of the polymers with aromatic group substituents had an MIC of 16, 8, 64 and 128 µg mL⁻¹ against *E. coli*, *S. aureus*, *M. tuberculosis* and *C. albicans*, respectively, while those with non-aromatic carbons had a higher MIC compared to those with aromatic carbons. The aromaticity of the repeat unit had impressive effects on hemolytic activities as well. Zeta potential measurements of *E. coli* incubated with active and inactive polymer concentration revealed a relationship between the MIC and membrane surface charge density. Polymers bearing aromatic groups killed the bacteria with widespread damage after the polymers, holding the threshold concentration, were added to the bacteria.

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1. Introduction

The evolution of antibiotic-resistant microorganisms is one of the most threatening global problem.^{1–4} In particular, the appearance of new pathogens and growing antibiotic resistant have spread out the research for new antibiotics. During the same period, new pathogens have appeared and antibiotic-resistant strains have evolved. The war against microbes is far from over.⁵ Hospitals by their very nature contain large numbers of sick people, many of whom carry infectious agents that can spread by direct or indirect contact. Therefore, hospital surfaces are subject to constant contamination events as patients and staff could move from one place to another.

Nowadays, hospital acquired, or nosocomial, infections have gradually expanded all over the world and the trend is increasing. Approximately two million people acquired hospital infections in United States and 99 000 died from those infections.⁶ Host-defence peptides (HDPs) and their synthetic mimics have prompted extensive studies for potential therapeutic applications.^{7,8} The exploration of HDPs and their chemical analogues as an antibacterial agent is currently

attracting interest for potential therapeutic applications.⁹ The distribution of hydrophilic (cationic) and hydrophobic groups in the peptide backbone is favorable for the amphiphilic structures; moreover, it enhance the interaction with the bacterial surface, which are one of the fundamental mechanisms of action. However, there are some drawbacks of using HDPs and their synthetic analogues as antimicrobial agents such as (i) the production of these types of compounds is extremely costly and (ii) long range resistance to enzymatic degradation for their possible usage in therapeutic applications.^{10,11} Synthetic cationic polymers have emerged as promising candidates for further development as antimicrobial agents.^{12–19} This class of polymeric compounds is rather inexpensive with respect to the HDPs and resistant to physiological conditions. There are several reported synthetic polymers that can display selectivity, *i.e.*, prokaryote-specific toxicity.^{12,13,20–22} Selectivity has typically been assigned by the hemolytic activity over antibacterial activity. Basically, antimicrobial cationic polymers and peptides target the phospholipid membranes of bacterial cells. Phospholipid bilayer of both Gram-positive and Gram-negative bacterial cell membranes predominantly consist of anionic phospholipid head groups, whereas those found on eukaryotic cells are neutral. These negatively charged outer surfaces enhance the electrostatic interactions for the cationic peptides and polymers with bacteria, thus allowing them to implement their antibacterial effect. Despite this action, hydrophobicity also plays a role in the enhancement of activity and toxicity. Thus, to improve the resistance to HDPs and

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cationic polymers, bacteria will require more complex changes including alteration of the cell membrane.

Biocidal cationic polymers possessing quaternary ammonium and phosphonium salts have better antibacterial activities compared to their small biocidal counterparts.²³ However, there are fewer studies on phosphonium based antibacterial polymers in the literature compared to ammonium salt derivatives. Tan and his group obtained phosphonium salts by reacting graphite surface with different concentrations of TTP (tetradecyl triphenyl phosphonium bromide).²⁴ The antibacterial activity of graphite that contains 33.7% phosphonium salt was determined to be 580 mg L⁻¹ and 285 mg L⁻¹ against *E. coli* and *S. aureus*. They also used montmorillonite instead of graphite and added phosphonium salts to Na-montmorillonite layers using an ion exchange method.²⁵ They found that phosphonium-containing montmorillonites are thermally more stable than ammonium-containing montmorillonites and their MIC values are 150 mg L⁻¹ and 50 mg L⁻¹ against *E. coli* and *S. aureus*. Guo and his group obtained different concentrations of water soluble chitosan that contains different concentrations of quaternary phosphonium salt (3.6% and 4.2%).²⁶ The toxicity of the obtained polymers was examined on L929 cells and no toxic effects were found. Endo and Kanazawa synthesized polymers using the monomer 4-vinyl benzyl phosphonium chloride as a phosphonium salt, which can be used for dental fillers and examined their antibacterial activity.²⁷ The activity of polymers was measured against *Streptococcus mutans* (*S. mutans*) and it was found that at 10 μmol mL⁻¹ within 24 hours, the polymers showed higher activity compared to poly (methylmethacrylate).

The ROMP (ring opening metathesis polymerization) method is one of the techniques used for controlled polymerization system. Norbornene backbone and functional group tolerance provide the ROMP in the preparation of biologically active well-defined polymeric materials.^{28–30} Recently, a large number of ROMP-based antibacterial polymers were synthesized.^{31,32} Coughlin and Tew synthesized polynorbornene derivatives of ammonium salts with ROMP method and evaluated their antibacterial activity. It was seen that when the hydrophobic parts on the polymer chain was increased, the antibacterial activity and toxicity increased as well.¹² In another study, pyridine salts were polymerized by the ROMP method and assessed for their antibacterial and hemolytic activity, and it was found that ethyl bromide based salt has the highest hemolytic activity.²² The structure–property relationship of those polymers might provide a new design for the biocidal polymer architecture. These polymers can also be synthesized on a large scale. One-pot synthesis with a control of the backbone is particularly attractive for the use of a new generation of biocidal polymeric antibiotics.^{33,34}

Herein, we present phosphonium containing antibacterial polymers that can be considered a subgroup of biocidal polymers. In the literature, there are no studies of phosphonium-containing polymers synthesized with ROMP, so we report the characterization and antibacterial evaluation of cationic charged phosphonium-containing polyelectrolytes synthesized by ROMP, which can be an alternative for cationic based peptidomimic. Herein, quaternary phosphonium functionality

Scheme 1 Monomer and polymers based on oxanorbornene derivatives.

attached to oxanorbornene monomers was then polymerized using a Grubbs' catalyst, as shown in Scheme 1.

2. Experimental section

All experimental procedures, including monomer and polymer synthesis, all spectroscopic data, as well as the biological assays, are included in the ESI.†

3. Results and discussion

3.1. Monomer synthesis

Phosphonium bearing monomer synthesis started with a Diels-Alder reaction between maleic anhydride and furan to yield product **1**.³⁵ Treatment of **1** with 3-bromopropylamine hydrobromide resulted in imide transformation in the presence of NaOAc/acetic anhydride mixture to obtain bromoderivative **2**.³⁶ The salts, **3a–f**, were synthesized by nucleophilic (S_N2) addition of respective phosphine reagents to compound **2**, as shown in Scheme 1. The average yield of all the compounds was between 11% and 90%; *tert*-butyl (**3d**) and triphenyl phosphine (**3e**) were the yield reducing step due to steric effects. All the monomers were typically creamy to pale yellow crystals. All the salts were hygroscopic and soluble in water except monomers **3e** and **3f**. These monomers were analyzed by NMR spectroscopy.^{37,38} The ³¹P {¹H} NMR spectra of the salts exhibited a singlet between δ 20.01 and 49.91 ppm. Monitoring the reactions was very straightforward as the used phosphine reagents showed signals in the δ –6 to –60 ppm range. In addition, traces of phosphine oxide side products for the ethyl (**3b**), phenyl (**3e**) and methoxyphenyl (**3f**) salts at δ 65.11, 29.21, and 27.03 ppm were observed. The presence of side products might result from the sensitivity of phosphines to oxidation and precaution and exceptional care in handling should be taken into consideration while the application in the synthesis. These side products can be eliminated easily by successive washing of the residue with the appropriate solvents (usually THF was sufficient for this

purpose). Proton and carbon-13 NMR spectra of **3a–f** are also assigned (ESI Fig. S1–S14†). The characteristic signals of oxanorbornene protons and carbons appeared at δ 6.57, 5.15 and 2.98 ppm in the ^1H NMR spectra and δ 176.65, 136.41, 80.45 and 47.21 in the ^{13}C NMR spectra. The protons of the substituent neighbour to the phosphor can be observed as a doublet due to the strong phosphorous–hydrogen coupling. For example, methyl groups in **3a** give rise to a doublet at δ 1.75 ppm with the characteristic 2J coupling of approximately 14.3 Hz. While the number of ($-\text{CH}_2-$) groups increases, *i.e.* **3a–c**, the shift of the methyl group ($-\text{CH}_3$) at the end of the alkyl functional group moves to a higher magnetic field. In addition, the typical doublet shifts due to hetero-coupling between the C-nucleus and the P-nucleus was also observed in ^{13}C NMR.³⁹ As an example, verification of the phosphorus–carbon bond in **3a** was observed as large 1J coupling (85.8 Hz) in the doublet of methyl carbons (CH_3-P^+) at δ 7.22 ppm (Fig. S4†). Methylene carbons next to the phosphonium cation ($-\text{CH}_2-\text{P}^+$) were observed at δ 20.02 ppm with a 1J coupling as 50.9 Hz. In addition, 2J coupling was also observed for methylene carbons ($-\text{CH}_2-\text{CH}_2-\text{P}^+$) at δ 20.45 ppm with $J = 5.6$ Hz. Longer range 2J and 3J couplings were also reliable through phosphorus–carbon bond formation.

3.2. Polymer synthesis

The polymers, **4a–f**, were synthesized from the respective monomers **3a–f** and used to evaluate the influence of the alkyl/aromatic side chain on the antibacterial and hemolytic activity of the polymers. Monomers **3a–f** were polymerized using Grubbs' third generation catalyst in 2,2,2-trifluoroethanol, *N,N*-dimethylformamide or dichloromethane at room temperature. A targeted number average molecular weight (M_n) of 3000 or 10 000 g mol^{-1} was applied using the proper monomer to catalyst concentration. After work up, complete conversion of polymerization was observed by the total disappearance of the monomer olefin proton peaks at δ 6.0–6.3 ppm and the appearance of the polymer backbone double bond protons as *cis* or *trans* signals at δ 5.1–5.6 ppm (Fig. S15–S20†). The appearance of vinylic hydrogens obtained by the ROMP pathway resulted in both *cis*- and *trans*-double bond formation and the integration of these two signals in ^1H NMR indicates the relative *cis/trans* ratio in the polymer backbone. The resulting homopolymers contained ~55–65% *trans* residue. In addition, long range coupling of phosphorus–carbon linkage was not observed due to the diminishing resolution of the polymeric system. However, the large 1J coupling of the *para*-aryl carbon to the phosphonium cation (present in **4d** and **4f**) was still evident in the doublet at δ 118.03 ppm ($^1J_{\text{CP}} = 86.2$ Hz). End group analysis of the purified polymer was applied to calculate M_n in comparison to the assumed molecular weight using ^1H NMR techniques.²² The phenyl end group connected to the original carbene moiety from the catalyst is visible at *ca.* $\delta \sim 7.20$ – 7.50 ppm. Integration of the ($-\text{CH}=\text{CH}_{\text{cis,trans}}-$) peaks relative to the styrenic end group from the Grubbs' catalyst in the ^1H NMR spectra of the polymer indicated the degree of polymerization. For example, theoretical molecular weight, 3000 g mol^{-1} , of

polymer **4a** has a degree of polymerization of 8, which compares well with the calculated value of 9 (Table 1). However, it is well known that integration values of the ^1H NMR peak refer to the solvent used for the analysis in NMR. The actual integration values of NMR peaks can be obtained correctly only if the micelle formation does not occur in the NMR solvent.⁴⁰ Because of these reasons, some deviation from the theoretical values could be observed. GPC measurements of all the polymers could not be carried out as **4d_10k**, **4e_3k**, **4e_10k**, **4f_3k**, and **4f_10k** are not soluble in PBS buffer solution. The theoretical M_n and observed M_n values by GPC do not comply well with each other (Table 1). GPC calibration with linear, non-ionic poly(ethylene oxide) standards resulted in different hydrodynamic volumes with respect to the cationic phosphonium polymers. However, the experimentally determined M_w/M_n for the polymers, as shown in Table 1, indicates that a controlled and well-defined system was achieved by the ROMP method with the polydispersity index (PDI) of ~1.

3.3. Biological activity of polymers

The activity of all polymers was explored against *E. coli*, *M. tuberculosis* (Gram-negative bacteria), *S. aureus* (Gram-positive bacteria) and *C. albicans* (fungus) as representative microbial communities. The biocidal activity obtained for these homopolymers is reported in Table 2. Polymer solutions were prepared by dissolution in water (**4a**, **4b**, **4c** and **4d_3k**) or DMSO (**4d_10k**, **4e** and **4f**) and further diluted with buffer. The results confirm that the phosphonium polymers with aromatic substituent (**4e** and **4f**) have better antibacterial activities than the alkyl substituent derivatives (**4a–4d**). The MICs of all the polymers against fungi are generally low ($\geq 128 \mu\text{g mL}^{-1}$). Increase in the alkyl substituents from methyl to *tert*-butyl (**4a–4d**) alone decreases the MICs against Gram-positive *S. aureus* but not against Gram-negative *E. coli* and *M. tuberculosis*. It should be mentioned that *M. tuberculosis* is related more to Gram-negative than to Gram-positive bacteria as revealed by the

Table 1 Summary of the theoretical M_n , measured M_n and M_w/M_n values for the homopolymers **4a–f**

Polymer	$M_{n,\text{the}}^a$	$M_{n,\text{NMR}}^b$	$M_{n(\text{ASEC})}^c$	M_w/M_n^c
4a_3k	3000	3864	6306	1.059
4a_10k	10 000	9430	38 137	1.102
4b_3k	3000	4475	7234	1.057
4b_10k	10 000	14 028	14 298	1.049
4c_3k	3000	4053	8869	1.057
4c_10k	10 000	15 534	17 277	1.008
4d_3k	3000	3698	30 911	1.06
4d_10k	10 000	n.d.	n.d.	n.d.
4e_3k	3000	n.d.	n.d.	n.d.
4e_10k	10 000	n.d.	n.d.	n.d.
4f_3k	3000	n.d.	n.d.	n.d.
4f_10k	10 000	n.d.	n.d.	n.d.

^a $M_{n,\text{the}}$ = mass of monomer g mol^{-1} of initiator. ^b As determined by ^1H NMR spectroscopy. ^c As determined by aqueous size exclusion chromatography in buffer 0.25 M NaBr. System was calibrated with narrow molecular mass poly(ethylene oxide) standards.

Table 2 Antimicrobial and hemolytic activities of polymers

Polymer	MIC ^a (μg mL ⁻¹)				HC ₅₀ ^b (% hemolysis)
	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>	<i>M. tuberculosis</i>	
4a_3k	512	512	512	>512	1
4a_10k	512	512	512	>512	1
4b_3k	>512	64	128	>512	2
4b_10k	>512	>512	>512	>512	2
4c_3k	>512	256	256	>512	1
4c_10k	>512	512	512	>512	0.5
4d_3k	>512	128	128	128	4
4d_10k	512	32	128	256	2
4e_3k	16	8	128	64	99
4e_10k	32	16	128	512	>100
4f_3k	32	8	128	64	85
4f_10k	64	8	128	128	46

^a Inhibitory activity towards bacterial growth of *E. coli*, *S. aureus* and *M. tuberculosis* bacteria and growth of *C. albicans* fungi (MIC₉₀ = minimal inhibitory concentration preventing 90% bacterial or fungi growth) and hemolytic activity towards red blood cells (HC₅₀ = concentration lysing 50% of blood cells). ^b Hemolysis caused by 512 μg mL⁻¹ polymer concentration.

sharing comparatively more genes for energy production and conversion with Gram-negative bacteria than with Gram-positive bacteria.⁴¹ The architecture of the entire cell-wall of *M. tuberculosis* mainly consist of a peptidoglycan layer with a phosphodiester linkage, which in turn is attached to mycolic acids.^{42,43}

Mycolic acids are long fatty acids found in the cell membrane and provide a lipid barrier that enhances the resistance to chemical damage and dehydration and prevent the activity of common antibiotics. All the polymer series were inactive against *M. tuberculosis*, except the MIC of polymer **4e_3k** and **4f_3k** containing aromatic units was 64 μg mL⁻¹. However, more rigid substituents on the polymer structure (entries **4e** and **4f**, Table 2) have dramatically lower MICs against *E. coli* and *S. aureus* (8–64 μg mL⁻¹), which are comparable to natural host defence peptide magainin MSI-78 possessing MIC of 12.5 μg mL⁻¹.¹² Polymer **4b_3k** shows a much lower MIC compared to **4b_10k** against *S. aureus*. Polymer **4d_10k**, containing the *tert*-butyl substituent, has an MIC of 512 μg mL⁻¹ against *E. coli* compared to 32 μg mL⁻¹ for *S. aureus*, resulting in a polymer selectivity (one bacterial type over another); it was 16 times more active against *S. aureus* than against *E. coli*. Such bacteria type selectivity was previously observed by Lienkamp *et al.*¹³ Herein, the reason for the lower biocidal activity to *E. coli* over *S. aureus* is unclear and further studies should have been applied to understand the mechanism. One possible explanation is that *E. coli* has a thin peptidoglycan cell wall sandwiched between an outer and inner membrane. Despite this, *S. aureus* has 20–80 nm thick peptidoglycan layers surrounding plasma membrane bearing a single phospholipid bilayer. Therefore, the biocidal polymers need to fracture two membranes to kill the *E. coli* envelope, whereas they can diffuse through the peptidoglycan layers of *S. aureus* and result in the destruction of its plasma membrane more efficiently. The weak interaction with phospholipid membrane of *E. coli* and strong adhesion to phospholipid

membrane of *S. aureus* could be a reason for the higher biocidal activity against *S. aureus* than *E. coli*.

The amphiphilicity of the ROMP polymers is an important factor for the selectivity and activity.^{12,13} Increasing the hydrophobicity of the polymer structure enhances the interaction between the phospholipid bilayer and result in an enhancement in activity. However, excessive hydrophobicity in the polymer backbone favors cytotoxicity and also might precipitate in the test conditions. It was determined that incorporation of the aromatic ring to the polymer backbone, **4e** and **4f**, has a significant effect on biocidal activity. It was hypothesized that the respective π electron density and rigidity of the aromatic ring would destabilize the cell membrane for tuning the activity for **4e** and **4f**. An increase in the aromaticity of the polymers enhances the various π interactions between the polymers and cell membrane.⁴⁴ However, phenyl rings with a highly hydrophobic rigid structure might be the key parameter here for better interaction and destabilization of the bacterial membrane and leakage of the cell.

Overall, we can conclude that adding alkyl-based hydrophobicity, *i.e.* methyl to *tert*-butyl (**4a–4d**), improves activity, even though polymers bearing aromatic groups show better biocidal activity than aliphatic groups.

3.4. Hemolytic concentration

The hemolytic activity (HC₅₀) of the polymers toward red blood cells (RBCs) was tested, as described previously.¹² HC₅₀/MIC₉₀ ratio is defined as the antibacterial selectivity. As can be seen from Table 2, the polymer with the highest selectivity for bacterial over mammalian cells is **4d_10k**, with a good selectivity of >30 for *S. aureus*. Moreover, magainin, one of the natural host defense peptides, has a selectivity of 10.⁴⁵ From **4a** to **4f**, the polymers became strongly hemolytic. Their high hydrophilicity prevents **4a–4d** from the activity against RBCs; however, polymers having hydrophobic rigid analogous (**4e–4f**) cause significant hemolysis. Polymer possessing the aliphatic

hydrophobic group was altered from a methyl group, **4a**, to a *tert* butyl, **4d**, and resulted in $\sim 1\%$ hemolysis at $250 \mu\text{g mL}^{-1}$ polymer concentration. An overly aromatic substituent (**4e–4f**) enhances the membrane interaction and as a result, is very hemolytic. For these series of polymers, they start to become more hemolytic ($\text{HC}_{50} \leq 250 \mu\text{g mL}^{-1}$). It was also determined that the nature of π -rich (**4f**) aromatic rings, methoxy substituents on the phenyl ring, did not have any significant difference on hemolysis data with respect to phenyl substituents (**4e**). It seems that the polymers have ideal facial amphiphilicities and rigidity to destroy the bacterial and RBCs membrane.

The effect of molecular weight on biocidal activities was also examined by 3000 g mol^{-1} and $10\,000 \text{ g mol}^{-1}$ for the polymer **4a–4f**. The molecular weights did not result in substantial deviation in activities and activity was described as mass/volume rather than molarity.²² It can be expected that high molecular weight polymers will be more active with a lower molar concentrations. We assume that polymers with higher molecular weight could then get stuck on the membrane surface and result in an improvement of the electrostatic and hydrophobic interactions with the phospholipid bilayer of the cell membrane. However, compared to their $M_n = 3000 \text{ g mol}^{-1}$ analogues of **4e** and **4f**, these polymers holding $M_n = 10\,000 \text{ g mol}^{-1}$ were slightly less active against *E. coli*. More notably, biological data for **4d** showed an increase in activity against *S. aureus* (from $128 \mu\text{g mL}^{-1}$ to $64 \mu\text{g mL}^{-1}$) with an increase in molecular weight.

3.5. Biophysical characterization

Several mechanisms, such as carpet, barrel-stave, and toroidal pore, have been suggested for the action of host-defense peptides and their synthetic analogues against bacteria and RBCs.⁸ Facially amphiphilic, described as the hydrophilic and hydrophobic side chain, enables the molecules to penetrate the cell membranes. This type of interaction locally changes the membrane association, causing pore formation, and the holes in the phospholipid bilayer result in crumbling of the cell membrane and the death of the cell. In general, antimicrobial peptides and polymers possess positively charged hydrophilic groups that enhance electrostatic interaction with the negatively charged bacterial cell membrane, whereas their hydrophobic groups interact with the phospholipid tail groups, resulting in membrane permeation and disruption.⁴⁶ This type of mechanism does not request a specific receptor-like cell target for the biocidal activity. Facial amphiphilicity could also provide selectivity by becoming less toxic towards mammalian cells. It is also argued that this type of nonspecific interaction with the bacteria membrane might diminish the bacterial resistance build-up, as associated to traditional antibiotics.⁸

An important development step in synthetic mimic of antimicrobial peptides design was to completely exclude the helical secondary structure. DeGrado and Tew developed aryl-amide based molecules with a phenylene-ethylene backbone with a primary amine salt to obtain facially amphiphilic molecules.⁴⁷ The rigidity of aryl-amide backbone induced a linear facially amphiphilic confirmation that enhances the action upon

contact with the cell membrane. However, Kuroda and DeGrado showed that the rigid nature of the backbone and the presence of aromatic groups were not crucial for obtaining the selectivities for bacteria over RBCs.²⁰ It was declared that facial amphiphilicity on the monomer level was an important parameter. Tew and coworkers observed that aromatic functionality provides better biocidal efficiency than polymers bearing aliphatic groups.^{48,49} It was proposed that aromatic functional groups may play a special role, beyond their general hydrophobicity. Despite these observations, each molecule should be considered separately and other parameters, such as molarity, solubility in buffer media, and conformations, should also be taken into consideration while the mechanism in action is described.

Biophysical techniques, such as AFM, fluorescence spectroscopy, confocal microscopy, and solid state NMR, are common techniques to investigate the mechanism and mode of action of antimicrobial substances.⁴⁵ Analysis of the polymer interactions at the membrane level is one of the important parameters to understand the antimicrobial mechanism. In this study, the mechanisms adopted by active and inactive polymers (**4b**, **4e** and **4f**) in disrupting the Gram negative *E. coli* bacterial envelope were investigated. Polymer bearing a positive charge with phosphonium groups enhances the electrostatic interaction with the anionic bacteria surface, whereas the hydrophobic groups, basically aromatic ones, support polymer insertion into the phospholipid bilayer of the cell membrane. We applied zeta potential measurements to establish a correlation between MIC and surface charge distribution of bacterial membrane. Herein, *E. coli* was incubated with active/inactive polymer concentration. As shown in Table 3, *E. coli*, in the absence of either polymer, displayed a zeta potential of $0.00161 \pm 0.0002 \text{ mV}$. Relative to *E. coli*, polymers are considerably more basic and positively charged except **4e_3k** in the buffer conditions used at the zeta experiment. Upon the addition of the active polymer **4e_3k** and **4f_3k**, *E. coli* zeta potential values decreased and then stabilized at approximately -17.1 mV and -15.1 mV , respectively. The interaction of **4e_3k** with *E. coli* can be attributed to hydrophobic interactions associated with membrane disruption.⁵⁰ The decrease in zeta potential may be explained by taking the release of negatively charged phospholipids, such as

Table 3 Effect of polymer treatment on the zeta potential properties of *E. coli*^a

Polymer	Zeta potential (mV)
4b_3k	0.048 ± 0.01
4f_3k	-6.59 ± 0.1
4e_3k	7.98 ± 0.1
<i>E. coli</i>	0.00161 ± 0.0002
<i>E. coli</i> /DMSO	0.0437 ± 3.0
<i>E. coli</i> / 4e_3k	-17.1 ± 0.1
<i>E. coli</i> / 4f_3k	-15.1 ± 0.1
<i>E. coli</i> / 4b_3k	-0.0303 ± 0.01

^a In each case, each value represents the mean of duplicate determination (mean $\pm 2 \text{ SDM}$, $n = 2$).

cardiolipin and phosphatidylethanolamine from the *E. coli* membrane disruption, into account. However, **4f_3k** interacts more strongly with the *E. coli* membrane by a hydrophobic interaction and also with the lipid head groups by electrostatic forces.

In contrast, addition of the inactive polymer **4b_3k** indicated a slight decrease in the zeta potential value to -0.0303 mV. Our results with **4b_3k** suggested that this polymer was not embedded or weakly partitioned to the membrane bilayer and was not effective in destabilizing the bacterial membrane. We believed that the membranes ultimately equilibrate with the polymer solutions. It is also possible that **4b_3k** can be adsorbed (reversibly) by the membrane, but does not intercalate. The effect of DMSO that is used to solubilize the **4e_3k** and **4f_3k** in buffer was also tested and a minor change in the zeta potential value of *E. coli* was observed.

Electrostatic properties of the *E. coli* surface after the incubation of respective polymers indicated the significant differences in zeta potential values. Each amphiphilic molecules have different electrostatic and hydrophobic interactions with the cell membrane. Based on the result in this study, the adoption of a carpet-like or detergent-like mechanism by the two polymer **4e** and **4f** investigated here appears to be likely. Other factors, such as polymer conformation in the buffer or the role of the membrane potential cannot be excluded while describing the brief mechanism. However, further biophysical techniques should have been used to analyse the membrane in action.

4. Conclusions

Using ring-opening metathesis polymerization techniques, phosphonium based cationic polymers were obtained. By careful tuning of the overall hydrophobicity/aromaticity and charge density of these molecules, the inactive and active properties were determined. The effects of certain design features, such as charge, hydrophobicity and aromaticity, on the properties across the polymer series, can be understood.

When the alkyl substituent methyl, ethyl propyl and *tert*-butyl are used, the polymers are weakly or moderately active (and not hemolytic), but when the aromatic based alkyl substituent are used, the polymers are quite potent (and generally toxic). A rigid backbone against to nonaromatic groups enhances the antibacterial activity. This suggests that other design parameters, such as overall hydrophobicity and cationic charge, have a less significant impact on the biocidal activity. Understanding these design principles encourage us to develop new antimicrobial polymers possessing aromatic groups on their structure. We anticipate that future research towards the new design selective polymer will make significant contributions to a new generation of antibiotics.

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