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ROYAL SOCIETY OF CHEMISTRY

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View Article Online
DOI: 10.1039/C6RA15545F

RSC Advances

ARTICLE

Received 00th January 20xx,

Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Antimicrobial Activities of Phosphonium Containing Polynorbornenes

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In this study, amphiphilic polyoxanorbornene with different alkyl and aromatic phosphonium side chains were synthesized. The biological activities of these polymers were determined by 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, triphenyl, tris 4-methoxyphenyl) and two types different molecular weight of 3000 g mol^{-1} and 10000 g mol^{-1} were prepared. It was observed that the biological activity of the polymers with an aromatic groups 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 when compared to those with aromatic carbons. The aromaticity of the repeat unit was observed to have impressive effects on hemolytic activities as well. Zeta potential measurements of *E. coli* incubated with active and inactive polymer concentration let the establishment of a relation between the MIC and membrane surface charge density. Polymers bearing aromatic groups killed the bacteria with a widespread damage after the polymers holding the threshold concentration addition to the bacteria.

1. Introduction

The evolution of antibiotic-resistant microorganism is one of the most threatening global problem.[1-4] Especially, appearing 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 the 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 be spread by direct or indirect contact. Hospital surfaces therefore are subject to a constant background incidence of contamination events as patients and staff could move from one place to another.

Nowadays, hospital acquired, or nosocomial, infections have gradually expended all over the world and the trend is increasing. Approximately two million people acquired hospital infections in United States and 99000 died from the infection.[6] Host-defense peptides (HDPs) and their synthetic mimics lead to way to extensive studies for potential therapeutic applications.[7, 8] The exploration of HDPs and their chemical analogues as antibacterial agent is currently attracting interest for potential therapeutic applications.[9] The distribution of hydrophilic (cationic) and hydrophobic groups in the peptide backbone enables to favor amphiphilic structures and 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) production of these types of compounds is extremely costly, (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 many reported synthetic polymers that can display selectivity i. e. prokaryote-specific toxicity.[12, 13, 20-22] Selectivity has typically been assigned by hemolytic activity over antibacterial activity. Basically, antimicrobial cationic polymers and peptides target the phospholipid membranes of bacterial cells. Phospholipids bilayer of both Gram-positive and Gram-negative bacterial cell membranes is predominanly consist of anionic phospholipid head groups, whereas those found on eukaryotic cells are neutral. These negatively charged outer surface enhance the electrostatic interaction for the cationic peptides and polymers with bacteria, thus allowing them to implement their antibacterial effect. In spite of this action, hydrophobicity also plays a role in the enhancement of activity and toxicity. Thus, to improve resistance to HDPs and cationic polymers, bacteria will require more complex changes including alteration of its cell membrane.

Biocidal cationic polymers possessing with quaternary ammonium and phosphonium salts are well known that have better antibacterial activities compared to their small biocidal counterparts. [23] However, there are less studies on phosphonium based antibacterial polymers in the literature compared to

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

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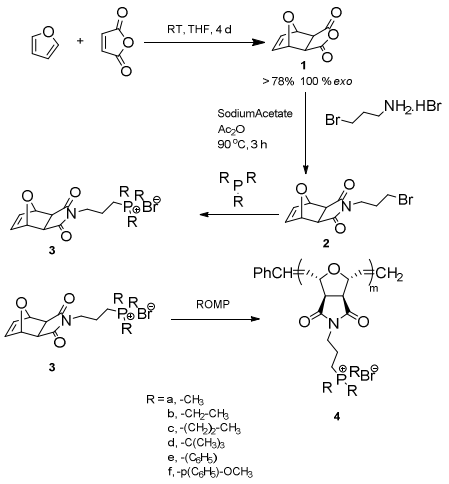
DOI: 10.1039/C6RA15545F

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 as 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 by using 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] Toxicity of obtained polymers were examined on L929 cells and no toxic effects was found. Endo and Kanazawa synthesized polymers by 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] Activity of polymers were measured against *Streptococcus mutans* (*S. mutans*) and it was found that at 10 µmol mL^{-1} concentration within 24 hours, polymers showed higher activity compared to the poly (methylmethacrylate).

ROMP (Ring opening metathesis polymerization) method is one of the technique for the 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 hydrophobic parts on the polymer chain was increased, antibacterial activity and toxicity increased as well.[12] In another study, pyridine salts were polymerized by the ROMP method and assessed for 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 in large scale. One-pot synthesis with a controlling the backbone are particularly attracting for the use of biocidal new generation of polymeric antibiotics.[33, 34]



Scheme 1. Monomer and polymers based on oxanorbornene derivatives.

Here, we presented phosphonium containing antibacterial polymers that can be considered a subgroup of biocidal polymers. In literature, there are no studies of phosphonium-containing polymers synthesized with ROMP yet, so here we report the characterization and antibacterial evaluation of cationic charged phosphonium-containing polyelectrolites synthesized by ROMP which can be an alternative for cationic based peptidomimic. Here, quaternary phosphonium functionality attached to oxanorbornene monomers were 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 electronic supplementary information (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 the compound **2**, shown in Scheme 1. Average yield for all the compounds was between 11-90 %, the tert-butyl (**3d**) and triphenyl phosphine (**3e**) being the yield reducing step due to steric effect. All the monomers were typically creamy to pale yellow crystals. All of the salts were hygroscopic and soluble in water except monomers **3e** and **3f**. These monomers were analyzed by using NMR spectroscopy.[37, 38] The ^{31}P {^{1}H} NMR spectra of the salts each exhibited a singlet between δ 20.01-49.91 ppm. Monitoring of 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 metoxyphenyl **(3f)** salts at δ 65.11, 29.21 and 27.03 ppm were observed. The presence of side products might resulted 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 easily be eliminated by successive washing of the residue with appropriate solvents. (Usually THF was enough for this purpose). Proton and carbon-13 NMR spectra of **3a-f** are also assigned. (Supplementary info. Figures S1-S14) Characteristic signals of oxanorbornene protons and carbons appeared at δ 6.57, 5.15 and 2.98 ppm in the ^{1}H 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** gives rise to a doublet at δ 1.75 ppm with the characteristic ^{2}J coupling of about 14.3 Hz. While the number of (-CH_2-) groups increases, i.e. **3a-c**, the shift of the methyl group (-CH_3) at the end of the alkyl functional group moves to higher magnetic field. Besides that, the typical doublet shifts due to the 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 ^{1}J coupling (85.8 Hz) in the doublet of methyl carbons (CH_3-P+) at δ 7.22 ppm (Figure S4). Methylene carbons next to the phosphonium cation (-CH_2-P+) observed at δ 20.02 ppm with a ^{1}J coupling as 50.9 Hz. In addition, ^{2}J coupling was also observed for methylene carbons (-

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CH_2-CH_2-P+) at δ 20.45 ppm with J = 5.6 Hz. Longer range ^2J and ^3J couplings were also reliable with through phosphorus-carbon bond formation.

3.2. Polymer Synthesis

The polymers, **4a-f**, were synthesized from respective monomers **3a-f**, were 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 10000 g mol^{-1} was applied with usuing 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 (Figure S15-S20). Appearance of vinylic hydrogens obtained by ROMP pathway resulted in both cis- and trans- double bond formation and the integration of these two signals in ^{1}H NMR indicate the relative cis/trans ratio in the polymer bakbone. The resulting homopolymers contained ~ 55-65 % trans residue. Additionally, long range coupling of phosphorus-carbon linkage was not observed due to the diminishing of the resolution of 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 ($^1J_{CP}$ = 86.2 Hz). End group analysis of the purified polymer was applied to calculate M_n in comparison to the assumed molecular weight using ^{1}H NMR techniques.[22] The phenyl end group connected to the original carbene moiety from the catalyst is visible at ca. δ ~ 7.20-7.50 ppm. Integration of the (-CH=$CH_{cis,trans}$-) peaks relative to the styrenic end group from the Grubbs catalyst in the ^{1}H 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 ^{1}H NMR peak extremely 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 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 indicate that a controlled and well-defined system was achieved by 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. Biocidal activity obtained for these homopolymers are 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 (≥128 μg 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 the Gram-negative *E. coli* and *M. tuberculosis*. It should be mentioned that *M. tuberculosis* is more related to Gram-negative than to Gram-positive bacteria as revealed by the sharing comparatively more genes for energy production and conversion with Gram-negative bacteria than with Gram-positive bacteria.[41] Architecture of the entire cell-wall of *M. tuberculosis* mainly consist of peptidoglycan layer with phosphodiester linkage, to which in turn, attached to mycolic acids.[42, 43]

Table 1. Summary of the Theoretical M_n, Measured M_n and M_w/M_n values for the homopolymers 4a-f.

Polymer	$M_{n,\,the}$ [a]	$M_{n,\,NMR}$ [b]	$M_{n\,(ASEC)}$ [c]	M_w/M_n [c]
4a_3k	3000	3864	6306	1.059
4a_10k	10000	9430	38137	1.102
4b_3k	3000	4475	7234	1.057
4b_10k	10000	14028	14298	1.049
4c_3k	3000	4053	8869	1.057
4c_10k	10000	15534	17277	1.008
4d_3k	3000	3698	30911	1.06
4d_10k	10000	n.d.	n.d.	n.d.
4e_3k	3000	n.d.	n.d.	n.d.
4e_10k	10000	n.d.	n.d.	n.d.
4f_3k	3000	n.d.	n.d.	n.d.
4f_10k	10000	n.d.	n.d.	n.d.

[a] $M_{n,the}$ = mass of monomer g mol^{-1} of initiator. [b] As determined by ^{1}H 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.

The mycolic acids are long fatty acids found in the cell membrane and provide a lipid barrier that enhance 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 defense peptide magainin MSI-78 possessing MIC of 12.5 μg mL^{-1}.[12] Polymer **4b_3k** shows 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] Here, the reason for the lower biocidal activity to *E. coli* over *S. aureus* is not clear 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. In spite of this, *S. aureus* has a 20-80 nm thick peptidoglycan layers surrounding plasma membrane bearing a single phospholipid bilayer. So, the biocidal polymers need to fracture two membranes in order to kill *E. coli* envelope, while they are able to diffuse through the peptidoglycan layers of *S. aureus* and result in destruction of its plasma membrane more efficiently. 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.*

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The amphiphilicity of the ROMP polymers is an important factor for the selectivity and activity.[12, 13] Increasing the hydrophobicity of the polymer structure enhance the interaction between phospholipid bilayer and result in enhancement in activity. However, excessive hydrophobicity in the polymer backbone favors cytotoxicity and also might precipitate in the test condition. 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 of activity for **4e** and **4f**. Increase in the aromaticity of the polymers enhances the various π interactions between polymer with cell membrane.[44] However, phenyl rings with bearing highly hydrophobic rigid structure might be the key parameters here for better interaction and destabilization of bacterial membrane and leakage of the cell.

Table 2. Antimicrobial and hemolytic activities of polymers.

Polymer	MIC (μg mL^{-1})[a]				HC_{50} (%hemolysis)[b]
	E.coli	*S.aureus*	*C.albicans*	*M.tuberculosis*	
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. (MIC_{90} = minimal inhibitory concentration preventing 90 % bacterial or fungi growth) and hemolytic activity towards red blood cells (HC_{50} = concentration lysing 50 % of blood cells). [b] Hemolysis caused by 512 μg mL^{-1} polymer concentration.

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

3.4. Hemolytic Concentration

Hemolytic activity (HC_{50}) of the polymers toward red blood cells (RBCs) were tested as described previously.[12] HC_{50}/MIC_{90} ratios 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*. Meanwhile, magainin, one of the natural host defense peptide, has a selectivity of 10.[45] From **4a** to **4f**, polymers became strongly hemolytic. Their high hydrophilicity prevents **4a-4d** from the activity against RBCs; but polymers having hydrophobic rigid analogous (**4e-4f**) cause significant hemolysis. Polymer possessing the aliphatic hydrophobic group incorporated was altered from a methyl group, **4a**, to a tert butyl, **4d**, and resulted in **~** 1 % hemolysis at 250 μg mL^{-1} polymer concentration. An overly aromatic substituent (**4e-4f**) enhances membrane interaction and as a result, is very hemolytic. For these series of polymers, they start to become more hemolytic ($HC_{50} \leq 250$ μg 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 polymers have the ideal facial amphiphicities and rigidity to destruct the bacterial and RBCs membrane.

The effect of molecular weight on biocidal activities was also examined by using 3000 g mol^{-1} and 10000 g mol^{-1} for the polymer **4a-4f**. The molecular weights did not result in substantial deviation in activities and activity was described as in 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 $M_n \approx 3000$ g mol^{-1} analogues of **4e** and **4f**, these polymers holding $M_n \approx 10000$ 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 μg mL^{-1} to 64 μg 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 analogous against bacteria and RBCs.[8] Facially amphiphilic described as the hydrophilic and hydrophobic side chain enables the molecules to penetrate of stucks into cell membranes. This type of interaction locally change the membrane association, causing the pore formation, and holes in the phospholipid bilayer result in the 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, while their hydrophobic groups interact with the phospholipid tail groups result in membrane permeation and disruption.[46] This type of mechanism does not request a specific receptor-like cell target for the biocidal activity. Facially amphiphilicity could also provide selectivity with 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]

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 enhance 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 selectivities for bacteria over RBCs.[20] It was declared that facial amphiphilicity on the monomer level was 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. In spite of these obervations, each molecules should be considered seperately and other parameters such as molarity, solubility in buffer media, conformations should also be taken into consideration while the mechanism in action is described.

Biophysical techniques such as AFM, fluorescence spectroscopy, confocal microscopy, solid state NMR etc. are common techniques

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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 parameter 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 positive charge with phosphonium groups enhance the electrostatic interaction with the anionic bacteria surface, while the hydrophobic groups, basically aromatic ones, supports polymer insertion into the phospholipid bilayer of the cell membrane. We applied zeta potential measurements for the establishment of a correlation between MIC and surface charge distribution of bacterial membrane. Here, *E. coli* 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 ± 0.0002 mV. Relative to *E. coli*, polymers are considerably more basic and positively charged except **4e_3k** in the buffer condition used at the zeta experiment. Upon 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 distruption.[50] Decrease in zeta potential values may be explained by taking the release of negatively charged phospholipids such as cardiolipin and phosphatidylethanolamine from the *E. coli* membrane disruption into account. However, **4f_3k** interacts more strongly with the *E. coli* membrane by hydrophobic interaction and also with the lipid head groups by electrostatic forces.

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
E.coli	0,00161 ± 0.0002
E.coli / DMSO	0,0437 ± 3.0
E.coli / 4e_3k	-17,1 ± 0.1
E.coli / 4f_3k	-15,1 ± 0.1
E.coli / 4b_3k	-0,0303 ± 0.01

[a] In each case, each value represents the mean of duplicate determination. (mean ±2 SDM, n =2)

In contrast, addition of the inactive polymer **4b_3k** indicated a slight decrease in the zeta potential value as -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 for the destabilization of bacterial membrane. We think of the membranes as ultimately equilibrating with the polymer solutions. It is also possible that **4b_3k** can be adsorbed (reversibly) by the membrane, but does not intercalate. Effect of DMSO that is used to solubilize the **4e_3k** and **4f_3k** in buffer was also tested and minor change in zeta potential value of *E. coli* were observed.

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

Conclusions

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

When the alkyl substituent methyl, ethyl propyl and tertbutyl 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 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 new generation of antibiotics.

Acknowledgements

This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) project no: 113S355. Support from the COST Action CM1302 SIPs is also gratefully acknowledged.

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