The synthesis, characterization and antibacterial activity of quaternized poly(2,6-dimethyl-1,4-phenylene oxide)s modified with ammonium and phosphonium salts

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\textbf{A B S T R A C T}

A new class of antimicrobial polymers consisting of PPO (polyphenylene oxide) was synthesized, and the antimicrobial activities of these polymers were investigated. This was accomplished by selective $\alpha$-bromination of PPO (BPPO) followed by quaternization reactions with various tertiary amines or phosphines. Two types of BPPO were prepared, and the antimicrobial activities of the quaternized polymers were tested against Gram-positive bacteria (\textit{S. Epidermidis}) and Gram-negative bacteria (\textit{Escherichia coli}). The triphenylphosphonium-modified polymer showed excellent antibacterial activity against both types of bacteria. Generally, the thermal stability of phosphonium-modified BPPO was superior to that of the ammonium analog, and the increase in the functionalization of the polymer backbone resulted in improved antimicrobial activity.

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1. Introduction

Nosocomial, or hospital-acquired, infection is a serious global public-health issue. The sterilization procedure in hospitals generally involves application of heat, chemicals, irradiation, high pressure or filtration. However, these conventional ways of hospital sterilization can alter the chemical structure and physical properties of materials and reduce equipment lifetime. There is a keen interest in materials capable of killing or preventing microorganisms. The previous approach to making materials bactericidal is to blend or coat materials with antibacterial agents such as antibiotics, silver ions, fluoroquinolones and quaternary ammonium compounds, but these antibacterial agents are gradually released over time [1]. Recent studies have focused on the non-release strategy for creating a permanent antibacterial effect, and various methods have been used to synthesize polymers with antimicrobial activity [2–5]. Besides the biguanide-containing polymers, which are known to exhibit intrinsic antibacterial activities [6,7], most polymers possessing biocides in their pendant groups are synthesized from the polymerization of acrylate [8–13], styrene [14–17], or norbornene [18,19] monomers. Immobilization of the antibacterial moiety through chemical anchoring by post-polymerization modification has also been applied in copolymer systems such as vinylbenzyl chloride cross-linked with 2-chloroethyl vinyl ether (CEVE) or with methylmethacrylate (MMA), poly(ethylene-co-vinyl alcohol) and poly(styrene-co-maleic anhydride) [20–27]. The antibacterial moieties in polymers include antibiotics, organotin, N-halamines, silver ions and various types of quaternary ammonium and phosphonium salts. Compared with conventional antibacterial agents of low molecular weight, polymeric antibacterial agents have advantages such as non-volatilization, inability to permeate the skin and reduced toxicity to the environment. Moreover, cationic polymers with quaternary ammonium or biguanide groups generally exhibit higher antimicrobial activities than the corresponding low-molecular-weight model compounds because of the much higher charge density carried by the polycations, which can initiate stronger interactions with the negatively charged bacterial cell surfaces [28,7,29].

Despite extensive research efforts, most of the resulting copolymers consist of an ethylene backbone, whereas only a few non-ethylene main chain skeletons, such as cellulose [30], chitosan [31–35], polysiloxanes [36,37] or polylamide [38] have been reported and little work has been conducted on their thermal stability. Poly(phenylene oxide) (PPO), also known as poly(phenylene ether) (PPE), is one of the most important engineering plastics and is widely used in electrical appliances due to their balanced physical, chemical and electrical properties. Because PPO is also a nontoxic, Food and Drug Administration (FDA) compliant material and readily available from the commercial market, it is of great interest to develop these non-ethylene-backbone polymers, which
lead to new antimicrobial materials with applications for which known polymers are unsuitable. In this study, a series of quaternary ammonium- and phosphonium-modified PPOs were synthesized, and the objective of the present study is to explore how this structural modification of the PPO polymer will influence material properties such as thermal stability and the antibacterial properties against Gram-positive and Gram-negative bacteria.

2. Experimental

2.1. Materials

All reagents and solvents were of reagent grade. Poly(2,6-dimethyl-1,4-phenylene oxide) (intrinsic viscosity = 0.4 × 10⁻³ m² kg⁻¹, i.e., 0.4 dl g⁻¹) in chloroform at 25 °C was obtained from General Electric Plastics and purified before use by precipitation from a chloroform solution into methanol. N-Bromosuccinimide (NBS; Acros), 2,2'-azobisobutyronitrile (AIBN; Showa), triphenylphosphine (PPh₃; Lancaster), triethylamine (TEA; RDH), tributylphosphine (PPh₃; Acros), dimethylsulfoxide (DMSO; Sigma), triethylenediamine (TETA; Sigma), triphenylphosphine (PPh₃; Kanto), toluene, chlorobenzene, diethyl ether, chloroform, n-hexane (all from Tedla), tetrahydrofuran (THF; J.T. Baker), Peptone (AMRESCO), beef extract (MP Biomedicals), LB broth (Lab M Limited) and agar (CONDA) were purchased from commercial companies and used as received.

2.2. Characterization

¹H spectra were recorded on a 300-MHz Varian–Mercury’300 spectrometer using deuterated solvent. Gel permeation chromatography (GPC) was carried out with a TGA Q50 (TA Instruments) thermogravimetric analyzer under a nitrogen atmosphere. Samples (10–15 mg) were placed in platinum pans and put in an oven at 30 °C. The temperature was raised from 30 to 105 °C at 10 °C/min, maintained for 20 min, and raised to 800 °C under nitrogen. Degradation temperature at 5% weight loss was measured (Td5%) and the char yield (Char %) at 800 °C was recorded on TGA.

2.3. Synthesis

2.3.1. Synthesis of methyl-brominated poly(2,6-dimethyl-1,4-phenylene oxide) (BPPO)

NBS (4.70 g, 26.6 mmol) and AIBN (0.20 g, 1.2 mmol) were added to a stirred solution of 4.80 g of PPO in chlorobenzene (200 mL). The mixture was stirred under refluxing conditions for 4 h, and the reaction mixture was added to a ten-fold excess of n-hexane to precipitate the product. After filtration and washing with methanol, the polymer was dissolved in chloroform and precipitated in a methanol solution. (BPPO-1, 0.51 g) was used. (BPPO-1, 0.52 g) and TEA (0.24 g) were used. (TEA-PPO-1, 0.47 g) was obtained from Tega, tetrahydrofuran (THF; J.T. Baker), and agar (CONDA) were purchased from commercial companies and used as received.

2.3.2. Synthesis of the triethylammonium salt of PPO (TEA-PPO)

TEA (0.14 g) was added to a stirred solution of BPPO-1 (0.52 g) in 15 mL of THF and 5 mL of MeOH. The mixture was stirred for 16 h at reflux temperature, and the reaction mixture was added to a sixfold excess of diethyl ether. The product was filtered, washed several times with diethyl ether, and dried under a vacuum at room temperature to give 0.49 g of 1. (TEA-PPO-1, ammonium salt ratio: 35.8%, yield: 83.2%). ¹H-NMR: Ar-CH₃ (2.02 ppm, s, 9H), Ar-H of a non-substituted PPO unit (6.51 ppm, s, 2H). (TEA-PPO-2, 0.51 g) and TEA (0.48 g) were used. (TEA-PPO-2, 0.36 g, yield: 69.6%, ammonium salt ratio: 19.8%).

2.3.3. Synthesis of the tributylammonium salt of PPO (TBA-PPO)

3: BPPO-1 (0.52 g) and TEA (0.30 g) were used. (TEA-PPO-1, 0.47 g, yield: 66.6%, ammonium salt ratio: 36.9%). ¹H-NMR of TBA-PPO (DMSO-d₆, δ ppm): Ar-CH₃ (2.02 ppm, s, 9H), Ar-H of a non-substituted PPO unit (6.51 ppm, s, 2H). (TBA-PPO-1, 0.55 g) and TEDA (0.77 g) were used. (TEDA-PPO-1, 0.47 g, yield: 73.1%, ammonium salt ratio: 19.8%).

2.3.4. Synthesis of the triethylenediamine ammonium salt of PPO (TEDA-PPO)

5: BPPO-1 (0.52 g) and TBA (1.16 g) were used. (TEA-PPO-1, 0.47 g, yield: 66.6%, ammonium salt ratio: 36.9%). ¹H-NMR of TEDA-PPO (DMSO-d₆, δ ppm): Ar-CH₃ (2.02 ppm, s, 9H), Ar-H of a non-substituted PPO unit (6.51 ppm, s, 2H). (TBA-PPO-1, 0.55 g) and TEDA (0.77 g) were used. (TEDA-PPO-1, 0.47 g, yield: 73.1%, ammonium salt ratio: 19.8%).

2.3.5. Synthesis of the triphenylphosphonium salts of PPO (PPh₃-PPO)

7: BPPO-1 (0.52 g) and PPh₃ (1.23 g) were used in 40 mL of methanol solution. (PPh₃-PPO-1, 0.70 g, yield: 87.2%, phosphonium salt ratio: 45.5%). ¹H-NMR of PPh₃-PPO (DMSO-d₆, δ ppm): Ar-CH₃ (2.02 ppm, s, 9H), Ar-H of a non-substituted PPO unit (6.51 ppm, s, 2H). (PPh₃-PPO-2, 0.64 g, yield: 97.6%, phosphonium salt ratio: 20.9%).

2.3.6. Synthesis of the tributylphosphonium salts of PPO (PBu₃-PPO)

8: BPPO-1 (0.52 g) and PPh₃ (0.93 g) were used in 40 mL of methanol solution. (PPh₃-PPO-2, 0.64 g, yield: 97.6%, phosphonium salt ratio: 20.9%).

2.3.7. Synthesis of the triethylphosphonium salts of PPO (PPh₂-PPO)

9: BPPO-1 (0.51 g) and PPh₂ (0.93 g) were used in 15 mL of THF and 5 mL of MeOH solution. (PPh₂-PPO-1, 0.42 g, yield: 58.0%, phosphonium salt ratio: 40.2%). ¹H-NMR of PPh₂-PPO-1 (DMSO-d₆, ppm): Ar-CH₃ (2.02 ppm, s, 9H), Ar-H of a non-substituted PPO unit (6.51 ppm, s, 2H). (PPh₂-PPO-2, 0.64 g, yield: 97.6%, phosphonium salt ratio: 20.9%).

2.3.8. Synthesis of the triphenylphosphonium salts of PPO (PPh₃-PPO)

10: BPPO-1 (0.51 g) and PPh₃ (1.23 g) were used in 40 mL of methanol solution. (PPh₃-PPO-1, 0.52 g, yield: 83.2%, phosphonium salt ratio: 35.8%). ¹H-NMR: Ar-CH₃ (2.02 ppm, s, 9H), Ar-H of a non-substituted PPO unit (6.51 ppm, s, 2H). (PPh₃-PPO-2, 0.55 g, yield: 86.2%, phosphonium salt ratio: 30.9%).
2.4.2. Media

The selected microorganisms were used to test the antimicrobial activity of the synthesized polymers. These microorganisms included the Gram-negative bacteria Escherichia coli BL21(DE3) (E. coli, BCRS No 51878, purchased from Bio Resource Collection and Research Center, Hsinchu, Taiwan) and the Gram-positive bacteria Staphylococcus Epidermidis PCI 1200 (S. Epidermidis, BCRS No 11030, purchased from Bio Resource Collection and Research Center, Hsinchu, Taiwan).

2.4.3. Antimicrobial activity test

Each bacteria suspension (0.25 mL, the initial bacteria density is 10^8 CFU/mL and 1.3 × 10^8 CFU/mL for S. Epidermidis and E. coli respectively) was mixed with 2.75 mL of the corresponding sterile broth to maintain the initial bacteria concentration. After 24 h, the colony-forming units were recorded. The surviving ratio was calculated for each tested polymer. Percent inhibition values were calculated for each tested polymer as the percent reduction in bacteria density or the growth rate relative to the control.

2.4.4. Measuring the minimum inhibitory concentration (MIC)

Each bacteria suspension (0.25 mL) was mixed with 2.75 mL of the corresponding broth media in a sterile test tube containing the tested polymers (30 mg/mL). The seeded tubes were then shaken at 150 rpm overnight. The 10^6-fold dilutions were carried out, and 1 mL of each dilution was spread into the agar plate of the corresponding media. Controls without the polymers were run, and the plates were incubated at 37 °C for 24 h. The colony-forming units were recorded. The surviving ratio was calculated for each tested polymer. Percent inhibition values were calculated for each tested polymer as the percent reduction in bacteria density or the growth rate relative to the control.

2.4.5. The antimicrobial activity of the polymer-immersed medium

The tested polymers (30 mg/mL) were added to 3 mL of a nutrient broth and then kept at 37 °C for 24 h. After the broths containing the tested polymers were centrifuged, the suspensions were removed and filtrated for an antimicrobial activity test. S. Epidermidis suspensions (1 mL) were mixed with 2 mL of the control or filtrated solutions and then shaken at 37 °C and 150 rpm overnight. The controls and samples containing tested polymers were separately measured at OD560. The results of the antimicrobial effects were expressed by the OD560 ratio (treated sample/control sample). The low value of the OD560 ratio represents the high level of the antimicrobial efficiency.

2.4.6. In Vitro cytotoxicity studies

The cytotoxicity analysis of quaternized PPO with different concentrations of active groups was performed by MTT assay. Polymer suspensions were prepared in non-serum-supplemented cell culture media and filtrated by a 0.45-nm membrane (Pall Corporation, USA) to exclude cytotoxic effects due to changes in osmolarity and pH value. Hs68 fibroblasts (Bioresource collection and Research Center, Taiwan) were plated into 24-well microtiter plates (Corning™) at a density of 10,000 cells/well. After 24 h, the culture medium was replaced by 1 mL of the filtrated media of the tested polymers and the cells were incubated for 24 h. Polymer solutions were aspirated and replaced by 200 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) reagent (0.5 mg/mL). After 4 h, the unreacted dye was removed by aspiration, and the insoluble formazan crystals were dissolved in 200 μL/well of DMSO. The absorbance was measured spectrophotometrically in an ELISA reader (BIO-TEK) at a wavelength of 570 nm.

2.4.8. Data analysis

The data analysis was performed by a paired t-test using the commercial software program MINITAB. p < 0.05 was considered to be a significant difference.

3. Results and discussion

3.1. Preparation and characterization of quaternized poly(2,6-dimethyl-1,4-phenylene oxide)s (1-10)

In the field of disinfection, quaternary ammonium and phosphonium salts are well known as effective antimicrobial agents that are used in a number of areas, such as cosmetics, common antiseptics, sanitizers in hospitals and disinfectants for contact lenses [39,40]. Although the antibacterial mechanism of these quaternary ammonium and phosphonium salts has not been clearly elucidated, the most common hypothesis is related to the polycationic nature of these compounds, which allows them to interact with negative charges on bacterial cell walls. Thus, their antimicrobial activity is conditioned by their surface properties. The antimicrobial PPOs were synthesized from the quaternization reactions of the methyl-brominated PPO (BPPO) with corresponding tertiary amines or phosphines, as outlined in Fig. 1. BPPO could be obtained in high yields from PPO with excellent selectivity using NBS under heat and irradiation in chlorobenzene, and hardly any phenylbrominated or methyl-dibrominated derivatives were found after isolation. To investigate the effect of different functionalization ratios on the antimicrobial properties, two types of BPPO with 46.4% (BPPO-1) and 24.8% (BPPO-2) degrees of bromination were prepared, and the analysis is listed in Table 1. TheGPC chromatograms of BPPO polymers show that unimodal peaks of Mn prepared, and the analysis is listed in Table 1. The GPC chromatographs of BPPO polymers show that unimodal peaks of Mn and 150 ppm overnight. The 10^6-fold dilutions were carried out, and 1 mL of each dilution was spread into the agar plate of the corresponding media. Controls without the polymers were run, and the plates were incubated at 37 °C for 24 h. The colony-forming units were recorded. The surviving ratio was calculated for each organism at different polymer concentrations and was compared to the control.
Gram-negative bacteria (*E. coli*). The structures of the polymeric quaternary ammonium or phosphonium salts were characterized by 1H and 13C NMR. For the ammonium or phosphonium salts of BPPO polymers, the 1H NMR spectra exhibit a broad hump from δ = 3.6 to 4.8 ppm (depending on quaternization reagents), which was assigned to the two benzylic protons (denoted as *a* in Eq. (1)), whereas multiplets at δ = 6.5–6.9 ppm (denoted as *b*, *b’*, *c*, respectively in Eq. (1)) correspond to the aromatic protons of PPO resonances. Therefore, the ratios of the functionalized groups (m-%) can be calculated by comparing the average integral of the benzylic proton concentration with the total area of the aromatic protons according to the following equation:

\[
m\% = \frac{a}{b + b’ + c} \times 100, \ n\% = 100 - m\%.
\]

A similar calculation is also applied to determine the bromination molar ratio (m-%) for BPPO. Further structural verification was confirmed by the 13C NMR spectra, which disclose all the resonances for phosphonium- or ammonium-salt moieties, revealing the successful nucleophile attack. The thermal stability of the resulting polymers was examined by TGA. The results of the quaternization ratio and the corresponding thermal stability measurements are listed in Table 2. A representative thermogravimetric trace is shown in Fig. 4, which reveals that the thermal decomposition of phosphonium-modified BPPO occurs in one step, whereas the decomposition of ammonium-modified polymers occurs in

![Image](57x270 to 279x392)

![Image](47x55 to 289x236)

![Image](312x464)

![Image](312x454)

![Image](312x443)

![Image](312x433)

![Image](312x422)

![Image](312x412)

![Image](312x401)

![Image](312x391)

![Image](312x381)

![Image](312x370)

![Image](312x360)

![Image](312x350)

![Image](312x340)

![Image](312x330)

![Image](312x320)

![Image](312x310)

![Image](312x300)

![Image](312x290)

![Image](312x280)

![Image](312x270)

![Image](312x260)

![Image](312x250)

![Image](312x240)

![Image](312x230)

![Image](312x220)

![Image](312x210)

![Image](312x200)

![Image](312x190)

![Image](312x180)

![Image](312x170)

![Image](312x160)

![Image](312x150)

![Image](312x140)

![Image](312x130)

![Image](312x120)

![Image](312x110)

![Image](312x100)

![Image](312x90)

![Image](312x80)

![Image](312x70)

![Image](312x60)

![Image](312x50)

![Image](312x40)

![Image](312x30)

![Image](312x20)

![Image](312x10)

![Image](312x0)

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**Table 1**

<table>
<thead>
<tr>
<th>Polymer</th>
<th>m (%)</th>
<th>Mw (g/mol)</th>
<th>Mn (g/mol)</th>
<th>PDI</th>
<th>Tg (°C)</th>
<th>T5%d (°C)</th>
<th>Char (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPPO-1</td>
<td>46.4</td>
<td>73,200</td>
<td>24,300</td>
<td>3.01</td>
<td>208.5</td>
<td>287.1</td>
<td>42.6</td>
</tr>
<tr>
<td>BPPO-2</td>
<td>24.8</td>
<td>65,300</td>
<td>22,500</td>
<td>2.90</td>
<td>213.9</td>
<td>305.2</td>
<td>43.8</td>
</tr>
</tbody>
</table>

Fig. 1. Preparation of quaternized poly(phenylene oxide)s.

Fig. 2. GPC chromatographs of BPPO polymers.

Fig. 3. TGA thermograms of BPPO under nitrogen.

Nu: = N(CH2CH3)3, N[(CH2)3CH3]3, N[(CH2)2]3N, P[(CH2)3CH3]3, P(C6H5)3
of empty d orbitals in the phosphorus atom. It is inferred that the counteranions might be associated with empty d orbitals via π interactions that enhance the association of the complex, thereby resulting in higher thermal stability. According to the hard-acid soft-base (HASB) theory, the more polarizable phosphorus atom would tend to react better with the soft bromide counteranion than the nitrogen atom and would therefore provide a stronger soft–soft interaction [41]. Moreover, TEDA-PPO exhibited much higher thermal stability than the TEA and TBA analogs, which could be interpreted by the steric effects on acid–base formation; when alkyl chains are linked into bicyclic rings, as in TEDA, the adduct formation is more favorable because of the significant decrease in the steric hindrance (B-strain). This effect therefore leads to higher thermal stability than the corresponding TEA and TBA adducts [42]. No measurable transition for $T_g$ or $T_m$ was detected for any of the quaternized PPOs due to the presence of salt moieties.

### 3.2. Results of antibacterial assessment

Five types of polymers (TEA-PPO, TBA-PPO, Teda-PPO, PBu$_3$-PPO and PPh$_3$-PPO) were investigated for their antibacterial activity. All of the results are from independent triplicate measurements for each tested polymer. The chosen strains, *E. coli* and *S. Epidermidis*, are the strains usually encountered in nosocomial infections. The growth-inhibiting effect was quantitatively determined by the ratio of the surviving bacteria in the medium with the polymer to those without the polymer (control). The capability of the quaternized PPO polymers to inhibit the growth of the tested microorganisms on agar media is shown in Fig. 5 and Table 3. The growth inhibition varied according to the active groups on the polymer and the test bacteria. The assay of the bacteria inhibition measurement showed that only PPh$_3$-PPO-1 (7) was active towards both microorganisms, whereas TEDA-PPO, TBA-PPO, TEA-PPO and PBu$_3$-PPO derivatives were only active towards *S. epidermidis* (Gram-positive bacteria). This difference might be attributed to the enhanced hydrophobicity of the PPh$_3$ moiety and the structural

![Fig. 4. TGA thermograms of quaternized BPPO. (a) BPPO-1 derived polymers. (b) BPPO-2 derived polymers.](image)

![Fig. 5. Growth inhibition of quaternized PPO polymers.](image)

### Table 2

<table>
<thead>
<tr>
<th>Polymer BPPO $m$ (%)</th>
<th>Reagent type</th>
<th>Salt ratio (%)</th>
<th>Yield (%)</th>
<th>$T_{5%,d}$ (°C)</th>
<th>Char (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 46.4 TEA</td>
<td>37.7</td>
<td>83.2</td>
<td>173</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>2 24.8 TEA</td>
<td>19.0</td>
<td>69.6</td>
<td>175</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>3 46.4 TBA</td>
<td>43.0</td>
<td>64.6</td>
<td>156</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>4 24.8 TBA</td>
<td>21.4</td>
<td>78.4</td>
<td>163</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>5 46.4 TEDA</td>
<td>39.2</td>
<td>66.6</td>
<td>279</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>6 24.8 TEDA</td>
<td>21.4</td>
<td>85.9</td>
<td>276</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>7 46.4 PPh$_3$</td>
<td>45.5</td>
<td>87.2</td>
<td>298</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>8 24.8 PPh$_3$</td>
<td>23.4</td>
<td>97.6</td>
<td>299</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>9 46.4 PBu$_3$</td>
<td>40.2</td>
<td>58.0</td>
<td>329</td>
<td>25</td>
<td></td>
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<tr>
<td>10 24.8 PBu$_3$</td>
<td>23.4</td>
<td>91.2</td>
<td>321</td>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Molar contents of salt (%)</th>
<th>MIC of <em>S. Epidermidis</em> (mg/mL)</th>
<th>MIC of <em>E. Coli</em> (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPh$_3$-PPO-1</td>
<td>45.5</td>
<td>2.5</td>
<td>20</td>
</tr>
<tr>
<td>PPh$_3$-PPO-2</td>
<td>20.9</td>
<td>12.5</td>
<td>–</td>
</tr>
<tr>
<td>TEDA-PPO-1</td>
<td>39.2</td>
<td>2.5</td>
<td>–</td>
</tr>
<tr>
<td>TEDA-PPO-2</td>
<td>21.4</td>
<td>&gt;15</td>
<td>–</td>
</tr>
<tr>
<td>TEA-PPO-1</td>
<td>35.8</td>
<td>10</td>
<td>–</td>
</tr>
</tbody>
</table>

Multiple steps. Interestingly, the degree of functionalization had little effect on the thermal decomposition temperature ($T_{5\%,d}$) for each ion pair. Because the thermal stability is generally a function of bond energy, the small difference in $T_{5\%,d}$ between BPPO-1- and BPPO-2-derived analogs could be ascribed to the similar energy required for bond rupture of each ion pair. Furthermore, the thermal stability of phosphonium-modified BPPO was found to be much superior to that of the ammonium analog. This might be attributed to the larger phosphorus molecule, which increases the steric resistance around the phosphorus atom, thus retarding the decomposition reactions. Another explanation could be based on the presence
differences between Gram-positive and Gram-negative bacteria. It has been reported that polymers preferably interact with cytoplasmic membranes with increased hydrophobicity [9,16,43]. The polymers act by penetrating the outer cell wall and disrupting the cytoplasmic membrane, leading to the potentially lethal event. Furthermore, the polycationic biocides are generally less active against Gram-negative bacteria than Gram-positive bacteria due to the potential barriers of the more complicated cell wall and bilayer Structures on Gram-negative bacteria (E. coli). PPh3-PPO-1 is also the most effective polymer for the inhibition of both Gram-negative and Gram-positive bacteria. These findings are in accordance with the results from Kenawy's studies [20], which indicate that the triphenyl phosphine salt (PPh3) is a better antimicrobial agent than the corresponding TEA, TBA and PBu3 analogs. As shown in Fig. 5, the growth inhibition of polymers differs among the bacterial strains. The results show that the inhibition becomes stronger in the order E. coli < S. Epidermidis, and the inhibition effect was improved by increasing the ratio of the active groups in PPO polymers. Overall, the potency of bacteria inhibition varies according to the polymer active groups and the tested strains.

Table 3 shows the MIC values of the tested polymers with S. Epidermidis and E. coli. PPh3-PPO, TEDA-PPO and TEA-PPO exhibited an effective antimicrobial activity against S. Epidermidis with MIC values between 2.5 and 20 mg/mL but only PPh3-PPO had a MIC value of 20 mg/mL for E. coli. This result is expected because Gram-negative bacteria are known to be particularly resistant to cationic biocides and to quaternary ammonium compounds in particular [44]. In all cases, the antimicrobial efficiency was improved against S. Epidermidis by increasing the percentage of active groups on the PPO backbone regardless of the nucleophile. For example, the MIC of PPh3-PPO-1 against S. Epidermidis decreased from 12.5 to 2.5 mg/mL with an increase in the salt molar contents from 20.9% to 45.5%. Furthermore, the results obtained from the immersed medium experiment suggest that the antibacterial mechanism of quaternized PPO polymers operates through the direct contact between the polymer particles and the bacteria. PPh3-PPO and TEDA-PPO were immersed in nutrient broths for 24 h and then filtrated to analyze the inhibition effect of the released materials. Fig. 6 shows that the immersed broth media of PPh3-PPO or TEDA-PPO polymers had no effect on the bacteria inhibition. Moreover, we performed the inhibition zone measurement in this study (unpublished data). Generally, a clear ring around the polymer disc on an agar plate reveals the inhibition zone of bacteria growth, but most of the tested polymers revealed no bacteria inhibition zones due to poor water solubility or a slow dissolution rate. Because measuring the diameter of the bacteria inhibition zone gave poor and inaccurate results, the tested polymers were immersed in bacteria suspensions for 24 h and then transferred to an agar plate for a colony counting assay. After 24 h of incubation on the agar plate, the bacterial colonies were counted to measure the antibacterial activity. This procedure is the standard medical test to study the effect of antibiotics on bacteria. Thus, the antibacterial mechanism of the quaternized PPO polymers is considered to be the direct contact with functional groups. Similar studies have been performed on chitosan, which is a natural polycationic saccharide [45,46]. From the images of transmission electron microscopy, the inhibition mechanism of polycationic polymers against bacteria is proven by the adherence of polymers to the outer membrane of the bacteria and the disruption of the bacteria membrane. Due to the effective antimicrobial
activity, many quaternary ammonium and phosphonium salts, such as benzalkonium chloride, have demonstrated acute cytotoxic effects on human epidermal keratinocytes and foreskin fibroblasts [47]. As a cytotoxicity test, the polymer was immersed in a cell culture medium for 24 h, and the effect of the released materials on cell viability was analyzed by MTT assay. This procedure is called "extract dilution" and is one of the common methods for measuring biomaterial cytotoxicity. The cytotoxicity analysis of synthesized antimicrobial PPO polymers is shown in Fig. 7. For all the quaternized PPO polymers, no significant cytotoxicity was observed in polymer-immersed media. Interestingly, some of polymer-immersed media demonstrated an improvement in cell and E. Coli growth (Figs. 5 and 7), which may be explained by the change of the medium osmolarity [48]. Therefore, the residual toxicity of the released polymers or free ammonium and phosphonium salts can be minimized.

4. Conclusion

A new class of antimicrobial polymers was synthesized through the chemical modification of polyphenylene oxide to allow various extents of antimicrobial activity on polymers with good thermal stability. For biomedical applications, a highly efficient antimicrobial polymer with good thermal stability is of great importance for the creation of new biomedical materials. The quaternized PPh3-PPO polymer showed superior thermal stability as well as antibacterial activity against both types of bacteria, whereas the TEDA-PPO, TBA-PPO, TEA-PPO and PBu3-PPO derivatives were only active against S. Epidermidis. The thermal stability of phosphonium-modified BPPOs is much superior to that of their ammonium analogs, and the salt molar concentration exhibits little effect on the thermal decomposition temperature in a comparison of each pair of resultant polymers. Regardless of the nucleophiles, the increment of pendant functionality on the PPO backbone can improve the antimicrobial activity, and the inhibition effect is due to the direct contact with the polymers. To the best of our knowledge, the compounds synthesized in this study have not been reported in the literature and this is the first paper to evaluate their antimicrobial activity, cytotoxicity and thermal stability.

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References