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Short communication

Quaternary ammonium poly(diethylaminoethyl methacrylate) possessing antimicrobial activity

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ABSTRACT

Quaternary ammonium (QA) methacrylate monomers and polymers were synthesized from a *N*-alkylation of *N*,*N*-diethylaminoethyl methacrylate (DEAEM) monomer. Linear copolymers, and for the first time reported crosslinked nanoparticles (NPs), based QA-PDEAEM were prepared by radical polymerization of the quaternized QA-DEAEM monomers with either methyl methacrylate (MMA) or a divinyl monomer.

QA-PDEAEM NPs of 50–70 nm were embedded in polyethylene vinyl acetate coating. QA-polymers with N-C_8 and N-C_{18} alkyl chains and copolymers with methyl methacrylate were prepared at different molar ratios and examined for their antimicrobial effectiveness. These coatings exhibited strong antibacterial activity against four representative Gram-positive and Gram-negative bacteria.

1. Introduction

The growing global concern about emerging infectious diseases has greatly stimulated research on polymeric biocides [1–3]. Chemical biocides play an important role in the preservation of products as diverse as cutting fluids, crop protection agents, foods and beverages, fabrics, cosmetics, and medicines [4–6]. *N*,*N*-diethylaminoethyl methacrylate (DEAEM) is a common vinyl monomer that can be copolymerized with acrylamide, acrylic acid, vinyl-2-pyrrolidone (NVP), etc., to prepare polymers with specific functions, such as flocculants and surfactants in waste water [7,8]. Furthermore, DEAEM is widely used to modify the properties of bulk plastic materials by grafting or copolymerization [7]. Polymers containing quaternary ammonium (QA) groups in a side chain or in a main chain have a broad spectrum of antimicrobial activity, and they are effective against both Gram-positive and Gram negative bacteria as well as against viruses, fungi, and algae [1,9–12]. There have been, however, very few reports on the antimicrobial polymeric ammonium salts prepared from quaternary DEAEM monomer [7,13,14].

Cationic polymers were obtained by the quaternization of the polymer, which limits the quaternization yield due to steric and electrostatic hindrance [15–17]. This polymeric group exhibits diversify of positive activity, while modifications and quaternarization degree improvement are highly encouraged for enhancing antimicrobial properties [18–20].

In this study, QA acrylate monomers were prepared from the alkylation of DEAEM, then they were polymerized. Coatings, and for the first time reported nanoparticles (NPs), based QA-PDEAEM were prepared by homo and copolymerizaion of these QA acrylate monomers, and their antibacterial activities were evaluated against four representative bacterial strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Heterotrophic plate count* (*HPC*). The study outcome provide a wide range of manipulation for developing NPs and modified polymeric coatings based QA-PDEAEM for preventing accumulation of micro-organisms onto device surfaces as well as in bioadhesives and self sterilizing surfaces.

2. Materials and methods

2.1. Materials and microorganisms

N,*N*-diethylaminoethyl methacrylate (DEAEM), 2,2′-azobis(2-methylpropionitrile) (AIBN), divinylbenzene (DVB), ethylene glycol

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dimethacrylate (EDGMA), methyl methacrylate (MMA), iodooctane and octyldodecyl iodide were purchased from Sigma-Aldrich (Rehovot, Israel). Sodium bicarbonate, ethanol, chloroform, ethyl acetate and methanol (HPLC grade) were purchased from J.T. Baker, Holland. All solvents and reagents were of analytical grade. Polysorbate 20 (Tween 20) and sorbitan monooleate 60 (span 60) purchased from Dexcel Pharma (Jerusalem, Israel). Polyethylene vinyl acetate 28 (28% VA) and 46 (46% VA), polyethylene methacrylic acid were purchased from DuPont^{TM} Elvax®, Switzerland.

Bacterial strains and growth conditions: clinically isolated *E. coli* and *P. aeruginosa* (Maurice and Gabriela Goldschleger School of Dental Medicine at Tel-Aviv University, Israel). *S. aureus* ATCC 8639 were used in this study. Heterotrophic plate count bacteria-HPC (mixed bacteria from tap water). The bacteria were cultured aerobically overnight in 5 ml of brain–heart infusion (BHI) broth (Difco, Detroit, MI), at 37 °C.

2.2. Methods

2.2.1. Quaternary ammonium—Polydiethylaminoethyl methacrylate synthesis (QA-PDEAEM)

QA-PDEAEM polymer were prepared using two methods, differ mainly in their steps order; monomer polymerization to form PDEAEM followed by quaternary ammonium formation (QA-DEAEM) followed by vinyl radical polymerization to form QA-PDEAEM, Scheme 1. In the first route, polymerization of DEAEM was carried out at reflux for 24 h in ethanol (40 g monomers per 100 ml ethanol) with 20 mg (0.05% w/w) of recrystallized AIBN with nitrogen continuously bubbled through the reaction medium. The reaction was quenched in cold water and the PDEAEM precipitate was isolated by decantation and dried by lyophilization. Then, 10 g of the dried polymer (PDEAEM) was dissolved in ethanol (10 ml) and *N*-alkylation was conducted using iodooctane at 1:1.5 mole ratio (monomer to alkylation agent, 14.62 ml). Alkylation step was carried out under reflux for 24 h. Excess of $NaHCO_3$ (1.25 equimolar, 0.065 mol, 5.7 g) was added to neutralize HI released for 3 h under the same conditions. Formed NaI salt and excess of unreacted $NaHCO_3$ were discarded by decantation, washed with hexane and double-deionized water (DDW), and lyophilized to form smooth powder, QA-PDEAEM. In the second route, 10 g of DEAEM monomers were first reacted with iodooctane at 1:1.5 ratio for 24 h in ethanol for monomer quarter ammonium formation, QA-DEAEM, then the resulted QA-monomer was decanted by hexane to discard unreacted iodooctane and dried completely at 50 °C. QA-DEAEM monomer polymerization and purification was carried out similar to route I first step, QA-PDEAEM.

2.2.2. Quaternary ammonium—polydiethylaminoethyl methacrylate nanoparticles synthesis (QA-PDEAEM NPs)

Ten grams of QA-DEAEM monomers (prepared in route II, step I) were dissolved in 100 ml ethanol and 1% w/w crosslinker DVB or EDGMA was added and the reaction mixture was stirred for 10 min followed by adding 10 mg (0.1% w/w) of recrystallized AIBN with nitrogen continuously bubbled through the reaction medium under reflux conditions for 24 h with stirring at 1000 rpm, Scheme 1. The reaction mixture was quenched in cold water, and the precipitate was isolated by decantation, lyophilized and well grinded using mortar and pestle followed by multiple washings with 100 ml methanol:DDW 20:80 mixture and DDW to remove unreacted AIBN and QA-DEAEM monomers, respectively, using center flask filtration followed second lyophilization for overnight. The final resultant bulky material was ground to obtain a fine powder, QA-PDEAEM NPs.

2.2.3. QA-DEAEM monomers, QA-PDEAEM polymers and NPs analysis

IR spectra were recorded on a Perkin Elmer System 2000 FT-IR. Particles size measured using a Zetasizer 2000 (Malvern, UK), measurements were done in double-deionized water (DDW) in triplicate. Hydrophobicity (C/N) was estimated by elemental microanalysis of nitrogen (% N), carbon (% C) and iodine (% I) using a Perkin-Elmer 2400/II CHN analyzer. Zeta-potential of QA-PDEAEM polymers and particles were measured at 1% w/v in water (pH 5.5) in triplicate using Zetasizer 2000 (Malvern, UK). For QA-PDEAEM NPs SEM visualization: particles were first Au/Pd coated to thickness of about 10 nm using a sputtering deposition machine (Polarone E5100) and were imaged using High resolution scanning electron microscope (HR SEM) Sirion (FEI company) at constant acceleration voltage of 2 kV. Linear polymers were characterized for their average molecular weight by GPC water-system. Monomers and polymers structure were confirmed ^{1}H NMR spectra (D_2O or $CDCl_3$) were obtained on a Varian 300-MHz spectrometer in 5 mm o.d. tubes. $D_2O/CDCl_3$-containing tetramethylsilane served as solvent and shift reference. Degree of quaternary ammonium formation was determined by ^{1}H NMR and elemental analysis as follow: $((\text{Halide content}_{\text{Found}}/\text{Halide content}_{\text{Calculated}}) \times 100)$ [21].

2.2.4. Preparation of quaternary ammonium (QA) coatings

For crosslinked QA-PDEAEM NPs different polymer coatings were applied and evaluated using polyethylene vinyl acetate (PEVA, 28% and 46% VA, DuPont^{TM} Elvax®) and polyethylene methacrylic acid (PEMA, DuPont^{TM} Elvax®) in 24 wells plate, QA-PDEAEM NPs were suspended in polymer solutions at 1% w/v in chloroform or ethyl acetate. Stable homogenous suspension with minimal particle aggregates was obtained by mixing the NPs with Tween 20 and Span 60 at 1:1 and 1:0.3 w/w, respectively, followed by 10 min probe sonication with an amplitude of 60 Hz. QA-particles suspension were sprayed over, using spray painting machine with constant air pressure of 0.4 atm and 20 cm distance from the sprayed object. Particles suspension were mixed during spraying process to avoid precipitation. Coatings with 5, 15 and 30% w/w QA-particles were prepared, while the same method were repeated without QA-particles for control coatings. For non-crosslinked QA-PDEAEM, coatings were prepared in wells by fast solvent evaporation. QA-PDEAEM was dissolved in chloroform (10 mg/ml) and each well was filled with 0.2 ml of the polymer solution. The well plate was evaporated in the fume hood for 5 min to form a stable film of polymer.

2.2.5. Antimicrobial activity analysis

The materials were tested in 24 wells plate, for each well 1 ml of bacterial suspension inlet fresh prepared (Appendix I 2.2.5) was transferred into each polymeric coated well (duplicate for each type of coating) and into control's wells, free QA coatings. Plates were incubated at 35 °C temperature for 6 days in different conditions: static and dynamic modes, without/or with stirring, respectively, and then results were collected using Count of Bacterial Concentration Method (Appendix I 2.2.5).

3. Results and discussion

Quaternary ammonium poly(diethylaminoethyl methacrylate) (QA-PDEAEM) was prepared either by polymerization of DEAEM monomers to form PDEAEM followed by quaternaization (Scheme 1, Route I) or monomer quaternaization (QA-DEAEM), followed by vinyl radical polymerization to form QA-PDEAEM, (Scheme 1, Route II). With the first method, polymerization of DEAEM was done at reflux for 24 h in ethanol with 0.05% w/w AIBN as an initiator. The reaction mixture was quenched in cold water to precipitate the polymer having a molecular weight of 32,000 Da.

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