Materials Science and Engineering C 70 (2017) 788–795

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Simple and versatile method for creation of non-leaching antimicrobial surfaces based on cross-linked alkylated polyethyleneimine derivatives

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ARTICLE INFO

Article history:
Received 24 May 2016
Received in revised form 1 September 2016
Accepted 15 September 2016
Available online 16 September 2016

Keywords:
Antimicrobial polymer
Quaternized polyethyleneimine
Cross-linked polyethyleneimine
Structure-activity relationship
Staphylococcus aureus
Pseudomonas aeruginosa
Non-leaching coating

ABSTRACT

Novel quaternized polyethyleneimine and cross-linked polyethyleneimine derivatives have been synthesized using both traditional and microwave-assisted techniques to create antimicrobial coatings, with octyl, dodecyl, or hexadecyl bromides as alkylating agent and various bifunctional electrophiles as cross-linkers. Quaternization has been performed using methyl iodide or dimethyl sulfate; it has been shown that methyl iodide has no advantages over dimethyl sulfate. Antimicrobial activity of the polymers against Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) bacteria has been evaluated. Antimicrobial activity declines with increase in the alkylating agent chain length. Equimolar ratio of the alkylating agent and the primary amino groups in polyethyleneimine is optimal. Although cross-linking decreases the antimicrobial activity of quaternized polyethyleneimines, it improves their "non-leaching" properties (i.e. minimizes undesirable water washout of the polymeric coatings).

1. Introduction

Microbial infections are among most serious concerns in food packaging and storage, hospital and dental surgery equipment, medical devices, shoe industry, textiles, water purification systems, etc. [1–3]. Bacterial contamination of implantable medical devices (e.g. permanent catheters or implants) is a major problem in clinic [4].

Quaternary ammonium compounds (QACs) are amphoteric surfactants that are widely used for suppression of bacterial growth in clinical and industrial fields [5]. Due to the broad range of antimicrobial and surface activity [6], various QACs have been recently recognized among most popular hygienic tools for disinfection [7,8].

Mechanism of antimicrobial action of QACs is based on destruction of cytoplasmic lipid bilayers and outer membrane via the interaction of the positively charged quaternary nitrogen atom with the polar head groups of acidic phospholipids [9]. A number of polycations possessing antimicrobial properties including ion exchange fibers [10], cationic alkoxysilanes [11], soluble and insoluble pyridinium-type polymers [12,13], polyionenes [14], polymer surfaces derivatized with poly(*N*-vinylpyridinium) [15,16], immobilized *N*-alkylated polyethyleneimine [17–19], hybrid systems [20], and other [21] suppressing bacterial growth have been developed. Polymeric surfaces with incorporated quaternary ammonium salts have been widely used as well [22].

Branched polyethyleneimine (PEI) is a typical polyamine compound applied for preparation of various polycationic polymers with excellent antimicrobial properties. It contains primary, secondary, and tertiary reactive amino groups (25% of primary amines, 50% of secondary amines, and 25% of tertiary amines) which enables versatile chemical modifications affording PEI derivatives with desired physicochemical and biochemical properties. Alkylated and quaternized PEI derivatives have already attracted attention as efficient antimicrobial agents. In view of this, "non-leaching" bactericidal surfaces can be created via covalent or non-covalent surface immobilization of different polyethyleneimines. Such polycation-containing coatings have been found efficient against a variety of Gram-positive and Gram-negative bacteria [23–25]. However, existing approaches still need to be improved, since covalent immobilization technology is time-consuming and costly [26], and non-covalent immobilization does not afford "non-leaching" coating [27].

A number of studies have been focused on the correlation of certain structure parameters of QPEI such as counter-ion nature, degree of alkylation [23], and chemical structure of the alkyl moiety [18,19,23–28] with their antimicrobial properties. Yet the relation between structural

Abbreviations: CFU, colony forming unit; MW, microwave; PEG, polyethylene glycol; PEI, polyethyleneimine; QAC, quaternary ammonium compound; QPEI, quaternized polyethyleneimine; *S. aureus*, *Staphylococcus aureus*; *P. aeruginosa*, *Pseudomonas aeruginosa*.

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http://dx.doi.org/10.1016/j.msec.2016.09.033
0928-4931/

features of QPEIs and their antimicrobial properties has not been generalized so far. In particular, the reports on the influence of the alkylating agent molecular weight on antimicrobial activity of QPEI have been controversial [19,24,27,28].

This study aimed to elaborate a method to create a "non-leaching" coating via simple and low-cost non-covalent immobilization. In detail, we evaluated antimicrobial properties of the coatings as a function of alkylation and cross-linking degree; furthermore, the relationship between antimicrobial activity of the coating and chemical structure of the alkylating as well as cross-linking agent was investigated.

2. Materials and methods

2.1. Materials

Highly branched polyethyleneimine (PEI) with M_w 50–100 kDa (50% w/w aqueous solution) was purchased from MP Biomedicals (Santa Ana, USA). Octyl bromide, dodecyl bromide, hexadecyl bromide, 1,5-dibromopentane, methyl iodide, glutaraldehyde (50% w/w aqueous solution), and polyethylene glycol with M_w 400 (PEG400) were obtained from Sigma-Aldrich (St. Louis, USA). Dimethyl sulfate and chloroacetic acid were purchased from Acros (Geel, Belgium). Ethylene glycol, diethylene glycol, triethylene glycol, potassium iodide, and chloroform were purchased from Component-Reaktiv (Moscow, Russia). Potassium carbonate, sodium hydroxide and sodium bicarbonate were purchased from Labtekh (Moscow, Russia). Toluene and *n*-butyl alcohol were obtained from Baum-lux (Moscow, Russia). *p*-Toluenesulfonic acid was purchased from Chemical line (Saint Petersburg, Russia). Ethyl alcohol was purchased from Medkhimprom (Roshal, Russia). The above-listed chemicals were of analytical grade and were used as received unless otherwise stated.

Bacterial strains, *S. aureus* (ATTC 6538p) and *P. aeruginosa* (clinical isolate 395) were obtained from the collection of Gause Institute of New Antibiotics (Moscow, Russia).

2.2. Methods

2.2.1. Spectroscopy

^{1}H and ^{13}C NMR spectra were recorded using Bruker Avance 300 (300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C), Bruker Avance 400 (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C), and Bruker Avance 600 (600.15 MHz for ^{1}H and 150.91 MHz for ^{13}C) spectrometers. ^{1}H and ^{13}C chemical shifts (δ, ppm) were reported relative to the residual solvent signals. Electronic absorption spectra were recorded using an Evolution 220 UV–Vis spectrophotometer. FT-IR spectra (600–4000 cm^{-1}, 32 scans, ATR mode) were recorded using a Nicolet iS10 FTIR spectrometer (Thermo Fisher Scientific, Inc.). Spectra were recorded over the wavenumber range between.

2.2.2. Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) was conducted using a TG 209 F1 Perseus thermogravimetric analyzer (NETZSCH, Inc.). Specimens (5–10 mg) were heated up to 300 °C (20 K/min) under inert atmosphere (argon). Aluminum crucibles (25 μL, no. 6.239.2-91.7, NETZSCH, Inc.) were used.

2.2.3. Microwave synthesis

Alkylation and cross-linking stages were carried out using MARS 6 multimode reactor (CEM, Inc.). The reactions were performed in Pyrex vessels according to the following program: heating up to 120 °C for 15 min followed by isothermal segment at that temperature during 30 min. Maximum power was 500 W.

2.2.4. Elemental analysis

Elemental analysis was performed at the Laboratory of Elemental Analysis, Institute of Organoelement Compounds, Russian Academy of Sciences.

3. Experimental

3.1. Synthesis of quaternary PEI

3.1.1. QPEI alkylated with octyl bromide (**1a**, **1a′**, **1a″**, **1a***)

PEI (1.0 g, 0.023 mol of monomer units) was dissolved in 6 mL of anhydrous ethanol. Then octyl bromide was added in 1:1 (**1a**), 1:0.5 (**1a′**), or 1:2 (**1a″**) primary amine of PEI to alkyl bromide molar ratio. The *N*-alkylation step was carried out at 120 °C for 30 min in a microwave oven. Quaternization was performed using dimethyl sulfate (7.0 mL, 0.074 mol; 1:3 monomer units of PEI to dimethyl sulfate molar ratio) or methyl iodide for **1a*** (4.6 mL, 0.074 mol). The reaction was conducted under reflux during 24 h. Then an equivalent amount of sodium hydroxide (0.92 g, 0.023 mol) was added to neutralize methyl sulfate formed during the reaction, and the mixture was stirred at room temperature for 24 h. The solid product was filtered off and washed sequentially with water and dilute hydrochloric acid to remove residual sodium hydroxide. If the product was a paste or viscous liquid, it was centrifuged off at 3000 rpm for 10 min, washed with water, and diluted with hydrochloric acid to neutral pH. Yield: 40–60%.

Typical spectral data were as follows (exemplified with compound **1a**).

^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.83–1.03 (m, 3H); 1.18–1.54 (m, 10H); 1.58–1.96 (m, 2H); 2.25–4.61 (m, 20H).

^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 14.08; 15.25; 22.64; 29.02–29.71; 31.39–31.80; 32.04; 54.58; 63.44.

FTIR spectrum (ATR), ν, cm^{-1}: 2955, 2926, 2856, 1468 (C—H), 1245, 1218, 1060, 1019 (C—N).

Elemental analysis, %: C 53.66; H 10.55; N 10.45 (**1a**).

3.1.2. QPEI alkylated with dodecyl bromide (**2a**, **2a′**, **2a″**, **2a***)

The compounds were synthesized similarly using dodecyl bromide as the alkylating agent instead of octyl bromide, the primary amine of PEI to alkyl bromide molar ratio being 1:1 (**2a**), 1:0.5 (**2a′**), or 1:2 (**2a″**). Yield: 50–65%.

Typical spectral data were as follows (exemplified with compound **2a**).

^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.74–0.99 (m, 3H); 1.22–1.59 (m, 18H); 1.64–1.88 (m, 2H); 2.15–4.93 (m, 21H).

^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 14.13; 15.32; 22.70; 27.33–27.77; 29.39–30.35; 31.93; 53.76; 63.23.

FTIR spectrum (ATR), ν, cm^{-1}: 2923, 2852, 1467 (C—H), 1247, 1222, 1061, 1023 (C—N).

Elemental analysis, %: C 57.21; H 10.99; N 9.43 (**2a**).

3.1.3. QPEI alkylated with hexadecyl bromide (**3a**, **3a′**, **3a″**, **3a***)

The compounds were synthesized similarly using hexadecyl bromide as the alkylating agent instead of octyl bromide, the primary amine of PEI to alkyl bromide molar ratio being 1:1 (**3a**), 1:0.5 (**3a′**), or 1:2 (**3a″**). Yield: 75–80%.

Typical spectral data were as follows (exemplified with compound **3a**).

^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 0.84–1.00 (m, 3H); 1.12–1.63 (m, 26H); 1.61–1.88 (m, 2H); 2.11–4.50 (m, 18H).

^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 14.13; 15.34; 22.70; 27.44–27.66; 29.38–30.44; 31.95; 53.10; 63.54.

FTIR spectrum (ATR), ν, cm^{-1}: 2931, 2821, 1459 (C—H), 1300, 1195, 1049 (C—N).

Elemental analysis, %: C 60.12; H 11.34; N 8.59 (**3a**).