Antibacterial activity of gemini quaternary ammonium salts

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Abstract

A series of gemini quaternary ammonium salts (chlorides and bromides), with various hydrocarbon chain and spacer lengths, were tested. These compounds exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria and were not mutagenic. The strongest antibacterial effect was observed for TMPG-10 Cl (against Pseudomonas aeruginosa ATCC 27853) and TMPG-12 Br (against Staphylococcus aureus ATCC 6538 and Escherichia coli ATCC 11229 and clinical ESBL(+) isolate 434) surfactants. These compounds inhibited the adhesion of Staphylococcus epidermidis ATCC 35984 to a polystyrene surface and eradicated biofilm formed by P. aeruginosa PAO1. The activity of studied compounds was dependent on hydrocarbon chain length.

Introduction

Quaternary ammonium salts (QAS) are widely used in many fields of medicine and industry. Due to their surface-active properties, QAS are commonly applied in cleaning agents, paints, emulsifiers, and moisturizers. Quaternary ammonium salts are highly active against microbial membranes and are thus exploited as disinfectants, fungicides, and biocides (Maillard, 2002; Hegstad et al., 2010).

Gemini surfactants are a class of compounds which have two hydrophilic head groups and two hydrophobic moieties per molecule, separated by a covalently bonded spacer. These compounds have better wetting and surface-active properties, as well as biodegradability, compared to corresponding monomeric surfactants (Shuckla & Tyagi, 2006). It is known that gemini surfactants are able to form aggregates such as micelles or bilayers (Sakai et al., 2011). Such positively charged aggregates might enclose DNA molecules and enhance transfection efficiency more than monomeric surfactants. What is more, DNA carriers built from gemini surfactants are able to deliver genes of almost any size (McGregor et al., 2001; Rosenzweig et al., 2001; Pullmannova et al., 2012).

Gemini quaternary ammonium salts exhibit strong antimicrobial activity with a much broader spectrum than the monomeric QAS. Their exact mode of action is not yet fully understood; however, it has been shown that pyridinium-based gemini-QAS cause membrane perturbations, probably by creating channels for ion and ATP leakage (Sumitomo et al., 2004; Shirai et al., 2006).

Many microorganisms are able to form biofilm, often causing drug-resistant infections. Biofilms are multicellular communities held together by an extracellular matrix. Bacterial cells within the biofilm have an altered metabolism and enhanced resistance to antibacterial agents. Biofilms are extremely dangerous in a hospital environment because they form on medical devices such as catheters, dialysis machines, or prostheses (Høiby et al., 2011). The chronic infections associated with biofilms formed on medical devices are often caused by Pseudomonas aeruginosa, an opportunistic pathogen which is especially dangerous for patients with cystic fibrosis. Its structural characteristics (such as fimbriae or capsules) facilitate adhesion to the surface (Mikkelsen et al., 2011). Another species causing nosocomial infections is Staphylococcus epidermidis, a natural inhabitant of human skin and mucosal membranes, but a real threat in the case of
medical device contamination by a patient or medical personnel (Otto, 2009). Bacterial biofilms are also problematic in some fields of industry, contaminating cooling systems, causing corrosion and limiting mass and heat flow in water supply systems (de Carvalho, 2007; Liu et al., 2009; Moritz et al., 2010).

The common uses of disinfectants contribute to the development of antimicrobial resistance to these agents, and there is therefore a need to search for new surfactants which efficiently overcome pathogenic microorganisms. The aim of this work is to study the biological activity against pathogenic bacteria of the series of gemini quaternary ammonium salts having betaine-based ester-type alkyl chain arrangements. The tested bacterial strains (E. coli, P. aeruginosa, S. aureus, S. epidermidis) are inhabitants of human that commonly contaminate industrial as well as clinical environment and might be the cause of digestive, urinary, or respiratory system infections.

Materials and methods

Chemicals

The structure of the gemini quaternary ammonium salts (QAS), synthesized at the Department of Chemistry, Technical University of Wroclaw, Poland, as described (Obłażk et al., 2013), is shown in Fig. 1.

Strains

In the present study, we used the following reference strains: Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 11229, Pseudomonas aeruginosa ATCC 27853, Staphylococcus epidermidis ATCC 35984 and Pseudomonas aeruginosa PAO1, Saccharomyces cerevisiae Σ1278b, Salmonella Typhimurium TA98 and TA100 as well as clinical E. coli ESBL(+) 434 isolated from the urinary tract infection.

Fig. 1. Chemical structures of the investigated gemini quaternary ammonium salts, derivatives of N,N,N',N'-tetramethylethylenediamine (TMG-n Cl: N,N'-Bis[2-(n-alkyloxy)-2-oxoethyl]-N,N,N',N'-tetramethylethylenediammonium dichlorides or TMG-n Br: N,N'-Bis[2-(n-alkyloxy)-2-oxoethyl]-N,N,N',N'-tetramethylethylenediammonium dibromides) or N,N,N'-tetramethyl-1,3-propanediamine (TMG-n Cl: N,N'-Bis[2-(n-alkyloxy)-2-oxoethyl]-N,N,N'-tetramethyl-1,3-propanediammonium dichlorides or TMPG-n Br: N,N'-Bis[2-(n-alkyloxy)-2-oxoethyl]-N,N,N',N'-tetramethyl-1,3-propanediammonium dibromides).

Minimal inhibitory concentration (MIC)

The antimicrobial activity of gemini-QAS was tested against the reference bacterial strains: E. coli ATCC 11229, S. aureus ATCC 6538, P. aeruginosa ATCC 27853, and the clinical strain E. coli 434 ESBL(+). The values of the MIC were determined using the dilution of microarrays recommended by CLSI (NCCLS, M7-A5). Overnight bacterial cultures were suspended in physiologic salt solution (0.5 McF) and diluted 1000 times (10^5 CFU mL^-1). Bacterial suspensions (10 µL) were added into the wells of the microplate containing serial dilutions (1–800 µM) of tested compounds and filled with 100 µL of LB medium (1% yeast extract, 1% tryptone, 0.5% NaCl). Plates were incubated aerobically for 24 h at 37 °C. As a growth control, a suspension of bacteria in LB medium without the compounds was used, sterile control – LB medium without microorganisms, and the blank control (cut off) – a suspension of bacteria in physiologic salt solution. Dilution of each control and tested compounds was performed in duplicate. The MIC values were determined spectrophotometrically [optical density at λ = 550 nm was measured using Asys Hitachi 340, Driver version: 4.02 (Biogenet, Poland)].

Adhesion assay

For adhesion inhibition, 100 µL of tested gemini-QAS in various concentrations was added to the microplate, incubated for 2 h at 37 °C with agitation (3.33 Hz), and washed with distilled water. One hundred microlitre of bacterial (P. aeruginosa, S. epidermidis) suspension (OD 0.6) was added to the microplate, incubated for 2 h at 37 °C with agitation (3.33 Hz), and washed twice. One hundred microlitre of 0.1% crystal violet (CV) was added, and after 5 min, microplate was washed three times. The absorbed CV was dissolved in the mixture of 100% isopropanol, 50 mM HCl, and 1% SDS. Absorbance was measured at λ = 590 nm.
Biofilm disruption test

To investigate whether gemini-QAS are able to disrupt bacterial biofilm, 100 μL of *Pseudomonas aeruginosa* or *Staphylococcus epidermidis* culture (OD 0.6) was placed on microplate, incubated for 24 h at 37 °C with agitation (3.33 Hz), and washed twice. The given compound was added at various concentrations to the microplate, incubated for 2 h at 37 °C with agitation (3.33 Hz), and washed. One hundred microlitre of 0.1% CV was added, and after 5 min, the microplate was washed three times. The absorbed CV was dissolved in mixture of 100% isopropanol, 50 mM HCl, and 1% SDS. Absorbance was measured at λ = 590 nm.

Biofilm viability assay

For *P. aeruginosa* and *S. epidermidis* biofilm viability, FilmTracer LIVE/DEAD BacLight Biofilm Viability Kit (Invitrogen) was used. To investigate whether gemini-QAS affect biofilm viability, bacterial biofilms were grown in glass chamber slides for 24 h at 37 °C, washed with distilled water, and incubated with tested compounds (120 μM). The freshly made LIVE/DEAD fluorescent dye (by mixing equal amounts of SYTO9 and propidium iodide) was added, and slides were incubated for 30 min in dark at room temperature. The red fluorescence indicates binding of propidium iodide to DNA of killed cells, and the DNA of the alive cells is bound by SYTO9 (propidium iodide is actively effluxed by alive cells). Untreated biofilms constituted control. For microscopic observations, Olympus BX51 fluorescence microscope was used.

Ames’ test

*Salmonella typhimurium* TA98 and *Salmonella typhimurium* TA100 strains, deficient in histidine synthesis, were used according to Ames et al. (1975). The tested gemini surfactant at a given concentration and 100 μL of bacterial culture (10⁸ CFU mL⁻¹) were added to 2 mL of top agar and spread on the plate with minimal Davis medium. The mixture without tested compound was used as negative control. Plates were incubated for 2 days at 37 °C, and colonies were counted. As a positive control, cisplatin was used (5 μg per plate). The mutagenic ratio (MR), ratio of the number of revertants incubated with the tested gemini surfactant to the number of spontaneously appearing revertants (negative control), was calculated. MR equal to or higher than 2.0 determines the mutagenic potential of the tested compound. An MR lower than 1.7 indicates a lack of mutagenic activity, while MR values between 1.7 and 2.0 designate the compound as a potential mutagen. The test was repeated three times.

Hemolysis assay

For hemolytic activity, gemini-QAS were tested as described in Falkingham et al. (2012). 5 mL of sheep blood was centrifuged to obtain erythrocytes (699 g, 15 min), washed three times in PBS (pH 7.4), and resuspended in PBS. The compound was mixed at various concentrations with 100 μL of erythrocytes and incubated for 1.5 h at 37 °C. Absorbance was measured at λ = 540 nm. PBS and 1% SDS (respectively) were used as negative and positive controls. The test was repeated three times.

Statistical analysis

The antimicrobial activity determined by the MIC values of the tested gemini surfactants (chlorides and bromides) against bacteria was changed to a six-point ordinal scale with the degrees of antibacterial activity, where 1 point means very good activity of the compound (0.001–0.1 mM), 2 points – good (0.101–0.2 mM), 3 points – medium good (0.201–0.3 mM), 4 points – sufficient (0.301–0.5 mM), 5 points – poor (0.501–0.8 mM), 6 points – very poor (> 0.801 mM). Data for each assay were compared by the nonparametric test of *ANOVA/MANOVA*. The Kruskal–Wallis test was used to evaluate the antimicrobial activity of the gemini surfactants against Gram-positive and Gram-negative bacteria as well as for the comparison of the antimicrobial effects between tested compounds. Additionally, the Friedman *ANOVA* test was used to estimate the antiadherent activity of selected gemini quaternary ammonium salts: TMEG-n Cl (*n* = 8, 10, 12 and 14 carbon atoms), TMEG-12 Br, TMPG-10 Cl, and TMPG-12 Br against *S. epidermidis* ATCC 35984 and *P. aeruginosa* PAO1. *P*-values from these tests are shown in the Results section. *P*-values < 0.05 indicate that the compared values are statistically significantly different at a 95% confidence level.

Results

Minimal inhibitory concentration

To determine the antibacterial activity of gemini surfactants, MIC were evaluated. Gram-positive *S. aureus* strain was the most sensitive to gemini-QAS, whereas Gram-negative rods, especially *P. aeruginosa* and *E. coli* 434 ESBL (+), were more tolerant (*P* = 0.035).
Among gemini-QAS with a shorter spacer (TMEG-n), the strongest activity against Gram-positive bacteria (\textit{S. aureus}) was exhibited by bromide with 12 carbon atoms within hydrophobic chains (TMEG-12 Br; Table 1). Gram-negative strains (\textit{E. coli}, \textit{P. aeruginosa}) were more resistant to this compound, however, altering the counterion to chlorine (TMEG-12 Cl) caused an increase in their sensitivity. The antibacterial effect of the remaining compounds against Gram-positive strain was similar (MIC of 40 \textmu M). Moreover, it was shown that chloride with 14 carbon atoms within alkyl chains (TMEG-14 Cl) exhibited the strongest activity against \textit{E. coli}, and chloride with 10 carbon atoms within alkyl chains (TMEG-10 Cl) caused an increase in the activity against \textit{P. aeruginosa} (MIC = 40 \textmu M; Table 1).

Hydrocarbon chains and spacer lengths of gemini-QAS are significant for their antibacterial activity. Generally, compounds with longer spacer ([\textit{CH}_2]_n) showed a much higher antibacterial effect against tested strains ($P < 0.05$). TMPG-n ($n = 10, 12$) possess much stronger antibacterial properties ($P < 0.03$), compared to TMEG-n ($n = 6$ and $8$; Table 1).

The results also showed that bromide with 12 carbon atoms within hydrophobic chains and longer spacer (TMPG-12 Br) inhibited growth of both Gram-positive and Gram-negative bacteria (\textit{E. coli}) on similar level (MIC of 20 \textmu M; Table 1). On the other hand, the activity of chloride (TMEG-12 Cl) was weaker against \textit{S. aureus} and \textit{E. coli}, but stronger against \textit{P. aeruginosa} (MIC = 40 \textmu M). Shortening of aliphatic chains in chlorides (TMPG-10 Cl) caused an increase in the activity against \textit{S. aureus} and \textit{E. coli} (80 \textmu M) and the strongest inhibition of \textit{P. aeruginosa} growth (10 \textmu M).

### Hemolysis

For further investigation, the three most active compounds against tested microorganisms (TMEG-12 Br, TMPG-12 Br, and TMEG-14 Cl) were chosen, and their hemolytic properties were examined, using sheep erythrocytes.

Among TMEG-n surfactants, TMEG-12 Br was the one with the highest hemolytic action (for 40 \textmu M of this compound almost 100% of hemolysis was observed). The change of counterion to chlorine (TMEG-12 Cl) caused about a twofold drop in hemolytic properties.

The elongation of the hydrophobic chains (TMEG-14 Cl) weakened membrane-disruptive activity, as 40 \textmu M caused only about 20% of hemolysis. What is more, the compound with a longer spacer (TMPG-12 Br) showed decreased hemolytic properties in comparison with TMEG-12 Br. Shortening of alkyl chains caused the decrease in hemolytic activity of surfactants (data not shown).

### Mutagenicity

Mutagenic potential was carried out in Ames’ test. Studied gemini surfactants were not mutagenic at the concentration range 10–40 \textmu M, as the observed mutagenic ratio (MR) never reached the 1.7 value (Table 2).

### Adhesion inhibition

The investigation of microbial adhesion and biofilm formation was carried out on two strains: \textit{P. aeruginosa}.

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**Table 1. Minimal inhibitory concentration of gemini quaternary ammonium salts**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Minimal inhibitory concentration (MIC) of tested compounds [\textmu M] for bacterial strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>\textit{S. aureus}</td>
</tr>
<tr>
<td>TMEG-14 Cl</td>
<td>40</td>
</tr>
<tr>
<td>TMEG-12 Cl</td>
<td>40</td>
</tr>
<tr>
<td>TMEG-12 Br</td>
<td>20</td>
</tr>
<tr>
<td>TMEG-10 Cl</td>
<td>40</td>
</tr>
<tr>
<td>TMEG-8 Cl</td>
<td>40</td>
</tr>
<tr>
<td>TMEG-6 Cl</td>
<td>40</td>
</tr>
<tr>
<td>TMPG-12 Br</td>
<td>20</td>
</tr>
<tr>
<td>TMPG-12 Cl</td>
<td>120</td>
</tr>
<tr>
<td>TMPG-10 Cl</td>
<td>80</td>
</tr>
</tbody>
</table>

**Table 2. Mutagenicity of gemini surfactant measured in the number of revertants and mutagenic ratio (MR)**

<table>
<thead>
<tr>
<th>Tested compound [\textmu M]</th>
<th>TA98 Colony number</th>
<th>TA100 Colony number</th>
<th>MR*</th>
<th>TA98 Colony number</th>
<th>TA100 Colony number</th>
<th>MR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC†</td>
<td>&gt; 400</td>
<td>&gt; 400</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC‡</td>
<td>34 ± 7.8</td>
<td>241.5 ± 2.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMEG-14 Cl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>46</td>
<td>1.18</td>
<td>1.03</td>
<td>46</td>
<td>1.18</td>
<td>1.03</td>
</tr>
<tr>
<td>80</td>
<td>53</td>
<td>1.36</td>
<td>1.13</td>
<td>53</td>
<td>1.36</td>
<td>1.13</td>
</tr>
<tr>
<td>TMEG-12 Cl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>56 ± 8.5</td>
<td>1.6</td>
<td>1.05</td>
<td>56 ± 8.5</td>
<td>1.6</td>
<td>1.05</td>
</tr>
<tr>
<td>40</td>
<td>33 ± 2.8</td>
<td>0.97</td>
<td>0.81</td>
<td>33 ± 2.8</td>
<td>0.97</td>
<td>0.81</td>
</tr>
<tr>
<td>TMEG-12 Br</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>24.5 ± 0.7</td>
<td>0.72</td>
<td>0.79</td>
<td>24.5 ± 0.7</td>
<td>0.72</td>
<td>0.79</td>
</tr>
<tr>
<td>40</td>
<td>27 ± 2.8</td>
<td>0.79</td>
<td>0.4</td>
<td>27 ± 2.8</td>
<td>0.79</td>
<td>0.4</td>
</tr>
<tr>
<td>TMPG-12 Br</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>21</td>
<td>0.82</td>
<td>0.93</td>
<td>21</td>
<td>0.82</td>
<td>0.93</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>0.78</td>
<td>0.95</td>
<td>20</td>
<td>0.78</td>
<td>0.95</td>
</tr>
</tbody>
</table>

*Mutagenic ratio (MR) – see Ames’ test in section Materials and methods. The values are average from at least three tests ±SD.
†As positive control (PC), cisplatin (5 \textmu g per plate) was used.
‡As negative control (NC), bacterial dilution was added to top agar without tested compounds.
PAO1 and *S. epidermidis* ATCC 35984. *Staphylococcus aureus* strain used in MIC assays lacked the ability to adhere and create biofilm, thus it was replaced by *S. epidermidis* with a documented adhesion and biofilm production (Jabbouri & Sadovskaya, 2010). Preincubation of a polystyrene plate with studied gemini surfactants strongly inhibited the adhesion of *S. epidermidis* to the surface, and the antiadhesive activity of these compounds was observed in low concentrations. The antiadhesive effect was significantly weaker for *P. aeruginosa* (data not shown).

Among tested surfactants, chloride with 14 carbon atoms within hydrocarbon chains and a shorter spacer (TMEG-14 Cl) blocked the attachment of *S. epidermidis* cells most effectively, as more than 90% of adhesion inhibition was observed at the concentration of 100 μM (*P* < 0.001; Fig. 2a). The shortening of aliphatic chains correlated with weaker antiadhesive properties of the compounds (TMEG-12 Br, TMEG-12 Cl), but bromides showed stronger antibacterial activity than chlorides. Both these compounds exhibited very good antiadhesive activity at concentrations of < 60 μM for TMEG-12 Br (*P* < 0.001) and 80 μM for TMEG-12 Cl (*P* < 0.01; Fig. 2b and c). The elongation of the spacer (TMPG-12 Br) significantly increased the adhesion inhibition of bacterial cells because 40 μM of this compound reduced 60% of cell adhesion (*P* < 0.001; Fig. 2d).

Antiadhesive activity toward *P. aeruginosa* PAO1 was very weak. TMEG-14 Cl which most efficiently blocked *S. epidermidis* adhesion at 100 μM reduced only 30% of *P. aeruginosa* attachment (data not shown). This compound had better antiadhesive properties against nonfermenting rods than other tested gemini surfactants and already blocked the adhesion of *P. aeruginosa* at a concentration of < 140 μM (*P* < 0.02).

**Biofilm**

Gemini-QAS were tested regarding their ability to eradicate *S. epidermidis* and *P. aeruginosa* biofilm formed on a polystyrene surface. The results showed that TMEG-12 Cl and TMEG-12 Br strongly dislodged biofilm produced by *P. aeruginosa* PAO1, as 20 μM of these compounds destroyed about 50% of biofilm and higher concentrations (120–160 μM) eradicated biofilm almost completely (Fig. 3a and c). The activity against staphylococcal biofilm was much weaker (Fig. 3b and d) because only high concentrations (200–240 μM) eradicated 50% of biofilm (data not shown). TMEG-14 Cl and TMPG-12 Br, despite the strong inhibition of bacterial adhesion, did not eradicate biofilms formed by both tested strains (data not shown).

The fluorescent staining of biofilms treated with 120 μM of TMEG-12 Cl and TMEG-12 Br showed that both these compounds affect the viability of *S. epidermidis* biofilm, as the amount of killed cells (red fluorescence) predominated over alive ones (green fluorescence). The microscopic observations also showed the dislodging of *P. aeruginosa* biofilm by TMEG-12 Br, however, no significant effect was observed for TMEG-12 Cl (Fig. 4).

**Discussion**

Quaternary ammonium salts (QAS) are commonly used in medicine and industry as disinfectants, fungicides, and biocides (Hegstad et al., 2010). Microorganisms have developed QAS resistance, there is thus a great need to search for new antibacterial surfactants. Gemini quaternary ammonium salts showed greater antimicrobial activity compared to conventional monomeric QAS (Shirai et al., 2006).
Our results showed that studied gemini-QAS generally exhibit strong antibacterial activity dependent on the hydrophobic chain and spacer lengths as well as counterion (Cl\(^-\) or Br\(^-\)). The strongest activity against both Gram-positive (S. aureus) and Gram-negative (E. coli) bacteria was shown by TMPG-12 Br. A slightly weaker antibacterial effect was observed for TMPG-10 Cl, however, this compound showed the strongest activity against P. aeruginosa. The bacterial sensitivity to gemini quaternary ammonium salts is connected with cell envelope structure. Guérin-Méchin et al. (2004) showed that P. aeruginosa spheroplasts were more sensitive to QAS in comparison with whole cells, which suggests that the outer membrane plays an important role in QAS resistance. Characteristic properties of outer membrane (such as numerous porins limiting the influx of hydrophobic compounds or LPS molecules that stiffen the outer membrane) prevent penetration of surfactants and their interactions with cell components. Resistance of Gram-negative bacteria to various compounds (such as biocides or surfactants) is also mediated by the additional envelope structures. Another common mechanism of QAS resistance, both in Gram-positive and Gram-negative bacteria, is the active export of these compounds by efflux systems (e.g. MexAB system in P. aeruginosa, Qac proteins in S. aureus or AcrAB system in E. coli; Russell, 1997; McDonnell & Russell, 1999; Poole, 2002).

Many bacterial species are able to form biofilms, multicellular communities, where bacterial cells are surrounded by a self-produced polymeric matrix, holding together biofilm structure and protecting it from drug penetration. Pseudomonas aeruginosa is an opportunistic pathogen, strongly connected with nosocomial infections. Biofilms formed by this species are a cause of chronic, drug-resistant infections (cystic fibrosis, burn wounds), as well as catheter- and ventilator-associated infections (Mikkelsen et al., 2011). Another species known as a strong biofilm producer is Staphylococcus epidermidis. It is a natural inhabitant of human epiderma; however, it can be a cause of medical device (catheter, prostheses) contamination where it can form biofilms hard in eradication (Otto, 2009). The adhesion of bacterial cells to a biotic or abiotic surface is the first stage of biofilm development, mediated by biological (cell envelope structure) and physicochemical (cell surface charge and hydrophobicity) factors. Conditioning of the surface (e.g. plastic or silicone) with antibacterial and anti-adhesive compounds (such as yttrium fluoride or silver nanoparticles) inhibits biofilm production (Lellouche et al., 2012; Marsich et al., 2013).
There is not much information about biofilm inhibition by quaternary ammonium salts. It was shown that loading of bone cement or titanium surface with quaternized chitosan significantly reduced staphylococcal biofilm formation (Peng et al., 2011; Tan et al., 2012). Similarly, the modification of silica nanoparticle surfaces with 3-(trimethoxysilyl)-propyldimethyl-octadecylammonium chloride strongly inhibited S. aureus adhesion (Song et al., 2011).

Our results showed that studied gemini surfactants inhibited the adhesion of S. epidermidis to the polystyrene surface, and this activity depends on the alkyl chain and spacer lengths. The strongest reduction in adhesion was observed for the compound with the longest hydrocarbon chains (TMEG-14 Cl), probably due to its hydrophobic nature. This might suggest that these compounds coat the polystyrene plate via hydrophobic interactions, however, whether the strength of the coating depends on the chemical nature of the compound needs to be verified by physicochemical studies. The cell surface of S. epidermidis is highly hydrophobic because of the presence of surface proteins such as Bap/Bhp, which mediate the adhesion to abiotic surfaces by hydrophobic interactions (Tormo et al., 2005). Thus, the exposition of hydrophilic moieties of gemini-QAS might cause the blockage of cell attachment to polystyrene. On the other hand, there was no antiadhesive effect of tested gemini surfactants against P. aeruginosa PAO1 cells. The cell surface of this strain is rather hydrophilic and negatively charged due to the presence of B form of O antigen in LPS structure (smooth LPS; Makin & Beveridge, 1996; Abu-Lail et al., 2007), which might explain the lack of adhesion blockade. Similar results, with P. aeruginosa ATCC 10145, were obtained for commonly used QAS – benzalkonium chloride. Coating the surface with this compound did not inhibit, and even promoted, biofilm formation by P. aeruginosa (Machado et al., 2011).

Bacterial biofilms are much more resistant to drugs than planktonic cells. This resistance is mediated by many factors, for example, the limitation of drug penetration by an extracellular matrix and changes in metabolism activity (Römling & Balsalobre, 2012). We investigated the ability of gemini-QAS to dislodge P. aeruginosa and S. epidermidis biofilms. The results showed that low concentrations of TMEG-12 Br and TMEG-12 Cl effectively eradicated P. aeruginosa PAO1 biofilm. The strong activity of TMEG-12 Br against P. aeruginosa biofilm was confirmed in fluorescence microscopy; however, this compound showed no effect against planktonic cells (MIC above 800 µM). These findings might point to specific interactions of gemini-QAS with biofilm structure dependent, for example, on the counterion. The dislodging activity of gemini-QAS against S. epidermidis biofilm was much weaker, but staining with LIVE/DEAD fluorescent dye showed high proportion of killed cells within the remaining biofilm. The differences in antibiofilm activity against these two species might be due to the distinct composition of the extracellular matrix surrounding biofilm. Glucose and mannose polysaccharides, as well as alginate, are dominant in P. aeruginosa biofilms, whereas an extracellular matrix of S. epidermidis biofilm contains mainly glucosamine polymers and teichoic acids, which contributes to the dissimilar elastic and viscous properties of these two species’ biofilms (Russell, 1997; Harmsen et al., 2010).

The mechanisms of gemini quaternary ammonium action is not fully understood; however, it is suggested that they influence the plasma membrane structure (Sumitomo et al., 2004). The hemolysis tests showed that gemini-QAS exhibit erythrocyte membrane-disruptive properties, and this activity depends on alkyl chain and spacer lengths as well as the counterion. The strongest hemolytic effect was observed for bromide with 12 carbon atoms within the hydrophobic chain and elongation or shortening of hydrocarbon chains caused a significant decrease in hemolytic activity. Similar observations were made for another group of cationic gemini surfactants, where the compounds with 12 carbon atoms in the chains also showed the strongest hemolytic action (Luczyński et al., 2013).

The results of our in vitro studies show that the gemini-QAS have a good antimicrobial activity against Gram-positive staphylococci and Gram-negative rods. They inhibit the adhesion of microorganisms to the surface and destroy their biofilms. After further studies, the gemini-QAS may have potential use in the future, in the control of microbial adhesion and colonization, important phenomenon in both medicine and industry.

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References