Poly(para-Phenylene Ethynylene) (PPE)- and Poly(para-Phenylene Vinylene) (PPV)-Poly[(2-(Methacryloyloxy)Ethyl) Trimethylammonium Chloride] (PMETAC) Graft Copolymers Exhibit Selective Antimicrobial Activity

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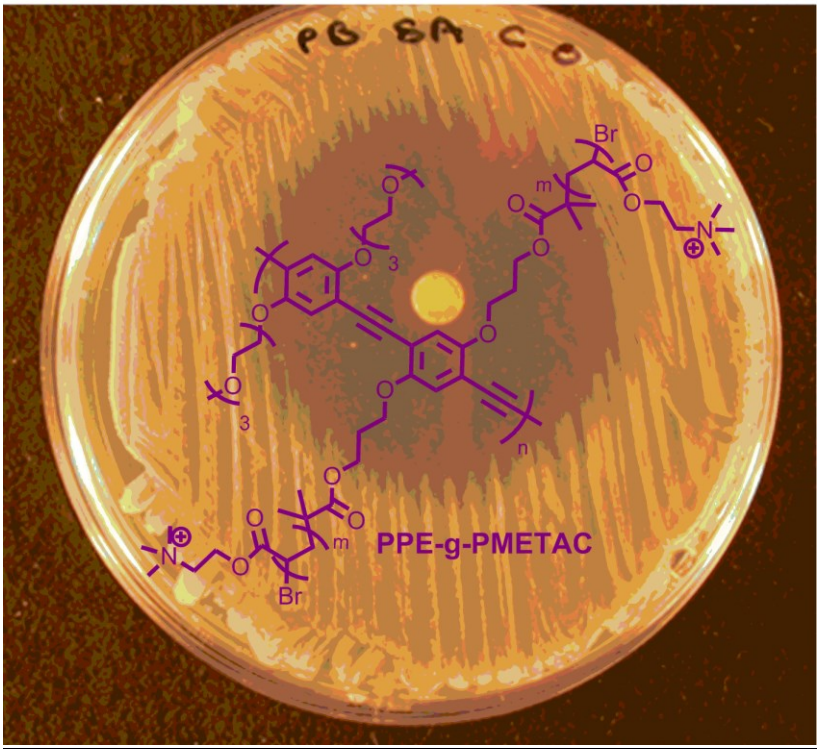


ABSTRACT

Antimicrobial resistance is becoming a global health concern; as such, the need for new effective treatments and preventive measures is increasing. Poly(para-phenylene ethynylene) (PPE)- and poly(para-phenylene vinylene) (PPV)-poly[(2-(methacryloyloxy)ethyl) trimethylammonium chloride] (PMETAC) graft copolymers were tested against a range of clinically and industrially relevant bacteria and results showed many of these conjugated polyelectrolytes (CPE's) to be active. Of all of the compounds tested, PPE-g-PMETAC (low molecular weight, LMW) had greatest antimicrobial activity, especially against *Enterococcus faecium*, Methicillin resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* and *Acinetobacter baumannii*.

KEYWORDS

PPE; PPV; Graft copolymer; ARGET ATRP; anti-microbial

INTRODUCTION

Antimicrobial resistance is a global health concern; as such, the need for new effective treatments and preventative measures is increasing. In Europe in 2007, 400,000 infections caused by multidrug-resistant (MDR) bacteria resulted in 25,000 attributable deaths and more than 1.5 billion dollars in annual spend to cover the extra hospital costs and productivity losses.[1] Both Gram negative and Gram positive bacteria are common causes of hospital acquired infections. In the clinical setting, there are a number of bacteria capable of 'escaping' the biocidal action of antibiotics. These bacteria include *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp., acronymically dubbed 'the ESKAPE pathogens'.[1] The normal flora of the skin is an important source of serious post operational infections with the involvement of skin

organisms such as *Staphylococcus epidermidis* being widely acknowledged.[2] Furthermore, modern food production facilitates the emergence and spread of resistance through the intensive use of antimicrobial agents and international trade of both animal and food products.[3] In order to control the spread of MDR bacteria, a strategy to reduce the use of antibiotics and prevent bacterial transmission between patients carriers is needed.[4] A number of bacteria are now also demonstrating an alarming increase in their reduced susceptibility to commonly used biocides e.g. chlorhexidine. For example, it is hypothesised that reduced susceptibility to chlorhexidine, the most widely used antiseptic skin cleanser, may contribute to the endemic nature of *Klebsiella pneumonia* ST258 that is resistant to almost all available antibiotics and is associated with high morbidity and mortality.[5] In other areas, for example the food and veterinary industries, alongside microorganisms such as *P. aeruginosa* and *S. aureus,* there are other persistent microorganisms such as *Listeria monocytogenes* and *Escherichia coli* which may cause food poisoning if they are not controlled or killed during food processing.

Such interventions include developing new classes of biocides that have enhanced antimicrobial efficacy and which can be used to decontaminate surfaces in both food and hospital environments. Cationic antimicrobials have been widely deployed in antisepsis for well over half a century without any apparent reduction in their effectiveness.[2] These molecules have been used in such items as medicated soaps, hand washes and bathing formulae. However, in recent years there has been movement towards the incorporation of these and other antimicrobial agents within polymer materials and coatings that comprise medical devices such as catheters and they have also been used in dressings and as topical antimicrobials.

The importance in using novel antibacterial compounds in order to combat bacterial transmission and contamination has resulted in the development of various antibacterial polymer systems.[6,7] In particular, conjugated polyelectrolytes (CPEs) have attracted much attention in recent years as a new class of materials.[6,8-10] CPEs have been shown to demonstrate

antibacterial efficiency, which is mainly attributed to their charged structure.[11,12] It has been shown that the addition of charged pendants to conjugated polymers has led to enhanced antibacterial activity, with both components seemingly necessary for potent acitvity.[13,14] In some instances it has been shown that the conjugated backbone allows UV activation pathways resulting in alternative killing mechanisms which in combination with the charged pendants increases activity.[12,14] Polyphenylene ethynylene (PPE) derivatives with positively charged quaternary ammonium (QA) or alkylpyridinium or negatively charged sulfonate pendants have been found to be active against both Gram-negative and Gram-positive bacteria.[8-10] It is hypothesized that such structural modifications enable these components to disrupt bacteria cell walls.[15,16] The antibacterial efficiency of modified polyphenylene vinylene (PPV) has also been demonstrated against *Bacillus subtilis* and *Escherichia coli* where an increase in antimicrobial activity was again attributed to the addition of charged pendants.[11] One strategy to further improve the antibacterial efficiency of CPEs is to amplify the overall charge which can be achieved by grafting of numerous charged sites onto a polymer backbone. Specifically, it may be advantageous to use amplified, positively-charged CPEs since charge increase has demonstrated a notably more efficient antimicrobial effect.[17,18]

Among the various grafting polymerisation methods, atom transfer radical polymerisation (ATRP) has been extensively used for the synthesis of polymeric brushes.[19,20] Moreover, these polymeric brushes have demonstrated antifouling efficiencies[21-24] one example being, neutral 2-(dimethylamino)ethyl methacrylate (DMAEMA) molecular brushes grafted onto glass or paper which showed significant antimicrobial efficacy against *E. coli* and *B. subtilis.*[25] Other examples demonstrating antimicrobial activity using ATRP, include microsphere surfaces grafted quaternized PDMAEMA or poly(butylmethacrylate) grafted with poly(Boc-aminoethyl methacrylate). An alternative to standard ATRP is Activator ReGenerated by Electron Transfer

Atom Transfer Radical Polymerisation (ARGET ATRP) which has been shown to allow the synthesis of grafted copolymers with improved controllability.[20,26]

We hypothesized that modification, via grafting, of the polymeric PPE and PPV macroinitiators may produce more efficient and effective biocidal materials. We therefore report on the preparation and properties of a range of antibacterial grafted conjugated-polymers.

EXPERIMENTAL DETAILS

Materials

All reagents were purchased as a reagent grade from Sigma and used without further purification. Dichloromethane (DCM), tetrahydrofuran (THF), dimethylformamide (DMF) and methanol were all used as an analytical grade and dried before usage. 2-(Methacryloyloxy)ethyl]trimethylammonium chloride (METAC) was used as a 30% solution in water. Solvents were dried using a solvent purifier (LC Technology Solutions Inc. SP-1 Standalone Solvent Purifier System).

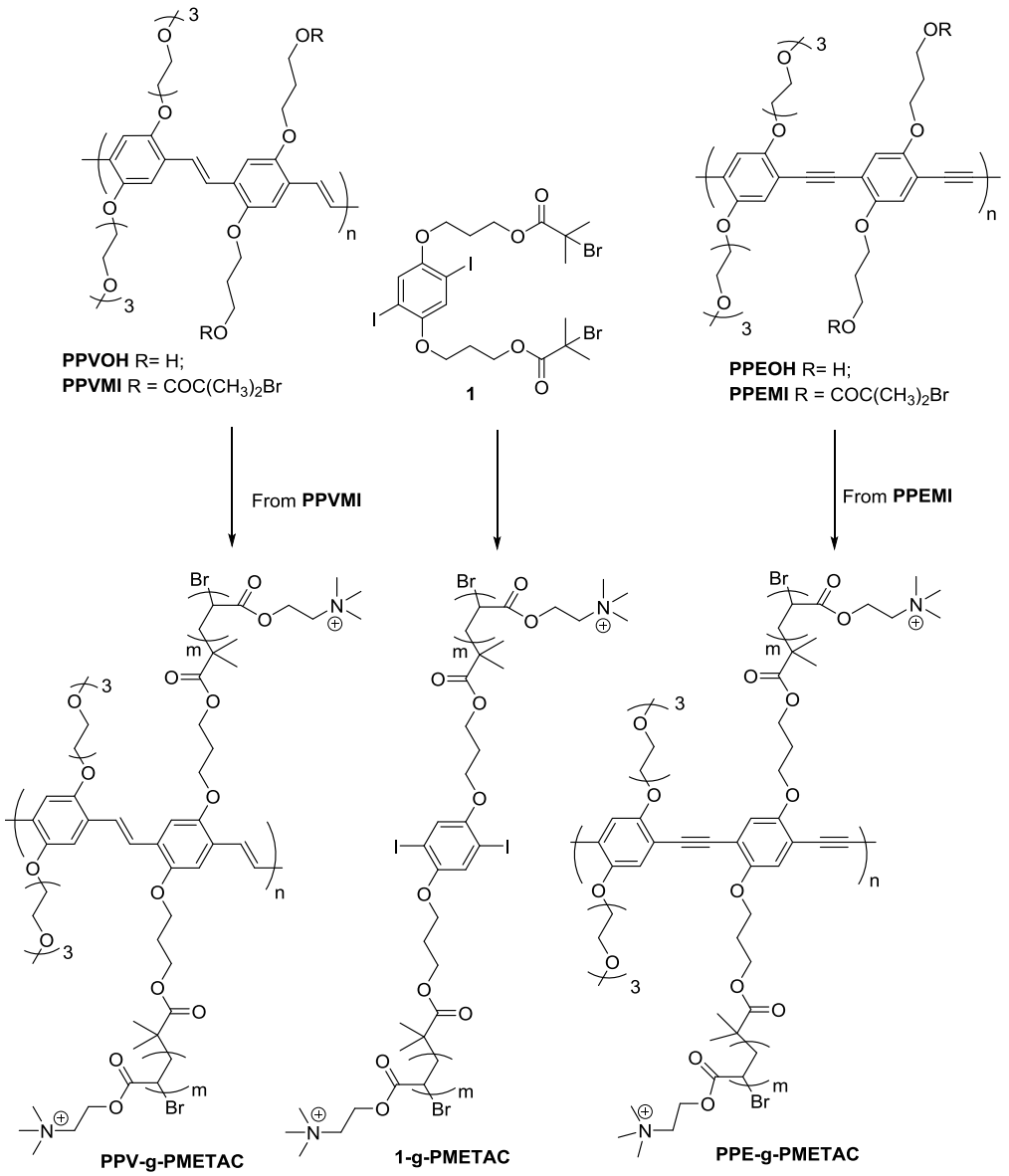
Instruments

A 300 or 400 MHz Bruker instrument was used for all NMR spectra recorded. IR spectroscopy was carried using a Perkin-Elmer Spectrum 1000 series Fourier Transform Infra-Red (FT-IR) ATR spectrometer, with a wavenumber range from 4000 to 400 cm^{-1}. Mass spectrometry was carried out on a VG 70-SE Mass spectrometer using an electron-spray ionization method. UV-Visible experiments were carried out on a Pharmaspec UV-1700, Shimadzu UV-Visible spectrophotometer using 3.5 mL quartz cuvettes. Solution-based fluorescence measurements were carried using a Perkin-Elmer LS 55 spectrophotometer with a 3-Q-10 mm rectangular quartz cell. The excitation wavelength was chosen as the maximum absorption wavelength of

the polymers. Molecular weight of the polymers were determined with a TDAmax GPC (Gel Permeation Chromatography) system (Malvern Instruments). GPC experiments were performed using the 2 x A5000 (300 mm x 8 mm each) Viscotech columns and A7Guard (50 mm x 8mm) Guard column. A 0.02% NaN_3 in water filtered through 0.02µm Nylon membrane filter (Grace) was used as an eluent with the flow rate of 1 mL/min. Before the injection all the samples were filtered through 0.22µm Nylon syringe filters (Grace). The columns and the detectors were maintained at 35 °C. Processing and acquisition of data was conducted using OmniSEC 4.7 software (Malvern Instruments) to achieve the calibration curve which was plotted using Dextran standards. The calibration curve was determined using dextran narrow standards purchased from Sigma-Aldrich.

CPE synthesis

The synthesis of dibromide **1** is given in the supporting information. The synthesis of **PPVMI** and **PPEMI** was performed as described previously, using Pd-catalyzed cross-coupling polymerization.[27-29] To maximize the PPE and PPV yields the grafting of cationic brushes was performed post-polymerization. It was decided to incorporate a spacer group into the conjugated polymer backbones to enhance antibacterial efficiency by improving the grafting polymerization process. This would occur by minimizing the steric effect of neighbouring cationic grafted units during the grafting process.



Scheme 1: Synthesis of the grafted copolymers.

To study the influence of the grafted cationic brushes, their density and the structure of the polymer backbones on the antibacterial efficiency, we grafted the cationic brushes onto dibromide **1**, PPVMI and PPEMI (Scheme 1). The cationic brush graft density was varied by using either a 250:1 (Low) or 500:1 (High) mass ratio of METAC monomer when PPEMI and PPVMI were used.

Initial attempts at grafting were made using traditional ATRP conditions, utilizing CuCl and *N,N,N′,N′,N″*-pentamethyldiethylenetriamine (PMDETA) but resulted in grafted copolymers

with low molecular weight for all of the initiators used. Therefore, to improve the molecular weight of the grafted polymers, ARGET ATRP was then attempted.[26] This method utilized $CuCl_2$ and PMDETA as the catalyst and ligand, with excess ascorbic acid as the reducing agent, to generate reactive Cu(I) species in solution. As expected in the ^{1}H NMR spectra signals corresponding to the initiators could not be observed after the grafting polymerization. This is consistent with previous reports as proton concentration of the polymer backbones becomes significantly lower than the grafted brushes.[30,31] The formation of the grafted brushes were confirmed by the broadening of the METAC signals and the disappearance of the (CH_2=CH) signals of the METAC monomer at 5.6-6.3 ppm, as well as the appearance of new peaks at 1.1-2.0 ppm due to the formation of CH_2-CH in the grafted polymer.

Preparation of copolymer from dibromide 1

The solution mixture of dibromide 1 (15 mg, 0.02 mmol) in DMSO (5 mL) was added to a solution of 2-(methacryloyloxy)ethyl]trimethylammonium chloride (METAC) (1.88 mL, 10 mmol) in DMSO (10 mL) and water (0.6 mL) and left at r.t. to achieve a clear solution. The ligand-catalyst complex was prepared by adding *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (PMDETA) (7.5 mg, 0.041 mmol) to $CuCl_2$ (2 mg, 0.0148 mmol) in anisole (1 mL) at 67 °C. The ligand-catalyst complex was added to the reaction mixture and heated to 60 °C. A mixture of ascorbic acid (980 mg, 5.56 mmol) in anisole (1 mL) and water (0.30 mL) was added slowly to the reaction and left for 24 h under an atmosphere of nitrogen at 60 °C. After this time the reaction was quenched with exposure to air and cooling the reaction flask in liquid nitrogen. The precipitated grafted polymer was then filtered and dissolved in water (5 mL). The grafted polymer was then re-precipitated into acetone (100 mL) and centrifuged to collect the product

as a white solid (78 mg, 42 %). δ_H (300 MHz; D_2O): 4.35-4.52 (2H, m, CH_2); 3.70-3.77 (2H, m, CH_2); 3.22 (9H, s, CH_3); 1.95-2.02 (1H, m, CH); 1.31-1.55 (2H, m, CH_2). GPC: M_w: 6.41×10^3, M_n: 4.98×10^3, M_w/M_n: 1.29

Preparation of copolymers from PPVMI

A solution of PPVMI (270 mg, 0.01 mmol) in DMSO (5 mL) was added to a solution of METAC (1.88 mL, 10 mmol) in DMSO (5 mL) and water (600 µL) whilst stirring, to give a colourless solution. Separately the ligand-catalyst complex was prepared by adding PMDETA (7.5 mg, 0.041 mmol) into a mixture of $CuCl_2$ (2 mg, 0.0148 mmol) mixture in anisole (1 mL) at 67 °C for 3 h. This complex was added to the reaction mixture at 60 °C. Then a solution of ascorbic acid (980 mg, 5.56 mmol) in anisole (1 mL) and water (300 µL) was added slowly to the reaction and left for 24 h under an atmosphere of nitrogen at 60 °C. The reaction was quenched by cooling the mixture with liquid nitrogen and exposing the mixture to air. The orange precipitate was collected and then dissolved in water. The product was re-precipitated using acetone (100 mL) and collected using centrifuge to give a bright orange solid (850 mg, 55 %). δ_H (400 MHz; D_2O): 1.01-1.10 (2H, m, CH_2), 1.95-2.01 (1H, m, CH), 3.25 (9H, br s, CH_3), 3.75-3.82 (2H, m, CH_2), 4.45-4.50 (2H, m, CH_2). GPC: M_w: 77.50×10^3, M_n: 46.19×10^3, M_w/M_n: 1.68. $\lambda_{max\ abs}$ = 435 nm. $\lambda_{max\ em}$ = 535 nm. The same procedure was used to achieve the low molecular weight PPV-g- METAC, except using METAC (950 µL, 5 mmol) in DMSO (5 mL) and water (300 µL) and ascorbic acid (600 mg, 3.40 mmol) in anisole (1 mL) and water (150 µL) to give a bright orange solid (180 mg, 40 %). δ_H (400 MHz; D_2O): 1.01-1.10 (2H, m, CH_2), 1.92-2.03 (1H, m, CH), 3.28 (9H, br s, CH_3), 3.77-3.85 (2H, m, CH_2), 4.43-4.48 (2H, m, CH_2). GPC : M_w: 22.87×10^3, M_n: 16.72×10^3, M_w/M_n: 1.37. $\lambda_{max\ abs}$ = 435 nm. $\lambda_{max\ em}$ = 537 nm.

Preparation of copolymer from PPEMI

A solution of PPEMI (220 mg, 0.02 mmol) in DMSO (5 mL) was added to a solution of METAC (1.88 mL, 10 mmol) in DMSO (5 mL) and water (600 μL) whilst stirring, to give a colourless solution. Separately, the ligand-catalyst complex was prepared by adding PMDETA (7.5 mg, 0.041 mmol) into a mixture of $CuCl_2$ (2 mg, 0.0148 mmol) in anisole (1 mL) at 67 °C for 3 h. This complex was added to the reaction mixture at 60 °C. Then a solution of ascorbic acid (980 mg, 5.56 mmol) in anisole (1 mL) and water (300 μL) was added slowly to the reaction and left for 24 h under an atmosphere of nitrogen at 60 °C. The reaction was quenched by cooling the mixture with liquid nitrogen and exposing the mixture to air. The orange precipitate was collected and then dissolved in water. The product was re-precipitated using acetone (100 mL) and collected using centrifuge to achieve a bright orange solid (870 mg, 63 %). δ_H (400 MHz; D_2O): 1.02-1.11 (2H, m, CH_2), 1.97-2.03 (1H, m, CH), 3.26 (9H, br s, CH_3), 3.77-3.82 (2H, m, CH_2), 4.47-4.51 (2H, m, CH_2). GPC: M_w: 77.48×10^3, M_n: 46.03×10^3, M_w/M_n: 1.68. $\lambda_{max\ abs}$ = 428 nm. $\lambda_{max\ em}$ = 493 nm. The same procedure was used to achieve the low molecular weight PPE-g-METAC, except using METAC (950 μL, 5 mmol) in DMSO (5 mL) and water (300 μL) and a solution of ascorbic acid (600 mg, 3.40 mmol) in anisole (1 mL) and water (150 μL) to give a bright orange solid (190 mg, 43 %). δ_H (400 MHz; D_2O): 1.04-1.13 (2H, m, CH_2), 1.95-2.01 (1H, m, CH), 3.24 (9H, br s, CH_3), 3.78-3.83 (2H, m, CH_2), 4.45-4.47 (2H, m, CH_2). GPC: M_w: 21.97×10^3, M_n: 16.20×10^3, M_w/M_n: 1.36. $\lambda_{max\ abs}$ = 428 nm. $\lambda_{max\ em}$ = 497 nm.

ARGET ATRP Kinetic Studies

As the grafting polymerisation was indicated to be successful within the ARGET ATRP the controllability of this reaction was studied whilst monitoring the polymerisation using ^1H NMR. Therefore at specific intervals, 100 μL samples were taken from the reaction. The monomer conversion was monitored using anisole as an internal standard.

Bacterial Preparation

All bacteria (Methicillin resistant *Staphylococcus aureus* (MRSA) NCTC 13552, *Escherichia coli* NCTC 10418*, Acinetobacter baumannii* NCTC 12156*, Klebsiella pneumoniae* NCTC 9633 and *Pseudomonas aeruginosa* NCTC 10332) were grown on tryptone soya agar (TSA) and in tryptone soya broth (TSB) at 37°C except *E. faecium* which was grown in brain heart infusion (BHI) broth on Columbia blood agar with added defibrinated horse blood in a CO_2 environment at 37°C. All microbiological agars and broths were purchased from Oxoid, (UK). A single colony of the bacteria was inoculated from agar into 100 mL of microbiological media. The inoculated culture was incubated at 3000 rpm in an air environment for 24 h, with the exception of *E. faecium* which was grown in a 5 % CO_2 incubator without shaking for 24 h. Cells were harvested by centrifugation (3500 × *g* for 10 min) and then washed with 10 mL sterile distilled water and vortexed to ensure even distribution of the cell suspension. The washed cells were again re-harvested. The pellet was re-suspended in 10 mL of broth, vortexed and the resultant cell suspension was adjusted to an optical density at 540 nm (OD_{540}) of 1.0 using a spectrophotometer (Jenway 6305 UV/Visible Spectrophotometer). The cell concentrations corresponded to 1.0 – 4.0 x 10^8 colony-forming units per mL (CFU mL^{-1}) at an OD_{540} of 1.0.

Zone of Inhibition (ZoI) assays

The appropriate agar (25 mL) was poured into sterile Petri dishes, which were then cooled and 100 µL of cell suspension was pipetted and spread across the entire area of the agar. Three equal wells (8 mm diameter) were cut out of the each agar plate using a sterile cork borer and a stainless steel needle. The borer and needle were sterilised in 70 % ethanol and flamed before

use. To each individual well, 100 µL CPE (5mg/L) was added. The plates were incubated under the appropriate conditions for 24 h. Following incubation, the ZoI was measured in mm from four sides of each well to determine an average mean value.

MIC and MBC assays

Bacteria were grown and prepared to an OD 1.0 as above. One millilitre of Triphenyl tetrazolium chloride (TTC) blue metabolic dye (Sigma-Aldrich, UK), was added into 9 mL of the cell suspension so that the working concentration of the dye was 0.15 % w/v. To determine the MIC, 100 µL of the test samples and acid controls were added to a 96 well flat-bottomed micro titre plate (MTP). One hundred microliters of bacterial suspension with the TTC dye was then added using a multi-channel pipette; the first column of cell/metal ion suspension was mixed, then 100 µL of the sample/bacterial mix was transferred to the column 2 wells. The dilution method was repeated until column 10 upon which 100 µL of the mixture was disposed of. To column 11, 100 µL of bacterial suspension without any CPE (positive control) was added and to column 12 and 100 µL of un-inoculated broth was added (negative control). After incubation, the MIC was taken as lowest concentration that inhibited the visible growth of the bacteria by comparison with the controls. Growth was indicated by a change of colour in the well to dark blue/purple. Twenty-five microliters of culture was taken from the first well that showed no growth and the last well that demonstrated growth and was pipetted onto agar plates using Miles and Misra methodology. After incubation, the lowest concentration well sample that showed no bacterial growth on the agar plate was determined to be the MBC for that test sample.

RESULTS AND DISCUSSION

PPV and PPE were chosen as the backbones for this investigation as a comparison of these two related backbones can provide an insight into how subtle changes affect the grafting of the brushes, and how their graft density/length can affect subsequent antibacterial efficiency.

Polymerization kinetics were studied using ^{1}H NMR spectroscopy to determine the controllability of polymerization and to evaluate the monomer conversion rate with both PPVMI and PPEMI using low and high monomer ratios. This was done by tracking the decrease in the integration of the (CH_2=CH) signals relative to integration of anisole (internal standard) (Figure 1). All of the kinetic studies demonstrate biphasic behaviour; with fast initial rates up to 35 min, followed by a slower rate. Such a fast initial rate is related to the higher monomer to initiator concentration ratio at the beginning of the grafting polymerization.[32] The slower kinetics beyond 30 min of the reaction time exhibits an almost linear behaviour which suggests a degree of controllability.

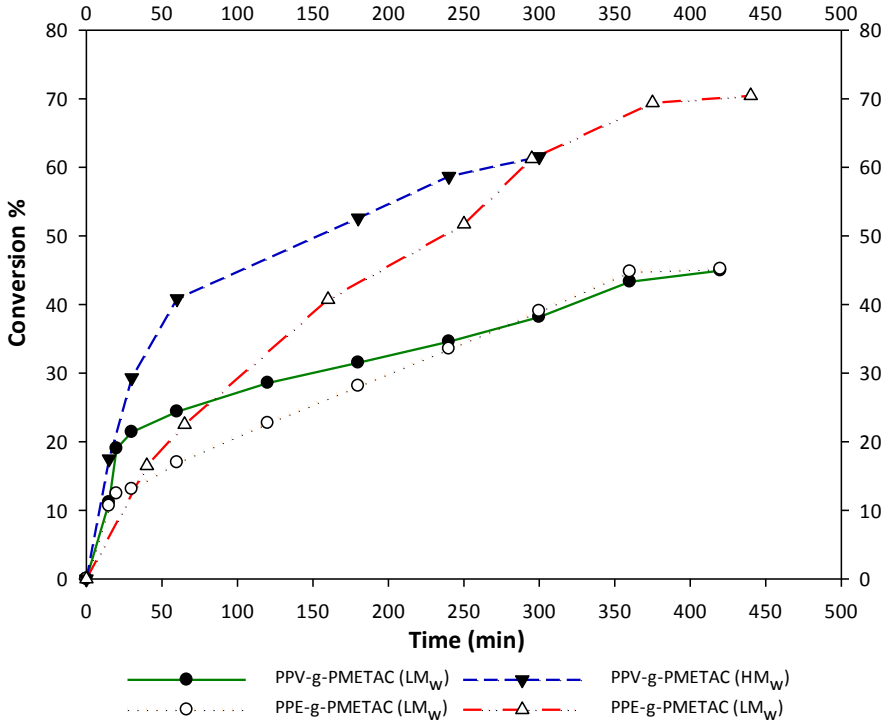


Figure 1. Kinetic model study of PPE-g-PMETAC and PPV-g-PMETAC with high and low molecular weight grafting polymerisation.

The anti-microbial activity of the synthesised cationic grafted polymers was assessed, using zone of inhibition (ZoI) measurements, against a range of pathogens including *Enterococcus faecium*, Methicillin resistant *Staphylococcus aureus* (MRSA), *Escherichia coli, Acinetobacter baumannii, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. It was determined that both the high and low molecular weight cationic compounds were particularly effective against all the Gram positive bacteria (*E. faecium* and MRSA) and also against Gram negative *E. coli* (Figure 2). Non-grafted backbones, PPVOH and PPEOH and 1-g-PMETAC did not demonstrate any antimicrobial activity. Thus, it may be speculated that the nature of side chain attachment to the polymer backbone influenced the antimicrobial efficacy of the compound

when tested using a semi-solid media method. The density of cationic grafts (high or low) did not affect the antimicrobial activity under these assay conditions.

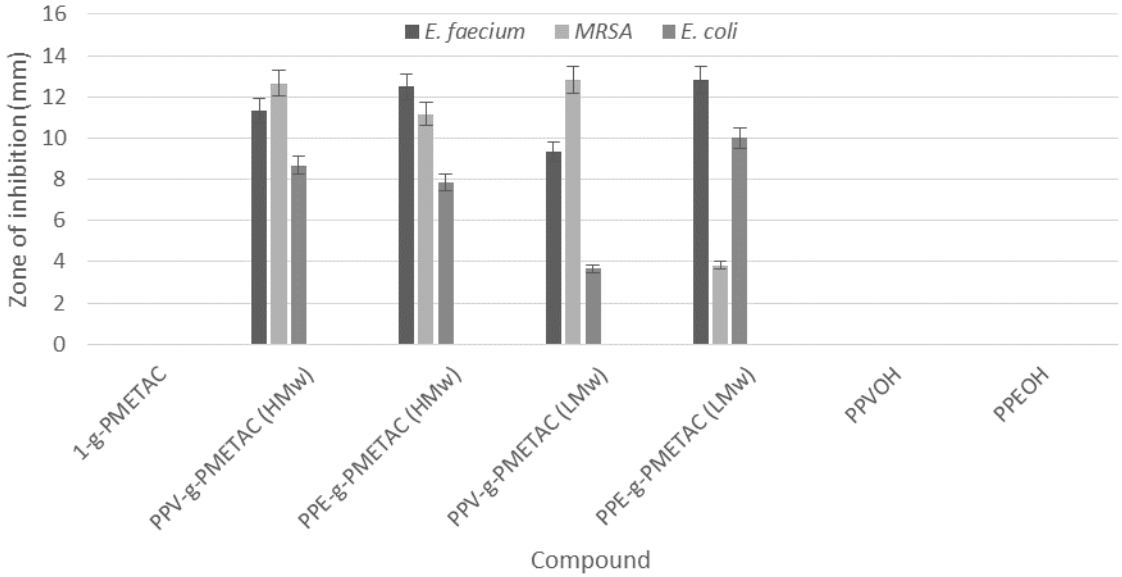
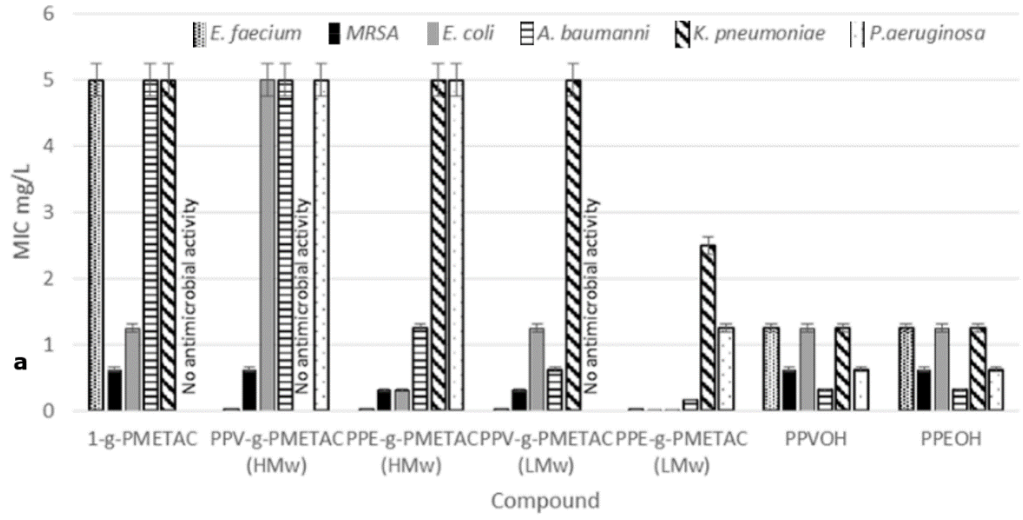


Figure 2. Antimicrobial activity of the compounds against a range of Gram negative and Gram positive bacteria using semi-solid agar tests. All compounds tested shown no activity against *A. baumanni*, *K. pneumoniae* and *P. aeruginosa* in this assay.

Next, minimal inhibitory concentration (MIC) and minimal bactericidal concentration assays (MBC) were carried out. The MIC of the compounds were determined to demonstrate the inhibition of the compounds on bacterial growth, whereas the MBC determine the bactericidal activity of the compounds. These antimicrobial assays were carried out in a microbiological media solution. Unlike the ZOI testing, MIC results demonstrated that PPVOH and PPEOH demonstrated good antimicrobial efficacy against all the bacteria tested (0.63 mg/L to 5.00 mg/L). Against *E. faecium* PPE-g-PMETAC (HMw), PPE-g-PMETAC (LMw), PPV-g-PMETAC (HMw) and PPV-g-PMETAC (LMw), that is all compounds combining conjugated backbone with cationic side chains, demonstrated excellent antimicrobial activity (0.02 mg/L). This was also demonstrated in the MBCs (0.16 mg/L – 0.31 mg/L). All the compounds inhibited and were bactericidal against MRSA and *E. coli* with PPE-g-PMETAC (LMw)

demonstrating the best antimicrobial activity (0.01 mg/L MIC and 0.02 mg/L MBC against MRSA and 0.02 mg/L MIC and 0.04 MBC against *E. coli*). Against *K. pneumonia* and *P. aeruginosa* similar trends were demonstrated whereby the PPVOH and the PPEOH demonstrated the greatest antimicrobial activities (MIC: 1.25 mg/L and 0.63 mg/L, MBC: 2.5 mg/L and 2.5mg/L for *K. pneumonia* and *A, baumannii*, respectively). *A. baumannii* was also most affected by PPVOH and PPEOH (MIC 0.31 mg/L, MBC 1.25 mg/L for both compounds), but the most antimicrobial compound against this bacteria was again PPE-g-PMETAC (LMw) (MIC 0.16 mg/L, MBC 0.63 mg/L).



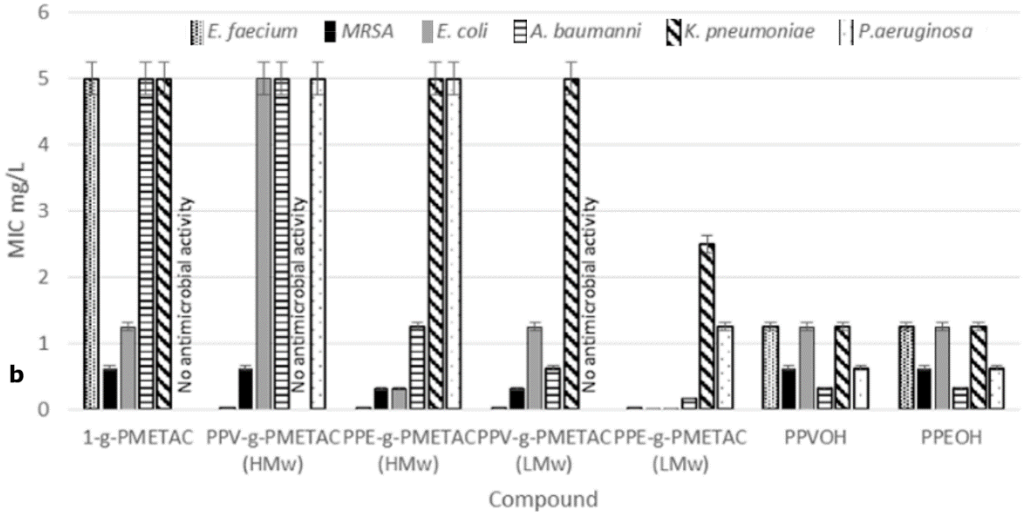


Figure 3. a) Minimal inhibitory concentration (MIC) and b) minimal bactericidal concentrations (MBC) of synthesised compounds against the bacteria investigated.

The positive results of these cationic compounds is comparable to other studies, where cationic compounds have been highly effective antimicrobials.[33] PPE derivatives containing short trimethylammonium pendants (total number of cationic sites ~14) have been shown to be active at concentrations of 1 mg/L against *E. coli,*[34] whilst PPE-g-PMETAC (LMw) had an improved MBC of 0.039 mg/L against the same bacteria.

The outermost surface of bacterial cells carries a net negative charge. This is associated with the teichoic acid and polysaccharide elements of Gram positive bacteria, the lipopolysaccharide of Gram-negative bacteria, and the cytoplasmic membrane itself, thus cationic agents have a high binding affinity for bacterial cells.[2] The results demonstrated that the PPE-g-PMETAC (LMw) was the most effective antimicrobial overall. In the ZOI assays, all the conjugated polymers bearing cationic side chains demonstrated antimicrobial activity against *E. faecium,* MRSA and *E. coli.* These results reflect the ability of these polymers to be dispersed in a semisolid media, most likely to their high aqueous solubility, resulting from extensive cationic groups. However, in the MIC and MBC assays, the compounds demonstrated a range of efficacies that were dependent on both the chemical structure of the compound and the cell type. Against *E. faecium* all the compounds with the cationic grafts were antimicrobial, whereas against MRSA and *E. coli* the most antimicrobial polymer was the PPE-g-PMETAC (LMw). In contrast against *K. pneumoniae* and *A, baumannii* the ungrafted PPVOH and PPEOH were the most antimicrobially effective.

Among vegetative bacteria, Gram-negative are deemed to be the least sensitive to biocides, followed by Gram-positive bacteria, however this classification is primarily based on the difference in permeability/impermeability of the micro-organism to an active agent.[35] This is

especially important in the resistant ESKAPE bacteria where differences in their cell wall structure and components leads to varying responses to biocides.[36] Certain cationic biocides (e.g. chlorhexidine) are considered to penetrate the cell by a mechanism of self-promoted uptake, which may lead to induce the formation of permeable pores in the bacterial envelope.[37] However, our work demonstrated that *E. coli* was particularly susceptible to the majority of the compounds tested, as was MRSA. *E. faecium* may have been more resistant than the MRSA to the compounds since it is difficult to inactivate due to high level recalcitrance and it can tolerate a wide range of growth conditions and can also survive for long periods of time on environmental surfaces.[38,39] This difference in the effect of the compounds may also be explained because the Gram negative bacteria used in this work have different physiologies. In *E. coli* the cell wall is thought to be only around 4 nm in thickness.[40] However, *K. pneumoniae* has a large polysaccharide capsule surrounding the bacterial cell, which both protects the bacteria and acts as a barrier to antimicrobial agents.[41] *A. baumannii* is an encapsulated Gram-negative coccobacilli bacteria whose features on the outer cell membrane include porins and efflux channels which contribute to antibiotic resistance. *A. baumannii* has fewer and smaller porins than other Gram-negative bacteria, thereby decreasing cell permeability and increasing antibiotic resistance.[42] It has been suggested that less than 5% of molecules are permeable to the cell membrane, which is less than that found in *Escherichia coli*.[42] This may be in part why *A. baumannii* was less affected by the biocides in our study than was *E. coli. P. aeruginosa* is intrinsically resistant to antimicrobial agents due to low permeability of its cell wall.[43] A characteristic feature of many *P. aeruginosa* strains is the production of a loosely associated layer of the anionic polysaccharide, alginate, which surrounds the cells and binds them together in aggregates and it has been shown that alginate can bind cationic antibiotics such as the aminoglycosides and restrict their diffusion.[44] The different results with these compounds

suggest their killing mechanism is more complex than just membrane disruption and this will be investigated in future work.

Conclusions

Whilst PPE and PPV derivatives have been previously found to be active against both Gram-negative and Gram-positive bacteria, previous molecules contained fewer charged sites.[8-10,11,13,14] By using ARGET ATRP, the antibacterial efficiency of these CPEs was amplified by grafting additional cationic sites onto the conjugated backbones. The benefit of this approach is that the same conjugated macroinitiator (such as PPVMI or PPEMI) can be used to add various cationic grafted brushes without the need for redesigning of the CPE backbone.

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