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# 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*.

## 1. 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 hypothesized 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

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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 polymerization methods, atom transfer radical polymerization (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 Polymerization (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.

## 2. Experimental details

### 2.1. 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).

### 2.2. 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 × A5000 (300 mm × 8 mm each) Viscotech columns and A7Guard (50 mm × 8 mm) Guard column. A 0.02% $NaN_3$ in water filtered through 0.02 μm 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 0.22 μm 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.

### 2.3. 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.

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 $^1H$ 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.

### 2.4. 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