The Influence of Biodegradable Gemini Surfactants, 
N,N’-bis(1-Decyloxy-1-Oxopronan-2-yl)-N,N,N’,N’-Tetramethylpropane-1,3-Diammonium Dibromide 
and N,N’-bis(1-Dodecyloxy-1-Oxopronan-2-yl)-N,N,N’,N’-Tetramethylethane-1,2-Diammonium Dibromide, on Fungal Biofilm and Adhesion

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Abstract: A group of biodegradable alanine-derived Gemini quaternary ammonium salts (bromides and chlorides) with various alkyl chains and spacer lengths was tested for anti-adhesive and anti-biofilm activity. The strongest antifungal activity was exhibited by bromides with 10 and 12 carbon atoms within hydrophobic chains (N,N’-bis(1-decyloxy-1-oxopronan-2-yl)-N,N,N’,N’-tetramethylpropane-1,3-diammonium dibromide and N,N’-bis(1-dodecyloxy-1-oxopronan-2-yl)-N,N,N’,N’-tetramethylethane-1,2-diammonium dibromide). It was also demonstrated that these Gemini surfactants enhanced the sensitivity of Candida albicans to azoles (itraconazole and fluconazole) and polyenes (amphotericin B and nystatine). Gemini quaternary ammonium salts effectively inhibited fungal cell adhesion to polystyrene and silicone surface. These compounds reduced C. albicans filamentation and eradicated C. albicans and Rhodotorula mucilaginosa biofilms, as it was shown in crystal violet and fluorescent staining. None of the tested compounds were cytotoxic against yeast mitochondrial metabolism.

Key words: Gemini quaternary ammonium salts, Candida albicans, Rhodotorula mucilaginosa, biofilm, adhesion

1 INTRODUCTION

Gemini surfactants are amphiphilic compounds, consisting of two monomeric surfactants linked by a spacer. Most of Gemini surfactants have symmetrical structure and possess two identical hydrophobic chains and two identical polar head groups. When compared to monomeric surfactants, Gemini ones generally exhibit lower critical micelle concentrations, higher surface activity and better foaming properties¹. Gemini surfactants are widely studied regarding formation of vesicles or liposomes. Such structures, when are additionally positively charged, might create complexes with DNA and be applied as non-viral gene delivery systems². Quaternary ammonium salts (QAS) are commonly used in many fields of medicine and industry, e.g., as disinfectants, biocides or preservatives³. Their antimicrobial activity manifests with the disruption of cell wall and plasma membrane resulting in cell lysis⁴. Gemini-QAS generally show stronger antimicrobial activity, in comparison with corresponding monomeric QAS, however their mode of action is not fully understood. It was shown that commercially available cationic Gemini surfactant (3DOBP-4,10) causes the leakage of cytoplasmic components (ATP, Mg²⁺ and K⁺), inhibits respiration and disrupts cell membranes but without observed cell lysis⁵.⁷. Fungal infections have become a large problem with regard to developing mechanisms of tolerance to commonly used drugs, like active efflux of drug molecules by ABC

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http://www.jstage.jst.go.jp/browse/jos/ http://mc.manuscriptcentral.com/jjocs
transporters\textsuperscript{8).} The solution might be finding new antifungal agents that would overcome drug resistance. Candida albicans is frequently isolated fungal pathogen, causing superficial and systemic infections. Colonization is often associated with the ability of this species to switch from yeast to filamentous form and production of biofilm, which is a multicellular structure, adhered to biotic or abiotic surface, where cells are surrounded by extracellular self-produced polymeric matrix. Generally biofilms exhibit elevated drug resistance, due to e.g., the reduction of drug penetration and alterations in cell metabolism\textsuperscript{9,10).} Gemini quaternary ammonium salts having betaine based ester type alky chain arrangements show strong antifungal activity against both planktonic and biofilm fungal forms\textsuperscript{11).}

In this work we describe the biological activity of the series of biodegradable gemini quaternary ammonium salts, molecular mimics of alanine derivatives with various hydrophobic chain and spacer lengths - their anti-adhesive and anti-biofilm effect on C. albicans and Rhodotorula mucilaginosa and cytotoxicity against yeast mitochondrial metabolism.

2 EXPERIMENTAL

2.1 Gemini quaternary ammonium salts

The studied Gemini quaternary ammonium salts, with cleavable ester bonds, were synthesized at the Wrocław University of Technology according to method described by Luczyński et al.\textsuperscript{12).} The structures of compounds were determined by means of spectral analysis: 1H NMR, 13C NMR, IR. NMR spectra were recorded with a Bruker Avance DRX-500 spectrometer in CDCl\textsubscript{3} solutions. IR spectra were measured with a Mattson PTR-300 Thermo Nicolet spectrometer.

The spectral data of TMEAL-12 Br are as follow: 1H NMR (500 MHz, CDCl\textsubscript{3}): \(\sigma (ppm): 0.85 [\text{H}, t, 2 (-\text{CH}_3)]; 1.24 -1.48 [28H, m, 2 (-\text{CH}_2-\text{C}(\text{CH}_3)-\text{C}(\text{CH}_3)]; 1.61 -1.65 [\text{H}, m, 2 (-\text{O}-\text{CH}_2-\text{CH}_2)]; 1.78 -1.79 [6\text{H}, t, 2 (-\text{CH}(\text{CH}_3)]; 2.68 -2.89 [2\text{H}, m, \text{N}(\text{CH}_2-\text{CH}_2-\text{CH}_2-N)]; 3.51 [12\text{H}, s, 2 (-\text{N}(-\text{CH}_3)); 3.91 [4\text{H}, t, 2 (-\text{N}-\text{CH}_2-\text{CH}_2-\text{CH}_2-N)]; 4.15 [4\text{H}, s, 2 (-\text{N}-\text{CH}_2-\text{COO}-)]; 4.13 -4.25 [4\text{H}, m, 2 (-\text{O}-\text{CH}_2)]; 4.58 -4.67 [2\text{H}, m 2 (-\text{N}-\text{CH}(\text{CH}_3)); 5.95 [4\text{H}, m, 2 (-\text{O}-\text{CH}_2)]; 7.52 [2\text{H}, s, 2 (-\text{N}-\text{CH}(\text{CH}_3)); 15.13 [\text{C} = \text{O}]]

IR (NaCl, cm\textsuperscript{-1}): 2460 (m), 1792 (s) 1224 (m), 1024 (s)

The structure of the tested compounds is shown in Fig. 1.

2.2 Strains

In the present study the following fungal strains were used: Candida albicans ATCC 90028, Rhodotorula mucilaginosa IHEM 18459 and Saccharomyces cerevisiae \(\Sigma 1278\).

2.3 Minimal inhibitory concentration (MIC)

To establish the antifungal activity of the tested compounds we determined the minimal inhibitory concentration (MIC) on 96-well polystyrene microtiter plates.

\[\text{Fig. 1 Structure of tested Gemini quaternary ammonium, derivatives of N,N',N'-tetramethylethylendiamine: TMEAL-n Br (N,N',bis(1-(n-alkyloxy)-1-ox propane-2-yl)-N,N',N'-tetramethylethylene-1,2-diammonium dibromide); TMEAL-n Cl (N,N',bis(1-(n-alkyloxy)-1-ox propane-2-yl)-N,N',N'-tetramethylethylene-1,2-diammonium dichloride) or N,N,N',N'-tetramethyl-1,3-propanediamine: TMPAL-n Br N,N',bis(1-(n-alkyloxy)-1-ox propane-2-yl)-N,N',N'-tetramethylpropane-1,3-diammonium dibromide; TMPAL-n Cl (N,N',bis(1-(n-alkyloxy)-1-ox propane-2-yl)-N,N',N'-tetramethylpropane-1,3-diammonium dichloride).}\]
Fungal strains were incubated with gemini-QAS in a range of concentrations 10-1200 μM for 48 h at 28°C in YPG medium (1% yeast extract Difco, 1% peptone Difco, 2% glucose) and optical density was measured using a microplate reader at A590nm (ASYS UVM 340 Biogenet). As growth controls strains incubated without cationic surfactants were used.

2.4 Cytotoxicity assay
For cytotoxicity against mitochondrial enzymes measurements alamarBlue Assay (Sigma) was used as described before. S. cerevisiae Σ1278b (final yeast absorbance at λ600 equaled 0.06) was incubated for 12 h with tested compounds (at concentrations of ¼ and ½ MIC) in YPG medium at 28°C. AlamarBlue was added at 10% of the well volume. Plates were incubated for 4 h, at 28°C in the dark. Reduction of resazurin to resorufin by mitochondrial enzymes (a change from blue to pink color) was observed. The experiment was repeated three times.

2.5 The influence of gemini quaternary surfactants on C. albicans susceptibility to azoles and polyenes
To determine the influence of the tested gemini-QAS on C. albicans susceptibility to antifungals (azoles and polyenes), C. albicans was diluted in YPG on microtiter plates to obtain optical density 0.08. Gemini cationic surfactants were applied at final concentrations of ¼ and ½ MIC and itraconazole and fluconazole at final concentrations of 7 μM and 490 μM, respectively were applied. From the polyene group 0.3 μM amphotericin B and 0.43 μM nystatin were used. Compounds were added separately or in combinations: gemini surfactant-azole or gemini surfactant-polyene. C. albicans incubated without the addition of any compound was used as growth control. Cells were incubated at 28°C for 24 h and optical density was measured using a microplate reader at A590nm (ASYS UVM 340 Biogenet). Test was repeated three times.

2.6 Adhesion assay
To investigate whether the gemini-QAS reduce fungal adhesion to the polystyrene surface, 96-well microtiter plates were incubated with tested surfactants (20-160 μM) for 2 h at 37°C with agitation (200 rpm). Plates were then washed with distilled water and 100 μL of C. albicans or R. mucilaginosa suspension (optical density 0.6) was added to the wells. After 2 h incubation at 37°C with agitation (200 rpm) plates were washed twice and 100 μL of 0.1% crystal violet (CV) was added to each well. After 5 min of staining plates were washed three times and adsorbed CV was dissolved with the mixture of 100% 2-propanol, 1% SDS and 50 mM HCl.

Additionally, the inhibition of C. albicans adhesion to silicone catheters was investigated. 2 cm catheter fragments were placed in test tubes and incubated with 3 mL of tested compound (80 and 160 μM) for 2 h at 37°C with agitation (200 rpm). Catheters were then washed and 3 mL of fungal suspension in YPG medium (optical density 0.06) was added and incubated for 2 h at 37°C with agitation (200 rpm). Catheters were washed twice and stained with 0.1% CV as described above. Absorbance was measured using a microplate reader at A590nm (ASYS UVM 340 Biogenet). All experiments were performed in triplicate.

2.7 Biofilm assay
For biofilm reduction test, CV staining was used as described before. C. albicans and R. mucilaginosa biofilms were grown on 96-well microtiter plates for 24 h at 37°C with agitation (200 rpm). Non-adhered cells were removed by washing and biofilms were incubated with gemini-QAS (20-160 μM). Plates were then washed twice and stained with 0.1% CV as described above.

Additionally, the reduction of C. albicans biofilm formed on silicone catheters was tested as follows. Fragments of catheters (2 cm) were placed in test tubes and 3 mL of fungal suspension in YPG medium (optical density 0.06) was added. Biofilms were grown for 24 h at 37°C with agitation (200 rpm). Catheters were washed twice, 3 mL of tested surfactants was added at final concentrations 80 and 160 μM and tubes were incubated for 2 h at 37°C with agitation (200 rpm). Next, the catheters were stained with 0.1% CV as described above. Absorbance was measured using a microplate reader at A590nm (ASYS UVM 340 Biogenet). All tests were performed three times.

2.8 Filamentous growth
To investigate if gemini surfactants influenced C. albicans filamentation, cell morphology was observed after 6 h and 24 h of incubation at 37°C in YPG medium with a final surfactant concentration of ¼ MIC. An AXIO Imager A2 (ZEISS) microscope was used for observations.

2.9 Fluorescence microscopy
For the visualization of the influence of gemini-QAS on C. albicans adhesion and biofilm, Filmtracer LIVE/DEAD BacLight Biofilm Viability Kit (Invitrogen) was used. Although this kit is generally applied for bacterial biofilms, it is also suitable for fungal biofilms. For the adhesion assay, 4-well glass Chamber Slides were preincubated with tested compounds (160 μM) for 2 h at 37°C with agitation (200 rpm). After washing they were incubated with C. albicans suspension (optical density 0.6) for 2 h at 37°C with agitation (200 rpm) and washed again two times. For biofilm assay, C. albicans biofilms were grown in 4-well glass Chamber Slides for 24 h at 37°C with agitation (200 rpm), washed twice and incubated with tested compounds (160 μM) for additional 2 h at 37°C. Freshly prepared LIVE/DEAD fluorescent dye (200 μL) (a mixture of equal volumes of SYTO9 and propidium iodide
according to Invitrogen Film Tracer LIVE/DEAD biofilm viability kit was added to each well and slides were incubated for 30 min in dark at room temperature. For microscopic observations Olympus BX51 fluorescence microscope was used.

3 RESULTS

3.1 Determination of minimal inhibitory concentration (MIC)

The series of gemini quaternary ammonium salts (bromides and chlorides) included surfactants with various alkyl chains and spacer lengths. Among the bromides with a shorter spacer, the compound with 12 carbon atoms within hydrocarbon chains (TMEAL-12 Br) appeared to be the most active, since its minimal inhibitory concentration against C. albicans and S. cerevisiae equaled 80 μM, while R. mucilaginosa cells were more sensitive to this compound (MIC of 40 μM) (Table 1).

The change of counterion in TMEAL-12 Br to chlorine caused some significant diminished activity against fungal strains, since C. albicans and S. cerevisiae showed a higher tolerance to this compound (MIC of 800 μM and 500 μM, respectively). However, we observed an increased sensitivity of R. mucilaginosa cells (MIC of 10 μM). Elongation and shortening of alkyl chains in this group of compounds caused a decrease in their antifungal activity (Table 1).

The compound with the strongest activity against fungal cells, among all of the tested gemini surfactants, was bromide with 10 carbon atoms within alkyl chains and longer spacer (TMPAL-10 Br). MIC against pathogenic fungi C. albicans and R. mucilaginosa equaled 80 μM and 10 μM, respectively, whereas MIC against non-pathogenic S. cerevisiae was 40 μM. Elongation of alkyl chain lengths in both chlorides and bromides (TMPAL-12 Cl, TMPAL-12 Br) caused a strong decrease in activity against C. albicans and S. cerevisiae (MIC of 500 μM and 800 μM, respectively). The antifungal effect (at a level comparable with TMPAL-10 Br) was observed only against R. mucilaginosa (Table 1).

The compounds with the strongest antifungal activity (TMPAL-10 Br and TMEAL-12 Br) were investigated further.

3.2 Cytotoxic activity of gemini surfactants against yeast mitochondria

The cytotoxicity against yeast mitochondrial metabolism of TMPAL-10 Br and TMEAL-12 Br was tested with alamarBlue assay. The solution of resazurin (alamarBlue) was reduced by yeast mitochondrial enzymes, which manifested in the color change (from blue to pink). The observations of the alamarBlue reduction by cells incubated with given compound (at ¼ and ½ MIC) showed no differences between treated and control cells.

3.3 Influence of gemini cationic surfactants on C. albicans sensitivity to azoles and polyenes

The tested surfactants, TMEAL-12 Br and TMPAL-10 Br, as well asazole compounds (itraconazole and fluconazole) applied separately at ½ MIC inhibited the growth of C. albicans at similar levels (about 40%). The combination of surfactants and azoles significantly decreased the growth of C. albicans. The joint application of TMPAL-10 Br and fluconazole or itraconazole was slightly more effective in comparison to TMEAL-12 Br, with about 80% of growth inhibition observed (Fig. 2c). The combination of TMEAL-12 Br and azoles inhibited the growth of C. albicans by about 70 and 60% (for itraconazole and fluconazole, re-

<table>
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<th>Compounds</th>
<th>Minimal inhibitory concentrations [μM] of gemini quaternary surfactants for tested strains</th>
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<td></td>
<td>Saccharomyces cerevisiae</td>
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<tr>
<td>TMEAL-6 Br</td>
<td>&gt;1200</td>
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<td>TMEAL-8 Br</td>
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<td>TMEAL-10 Br</td>
<td>320</td>
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<tr>
<td>TMEAL-12 Br</td>
<td>80</td>
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<td>TMEAL-12 Cl</td>
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<td>TMEAL-14 Br</td>
<td>240</td>
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<td>TMPAL-10 Br</td>
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<tr>
<td>TMPAL-12 Br</td>
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<td>TMPAL-12 Cl</td>
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The second tested class of antifungal drugs was polyenes (nystatin and amphotericin B), which when applied solely, inhibited the growth of *C. albicans* by 60 and 40%, respectively. The combination of polyenes and cationic surfactants was much more effective, especially for TMPAL-10 Br, which in the presence of nystatin reduced growth almost completely, whereas TMEAL-12 Br inhibited growth by about 80%. In the presence of amphotericin B, the growth inhibition by more than 70% was observed for both studied gemini-QAS (Fig. 2b and 2d).

### 3.4 The reduction of fungal adhesion and biofilm – CV staining

The deposition of gemini-QAS on the polystyrene plate inhibited the adhesion of *R. mucilaginosa* cells, however the antiadhesive effect was significantly stronger in the case of TMPAL-10 Br, as 50% inhibition of adhesion was observed at much lower concentrations (40 μM) in comparison to TMEAL-12 Br (Fig. 3c). The inhibition of *C. albicans* adhesion to polystyrene surface was also observed for both tested gemini surfactants, however 50% of the reduction was noted only at higher concentrations (160 μM) (Fig. 3a).

*R. mucilaginosa* biofilm disruption was efficient for the two tested gemini-QAS, with slightly stronger effect in the case of TMEAL-12 Br (Fig. 3d). TMEAL-12 Br was also more effective against *C. albicans* biofilm formed on a polystyrene plate, since 160 μM of this compound reduced biofilm by about 80% whereas for 160 μM TMPAL-10 Br only 50% of biofilm reduction was observed (Fig. 3b).

The influence of gemini-QAS on *C. albicans* adhesion to silicone catheter was investigated with CV staining and the results showed that both tested surfactants (TMEAL-12 Br and TMPAL-10 Br) exhibited anti-adhesive properties to some extent. Slightly stronger activity against cell adhesion was shown by TMEAL-12 Br (Fig. 4a). The reduction of already formed biofilm was exhibited only by TMEAL-12 Br, which at the concentration of 80 μM dislodged about 60% of *C. albicans* biofilm. The 2-fold increase of the concentration of this compound caused 80% of biofilm reduction (Fig. 4b).

### 3.5 Effect of gemini surfactants on *C. albicans* filamentous growth

The ability to switch to a filamentous form of growth is one of the factors facilitating the adhesion of *C. albicans* cells. The studied gemini surfactants inhibited adhesion at a preadhesive level (by coating the polystyrene plate), thus we investigated the influence of these compounds on filament formation by *C. albicans* cells. It was shown that TMPAL-10 Br at the concentration of 20 μM had already
completely inhibited filamentation after 6 h of incubation, whereas 20 μM of TMEAL-12 Br reduced this process, but even after 24 h singular filamentous forms were observed (Fig. 5).

3.6 Influence of gemini-QAS on \textit{C. albicans} adhered cells and biofilm viability – fluorescence microscopy

The influence gemini surfactants on \textit{C. albicans} adhesion and biofilm was tested with LIVE/DEAD viability kit, which comprises two DNA-binding fluorescent dyes SYTO9 and propidium iodide (PI). SYTO9 penetrates both alive and dead cells, whereas PI enters the cells with damaged membranes, where it replaces SYTO9 due to stronger affinity to DNA. As a result green or red fluorescence is shown by alive or dead cells, respectively. The staining with LIVE/DEAD fluorescent dye showed that gemini surfactants coating not only reduce \textit{C. albicans} cell adhesion but also kill some of the adhered cells (Fig. 6). The comparison of the two tested gemini-QAS indicated TMEAL-12 Br as stronger adhesion inhibitor at the concentration of 160 μM, however deposition of TMPAL-10 Br caused higher proportion of killed cells (Fig. 6).

The exposition of \textit{C. albicans} 24 hour biofilm to gemini-QAS effected in biofilm reduction, in the case of both
tested compounds. Slightly stronger antibiofilm activity was observed for TMEAL-12 Br, with high level of killed cells. The dislodging of C. albicans biofilm by TMPAL-10 Br was also effective, but within the remaining biofilm structure great amount of cells showed yellow fluorescence (Fig. 6). Some authors indicate that this effect might be caused by some perturbations in the plasma membrane, which allow some amount of PI enter the cell and bind DNA. In such scenario not all of the SYTO9 is excluded and merging the photographs from two channels reveals overlaid green and red color.

4 DISCUSSION

Quaternary ammonium salts are commonly used as disinfectants. These compounds exhibit activity against a wide range of microorganisms: Gram-positive and Gram-negative bacteria, fungi and enveloped viruses (including human immunodeficiency virus (HIV) and human hepatitis B virus (HBV)) but not non-enveloped viruses. Frequent usage of antibacterial and antifungal drugs causes the acquisition of various mechanisms of resistance to antibiotics and disinfectants. Thus, the search for new antimicrobials is nowadays in large demand in medicine and industry. Gemini quaternary ammonium salts, due to their unique structure, show stronger activity against microorganisms. The introduction of cleavable, "weak" bond (like amide or ester) between hydrocarbon chains and head groups improves the surfactant biodegradability of and makes it more environmental friendly.

Antifungal activity was dependent on the chemical structure of cationic gemini surfactants. Among tested compounds, the most active against fungal strains was bromide with 10 carbon atoms within alkyl chains and a longer spacer (TMPAL-10 Br). The changes in hydrocarbon chain lengths, both elongation and shortening, caused a decrease in biological activity. The importance of the counterion was also shown, since bromide was more effective against fungi than the chloride (TMPAL-12 Br and TMPAL-12 Cl), suggesting that bromide counterion might be more reactive towards cell membranes than the chloride one. This theory is supported by the studies of the interactions of counterions with model membranes, where it was shown that bromide ions can modify electrostatic properties of membrane surface more effectively than chlorides. It was also suggested that the role in weakening electrostatic interactions plays the effective radius of bromide ion, which is smaller than chloride and allows bromide to bind more strongly to positively charged groups of membrane surface. On the other hand, the studies regarding antifungal activity of gemini-QAS with betaine based ester type alkyl chain arrangements showed that the chlorides caused greater growth inhibition, what suggests that the counterion does not determine antifungal activity of the compounds. The comparison of alanine- and betaine-derivatives showed that in both cases the compounds with 10 carbon atoms within alkyl chains strongly inhibited fungal growth, however the activity of alanine-derived gemini-QAS is slightly weaker. Despite the fact that the mecha-
nism of action of gemini quaternary ammonium salts is not yet fully understood, it is suggested that the pyridinium-based compounds influence the plasma membrane by creating pores for cation and ATP leakage from *E. coli* cells. The effect of gemini-QAS on mitochondrial and vacuolar membranes, as well as respiration inhibition in yeast, has also been observed, indicating inflow of these compounds into the cell. The conventional quaternary ammonium salts additionally cause cell lysis, although for gemini-QAS this phenomenon has not been observed.

The pores created by gemini surfactants in fungal plasma membrane might provide the gate for cell penetration by antifungal drugs. There are few classes of drugs used for treatment of fungal infections, however pathogenic mi-

![Fig. 6](image_url) Fluorescence microscopy - *C. albicans* adhesion reduction (A, B, C) and biofilm killing (D, E, F) by gemini-QAS. A – control; B – TMEAL-12 Br (160 μM); C – TMPAL-10 Br (160 μM); D – control; E – TMEAL-12 Br (160 μM); F – TMPAL-10 Br (160 μM). Scale bar = 200 μm.
croorganisms have developed several mechanisms of resistance to overcome this barrier. An example is being active efflux by ABC and MFS membrane transporters or alteration in plasma membrane lipid composition. A combination of gemini surfactants and common fungicides could contribute to overcoming fungal infections at much lower concentrations.

In present work we have shown that gemini surfactants significantly decrease *C. albicans* growth in the presence of azoles (60-80%) and polyenes (70-100%), which are the most common antibiotics used in the treatment of candidosis. Strong inhibitory effects were observed particularly in the case of the combination of gemini-QAS and nystatin, which mode of action (as polyene antibiotic) concerns embedding plasma membranes and creating pores.

The most common fungal pathogen responsible for diseases of humans is *C. albicans*. This microorganism is the cause of superficial and systemic infections, especially dangerous to immune-compromised patients. This species has the ability of switching from yeast to a filamentous form of growth, which facilitates the adhesion of *C. albicans* to biotic and abiotic surfaces. *R. mucilaginosa*, another opportunistic pathogen, is considered less virulent, however endocarditis, meningitis, peritonitis, and eye infections of humans is *C. albicans*. This microorganism is the cause of superficial and systemic infections, especially dangerous to immune-compromised patients. This species has the ability of switching from yeast to a filamentous form of growth, which facilitates the adhesion of *C. albicans* to biotic and abiotic surfaces.

The immobilization of quaternary ammonium methacyloxy-silicate to dental resin caused the inhibition of *C. albicans* adhesion in a dose-dependent manner. What is more, gemini-QAS with betaine based ester type alkyl chain arrangements blocked fungal adhesion at high concentrations. Our results indicated that gemini-QAS coating of the surface reduced the adhesion of *C. albicans* cells. Moreover, some proportion of adhered cells was killed. Similarly, deposition of gemini surfactants on polystyrene surface inhibited the adhesion of *R. mucilaginosa* cells, although this process was much more effective for TMPAL-10 Br. Additionally, filament formation, which facilitates the adhesion of *C. albicans*, was also inhibited by the studied gemini surfactants (TMPAL-10 Br and TMEAL-12 Br).

Research regarding the influence of quaternary ammonium salts on biofilm concerned mainly bacterial biofilms. Interesting results were obtained by Campanac et al., where the activity of alkylbenzyldimethyl chloride against *Pseudomonas aeruginosa* biofilm was shown. Another study showed a significant reduction of oral biofilm viability by the combination of quaternary ammonium dimethacrylate and nanoparticles of amorphous calcium phosphate. Some groups of gemini quaternary ammonium salts exhibit biofilm eradicating properties, both against fungi and bacteria. Since the studied gemini-QAS inhibited biofilm formation at the preadhesive stage, the influence of these compounds on already formed biofilm needed to be determined. Our results showed that tested gemini surfactants (TMEAL-12 Br and TMPAL-10 Br) strongly reduced *C. albicans* biofilm. Additionally, a high level of the remaining cells within the biofilm structure was killed, especially after TMEAL-12 Br treatment. The activity of gemini surfactants against *R. mucilaginosa* biofilm formed on the polystyrene surface was observed already at low concentrations.

Results of the presented studies indicate that cationic gemini surfactants might be in the future applied as compounds eradicating fungal biofilms from medical devices, human tissues and industrial installations (such as cooling systems), as well as biocides against planktonic fungal forms.

5 CONCLUSIONS

In present study, it was shown that gemini quaternary ammonium salts 

\[ N,N'-\text{bis}(1\text{-decyloxy-1-oxopronan-2-yl}) \]

-\[ N,N',N'\text{-tetramethylethane-1,3-diammonium dibromide} \]

and 

\[ N,N'-\text{bis}(1\text{-decoxyloxy-1-oxopronan-2-yl}) \]

-\[ N,N',N'\text{-tetramethylethane-1,2-diammonium dibromide} \]

exhibit strong antifungal activity. These compounds inhibited growth of planktonic yeast forms and reduced *C. albicans* and *R. mucilaginosa* biofilms. What is more, the studied gemini surfactants inhibited *C. albicans* filamentation and blocked cell adhesion to the polystyrene and silicone surface. Gemini-QAS showed no cytotoxic effect against yeast mitochondrial metabolism and, after further studies, might be in the future applied as compounds eradicating fungal biofilms from medical devices, human tissues and industrial installations (such as cooling systems), as well as fungicides against planktonic fungal forms.

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