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Bismuth–thiol incorporation enhances biological activities of liposomal tobramycin against bacterial biofilm and quorum sensing molecules production by *Pseudomonas aeruginosa*

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ABSTRACT

Recurrent pulmonary infection and inflammation are major risk factors for high morbidity and mortality in patients with cystic fibrosis (CF). As such, frequent antibiotic use and drug resistant bacterial strains are main concerns in individuals with CF. Bacterial virulence and resistance are influenced by unique CF airways fluid lining and *Pseudomonas aeruginosa* quorum sensing (QS) and biofilm formation. We have developed a novel liposome formulation consist of bismuth–thiol and tobramycin (LipoBiEDT–TOB) that is non-toxic and highly effective against planktonic bacteria. In this study, we examined the effect of LipoBiEDT–TOB on QS molecule *N*-acyl homoserine lactone (AHL) secretion by *P. aeruginosa* isolates in the presence of *Agrobacterium tumefaciens* reporter strain (A136). LipoBiEDT–TOB activity against biofilm forming *P. aeruginosa* was compared to free tobramycin using the Calgary Biofilm Device (CBD). Our data indicate that LipoBiEDT–TOB prevents AHL production at low tobramycin concentration (as low as 0.012 mg/l) and stops biofilm forming *P. aeruginosa* growth at 64 mg/l. The formulation is stable in different biological environments (biofilm, sputum, and bronchoalveolar lavage) and is able to penetrate CF sputum. Taken together, co-encapsulation of bismuth–thiol metal with tobramycin in liposome improves its antimicrobial activities *in vitro*.

1. Introduction

Lung infection in cystic fibrosis patients with antibiotic resistant bacteria are difficult to treat resulting in high morbidity and mortality rates (Moreau-Marquis et al., 2008b). Over the period of chronic exacerbations due to a defect in cystic fibrosis transmembrane conductance regulator (CFTR), thick layers of mucus cover the lumen of the airways (Reiniger et al., 2007). CF sputum contains high concentrations of glycoproteins and neutrophil derived polyanions including DNA and F-actin, which provide an ideal environment for bacterial growth and act as a barrier to host defense and therapeutics (Bucki et al., 2007; Palmer et al., 2007; Sutherland, 2001; Yang et al., 2007).

Pseudomonas aeruginosa, a common pathogen in CF lungs, regulates its population density, virulence, and biofilm formation by several mechanisms (Moreau-Marquis et al., 2008b). In addition, *P. aeruginosa* can live in a biofilm mode within hypoxic mucus in the airways of patients with cystic fibrosis (Worlitzsch et al., 2002). Mutated CFTR also can enhance *P. aeruginosa* growth and biofilm formation by elevating the iron content in the apical domain of epithelia (Moreau-Marquis et al., 2008a). Furthermore, *P. aeruginosa* exploit the quorum sensing (QS) phenomenon by producing *N*-acyl homoserine lactones (AHL) signaling molecules that regulate expression genes that control biofilm formation, thus contributing to *Pseudomonas* tolerance to antimicrobial chemotherapy and host innate system (De Kievit et al., 2001; De Kievit and Iglewski, 2000; Stickler et al., 1998; Waters et al., 2008).

Aminoglycosidic antibiotics including tobramycin are broad antimicrobial agents and are commonly used to treat *P. aeruginosa* pulmonary infections (Burkhardt et al., 2006). Drug toxicity, however, limits their frequent use in high concentrations. (Pedersen et al., 1987). Encapsulation of chemotherapeutic agents in inert nanoparticles appears to be a plausible solution for alleviating drug toxicity (Omri et al., 1994). Liposomes are being used as the nanoparticle of choice because of their non-immunogenic and biodegradable characteristics. (Beaulac et al., 1997; Mehta, 1996).

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In our previous study, we optimized the activity of a liposomal-tobramycin formulation by incorporating bismuth–ethanedithiol and had confirmed its bactericidal activity against planktonic bacteria (Halwani et al., 2008). The inclusion of bismuth–thiol combination improves bactericidal properties of the liposomal-tobramycin formulation in several ways including reduction in biofilm formation, iron uptake, lipopolysaccharide production, and obstruction of bacterial redox enzymes (Domenico et al., 2001, 2004; Huang and Stewart, 1999; Zhang et al., 2001). Free bismuth–thiols cytotoxicity, however, is a major concern in its medical application (Halwani et al., 2008; Wu et al., 2002).

In the present study, we report some of the properties of LipoBiEDT–TOB, a liposomal drug formulation consisted of bismuth–thiol and tobramycin, in terms of its: (a) stability in biofilm extracts, CF sputum components, and BAL fluids; (b) antibacterial effect on biofilm forming strains of clinical isolates of *P. aeruginosa*; (c) ability to penetrate CF sputum samples and inhibiting AHL production by *P. aeruginosa*; and (d) bactericidal capacity in the presence of polyanions such as DNA, F-actin, lipopolysaccharides (LPS), and lipoteichoic acid (LTA).

2. Materials and methods

2.1. Chemical and media

The liposomes were composed of 1, 2-Distearoyl-*sn*-glycero-3-phosphocholine (DSPC) (Northern Lipids, Vancouver, BC, Canada). Common laboratory chemicals, cholesterol, bismuth nitrate [$Bi(NO_3)_3{\cdot}5H_2O$], X-gal, latex beads (amine-modified polystyrene fluorescent orange (1 μm)), and PKH2-GL kit were all obtained from Sigma–Aldrich (Oakville, ON, Canada). Antibiotics, DNA (human placental), lipopolysaccharide (LPS, *Escherichia coli*), and lipoteichoic acid (*Staphylococcus aureus*) were all obtained from Fisher Scientific (Ottawa, ON, Canada). For F-actin preparation, actin from rabbit muscles (G-actin powder in10 mM TRIS, pH 7.4, 0.2 mM $CaCl_2$, 0.2 mM ATP, 1 mM dithiothreitol) was obtained from Sigma–Aldrich (Oakville, ON, Canada) and was polymerized in 2 mM $MgCl_2$ and 150 mM KCl for 1 h at room temperature.

2.2. Organisms

Clinical strains of *P. aeruginosa* (PA-48912-1, PA-4892-2, and PA-48913) isolated from CF patients were obtained from the Clinical Microbiology Laboratory of Memorial Hospital (Sudbury, ON, Canada). *S. aureus* (ATCC 29213), an indicator strain for tobramycin activity and the laboratory strain *P. aeruginosa* (ATCC 27583) were obtained from PML Microbiologicals (Mississauga, ON, Canada). All strains were stored at −80 °C in Mueller Hinton broth supplemented with 10% glycerol. An 18-h fresh bacterial culture in Mueller Hinton broth alone was prepared for daily use. *Agrobacterium tumefaciens* strains A136 (Ti^-) (pCF218) (pCF372) and KYC6 (kindly provided by Dr. Fuqua, Indiana University, USA) were used for monitoring the QS signaling molecules production. The strains were cultured in Luria-Bertani (LB) broth supplemented with spectinomycin (50 μg/ml) and tetracycline (4.5 μg/ml) at 30 °C for 24 h and stored in 15% of glycerol at −80 °C. For QS experiments, the A136 and KYC6 were subcultured in LB broth without antibiotics for 18 h at 30 °C.

2.3. Sputum and bronchoalveolar lavage (BAL) samples collection

The sputum samples were collected from CF patients with *P. aeruginosa* infection by spontaneous expectoration during their visits to the CF clinic (the protocol and the informed consent were approved by the Research Ethics Committee of Sudbury Regional Hospital, Sudbury, ON, Canada). The samples were pooled and frozen at −80 °C in aliquots and were diluted 1:10 (w/v) in PBS for experimentation.

Male Sprague Dawley rats (6–8 wk old; Charles River Laboratories, Montreal, PQ, Canada) were used for BAL collection as described earlier (Halwani et al., 2007) and according to the approved protocol by the institutional Animal Care Committee at Laurentian University.

2.4. LipoBiEDT–TOB formulation preparation and labeling

The dehydration–rehydration technique was utilized to prepare multilamellar vesicles containing bismuth–ethanedithiol with entrapped tobramycin as described previously (Halwani et al., 2008). Briefly, DSPC and cholesterol (2:1 molar ratio) were dissolved in chloroform containing bismuth and ethanedithiol in 1:1 molar ratio. Chloroform was evaporated from the mixture by a rotary evaporator and the resultant lipid film was mixed with distilled water containing PG (1:1 w/v). The liposomal mixture was sonicated once for 5 min before and after the addition of tobramycin (8 mg/ml). The liposomal formulations with encapsulated tobramycin (LipoBiEDT–TOB) was lyophilized and kept at −80 °C until use. The formulation was rehydrated with PBS and washed twice to remove free bismuth and tobramycin (100,000 × *g* for 20 min at 4 °C, Beckman L8-M Ultracentrifuge). LipoBiEDT–TOB vesicles diameters were in the range of 908 ± 42.7 nm as measured by Submicron Nicomp particle sizer (Model 270, Nicomp, Santa Barbara, CA, USA). The final preparation contained 0.2 mg/ml tobramycin and 10.6 ± 0.7 μM bismuth.

The manufacturer's protocol was used in order to label the liposomes with PKH2-GL dye (a fluorescent cell membrane linker). Briefly, lyophilized liposomes were rehydrated with PBS and centrifuged (62,000 × *g*) at 4 °C for 15 min. The samples were resuspended in 1 ml of Diluent A (iso-osmotic aqueous solution to maximize dye solubility and staining efficiency) at room temperature. PKH2-GL (8 μl) was then added and the final volume, and then was brought to 2 ml by Diluent A and incubated for 5 min with gentle agitation prior to addition of bovine serum albumin (2 ml; 1% (w/v) in PBS, 1 min) to terminate the reaction. Labeled liposomes were then washed twice in PBS (62,000 × *g*, 4 °C, 15 min) to remove free PKH2-GL.

2.5. LipoBiEDT–TOB stability

LipoBiEDT–TOB formulation was tested for the retention of its entrapped tobramycin for intervals of 0.5, 1, 3, 6, 12, 24, and 48 h. LipoBiEDT–TOB samples were diluted in cold PBS (1:1 v/v, 200 μl final volume) and incubated at 4 °C, or mixed in CF sputum, rat BAL fluid, and biofilm suspension of *P. aeruginosa* PA-48913 (adjusted to 1×10^8 cfu/ml and disrupted by sonication) at 37 °C. Samples were centrifuged (62,000 × *g*, 20 min, 4 °C) after designated time periods and the supernatants were tested for tobramycin release using a microbiological method of agar diffusion (Halwani et al., 2007; Ravaoarinoro et al., 1993). In brief, a MIC standard curve constructed for tobramycin antibiotic. Supernatants of the liposomal samples were transferred in duplicate into the holes of an agar plate prepared with appropriate bacterial culture (*S. aureus* ATCC 29213 for tobramycin). Plates were incubated at 37 °C for 24 h and the inhibition zones were then measured. Encapsulation efficiency was calculated as follows:

$$\text{E.E.} = \left(\frac{\text{Released antibiotic Conc.}}{\text{Initial antibiotic Conc.}}\right) \times 100$$

2.6. LipoBiEDT–TOB sputum penetration

A modified Meers et al. technique was used to test the effectiveness of the liposomal formulation on sputum penetration (Meers et al., 2008), sputum suspensions were placed on the apical surface of polystyrene inserts in a 24-well plate (24 Transwell well plates with polycarbonate tissue culture porous membrane inserts (8.0 μm) Costar, Fisher Scientific, Ottawa, ON, Canada). PKH2-GL liposomes (green) samples or latex fluorescent beads with similar diameter were added in 1:1 (v/v) ratio on the sputum at different concentrations. The inserts were incubated with ~3.8×10^8 liposome particles/ml or ~4.5×10^8 beads/ml at 37 °C. After 1 or 3 h, the apical surfaces of the inserts were swabbed and the basal surfaces of the membranes were examined under a fluorescence microscope (Zeiss Axiovert 100) at 490 nm and 520 nm to detect PKH2-GL liposomes and latex beads, respectively. The bright fluorescent dots were considered as penetrated particles.

2.7. Quorum sensing signal molecules assay

The monitoring of AHL molecules as an indicator of QS signaling by *P. aeruginosa* PA-48913 were measured by a modified McLean et al method (McLean et al., 2004). Bacterial culture, grown in MH broth for 18 h, was standardized to 1.0 McFarland and treated with various concentrations of free tobramycin (TOB), combination of bismuth and ethanedithiol (BiEDT) alone, combination of BiEDT and tobramycin (BiEDT–TOB), encapsulated tobramycin in liposomes (Lipo-TOB), encapsulated bismuth–ethanedithiol in liposomes (LipoBiEDT), or liposome encapsulated tobramycin and bismuth–ethanedithiol (LipoBiEDT–TOB) for 1 h at 37 °C. Supernatants were collected after centrifugation for 15 min (18,000 × *g*, 4 °C) to assess AHL presence as follows: *A. tumefaciens* (A136) (Ti$^-$) (pCF218) (pCF372) in LB agar poured into rectangle glass plate containing 1 ml of X-gal reagent (20 mg/ml in dimethylformamide). The agar was then punctured to make holes by using a sterile vacuum device; and aliquots (25 μl) from control- and formulations-treated *P. aeruginosa* culture supernatants were transferred into the wells. The blue pigmentation on edges of the wells indicated AHL presence. The plates were incubated for 24 h at 37 °C and the competitive inhibition of AHL by exposing the reporter strain *A. tumefaciens* (A136) to its own supernatant was used as a negative control. AHL production by untreated *P. aeruginosa* PA-48913 served as a positive control. To rule out the killing effects of the formulations containing the drugs on AHL production, we compared the growth of 1.0 McFarland standard of untreated *P. aeruginosa* PA-48913 to those treated with the lowest inhibitory concentration of LipoBiEDT–TOB for 1 h at 37 °C. Aliquots of 100 μl were then plated on MH agar and incubated for 24 h at 37 °C for cell count (cfu/ml).

2.8. Antibacterial activity of LipoBiEDT–TOB against P. aeruginosa isolated from a biofilm community

Aliquots of standardized bacteria (0.5 McFarland standards) of clinical isolates of *P. aeruginosa* including PA-48912-1, PA-4892-2, and PA-48913 were transferred to MBEC^{TM} plate (Calgary biofilm plates, Innovotech, Edmonton, AL, Canada) and incubated at 37 °C for 72–96 h. We replaced the broth every 24 h to remove unadhered bacteria to the plate lid pegs. Biofilms were harvested from the pegs by sterile plier and suspended in 900 μl cation-adjusted MH broth (CAMH). Sonication (4 s) was applied to detach bacteria from the pegs. Bacteria were adjusted to 1×10^8 cfu/ml and then treated with different concentrations of the drugs for 24 h at 37 °C. Aliquots (100 μl) of each sample were plated on CAMH agar plates for 24 h at 37 °C for cfu determination. Samples of untreated bacterial cultures and CAMH alone served as positive and negative controls, respectively.

2.9. Bactericidal activity in the presence of polyanions

The bactericidal activity of LipoBiEDT–TOB formulations was examined on susceptible strain of *P. aeruginosa* (ATCC 27853) in the presence or absence of DNA, F-actin, LPS, and LTA. Bacterial cultures in CAMH broth (1×10^6 cfu/ml) were exposed to 125, 250, or 500, mg/l of the above molecules alone or in combination and to either free TOB, BiEDT–TOB, or LipoBiEDT–TOB carrying 4 mg/l tobramycin (the 2 MIC for sensitive *P. aeruginosa* ATCC 27853) for 6 h at 37 °C. Aliquots of 100 μl were then subcultured on CAMH agar plate for 24 h at 37 °C and MBC values were determined in three independent experiments.

2.10. Data analysis

The data presented as mean ± S.E.M. of three independent experiments. Comparisons of multiple groups were compared by two-way ANOVA using GraphPad Prism.

3. Results

3.1. LipoBiEDT–TOB stability

Stability of LipoBiEDT–TOB *in vitro* in the presence of biofilm suspension of *P. aeruginosa* was comparable to that of the PBS-treated control for 48 h at 37 °C. Although tobramycin gradually leaked from the formulation in the BAL and CF sputum samples, the formulation retained a significant amount of the drug during the first 48 h (79.1–84.6% of tobramycin; $P < 0.05$, as depicted in Fig. 1).

3.2. Sputum penetration

We confirmed the LipoBiEDT–TOB ability to penetrate sputum layers at the physiological temperature during incubation period of 1 and 3 h. Penetration of latex beads of a similar size (1000 nm) was used for comparison. The formulation penetrated the diluted sputum layers and reached the basal surface of the porous membrane as shown in Fig. 2, panels A and B. Panels C and D represent penetration of the CF sputum by the latex beads.

3.3. QS molecules reduction

Since LipoBiEDT–TOB composed of different active components, we examined the effect of these molecules alone and

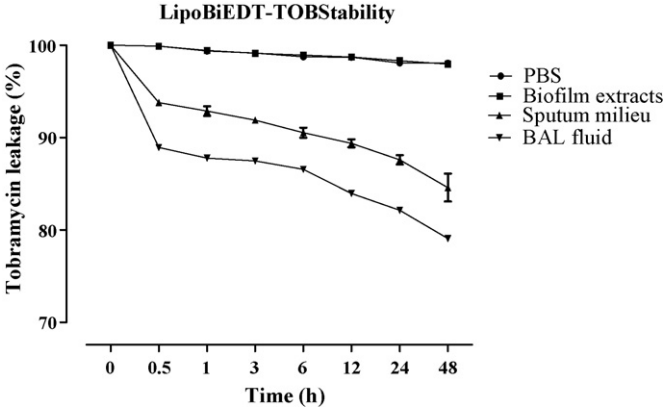

Fig. 1. Stability of LipoBiEDT–TOB formulation in PBS at 37 °C (circle) as control, in biofilm extracts (square) at 37 °C, sputum milieu (triangle up) at 37 °C, and in BAL fluid (triangle down) at 37 °C. Data represent the mean ± S.E.M. of three independent experiments. LipoBiEDT–TOB incubated in PBS and biofilm extracts at 37 °C retained significantly more antibiotics than those incubated in sputum ($P \leq 0.05$), and BAL fluid ($P \leq 0.05$) at 37 °C.

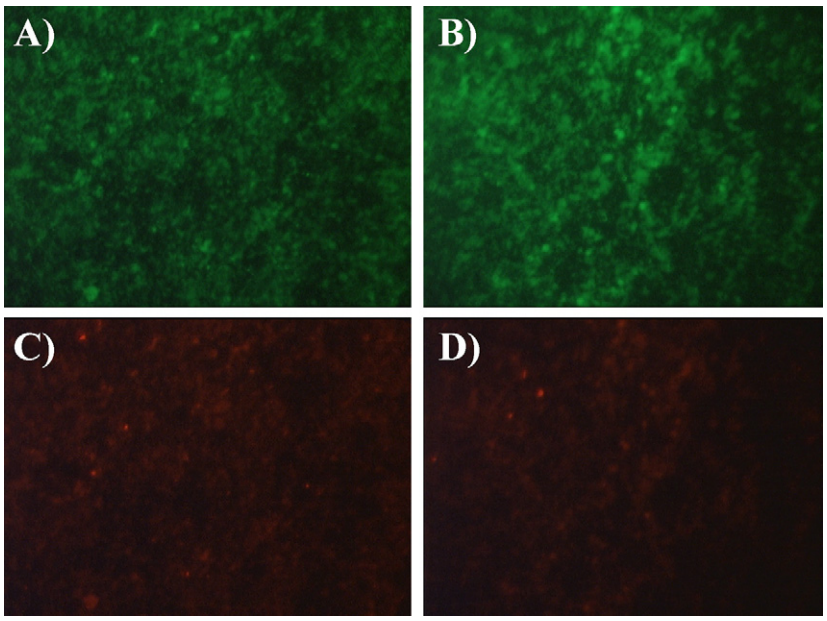

Fig. 2. Sputum penetration study: panel (A and B) 1 and 3 h incubation of labeled LipoBiEDT–TOB by PKH2-GL (green fluorescence) with sputum layer; panel (C and D) 1 and 3 h incubation of latex beads (orange fluorescence) with sputum layer. Experiment repeated three times independently. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

in combinations against QS signaling molecule production. The LipoBiEDT–TOB formulation containing non-inhibitory concentrations of TOB (0.012 mg/l) and Bi (0.0625 μM) was the only complex that interrupted AHL production by *P. aeruginosa* PA-48913. Neither the free TOB, BiEDT, or BiEDT–TOB, nor the Lipo-TOB or LipoBiEDT formulations killed the organism or inhibited AHL production (Fig. 3)

3.4. Bactericidal activity on biofilm forming strains

Among the formulations, the LipoBiEDT–TOB was the only drug that eradicated the growth of biofilm forming *P. aeruginosa* PA-48912-1, PA-48912-2, and PA-48913 at 64, 256, and 512 mg/l (Fig. 4). The Lipo-TOB formulation reduced the growth of all three strains significantly ($P < 0.001$). Tobramycin, free BiEDT and Lipo-BiEDT reduced the growth of PA-48912-2 and PA-48913 strain ($P < 0.01$–0.05) but had no effect on PA-48912-1. Lipo BiEDT was more effective than the free BiEDT against PA-48913 and PA-48912-1 ($P < 0.001$), however, no significant difference was observed between the two forms of the drug against PA-48912-2 strain.

As for the effect of polyanions on bactericidal activity of the formulations, we exposed a susceptible *P. aeruginosa* strain, ATCC 27853, to free TOB, BiEDT–TOB, and LipoBiEDT–TOB in the presence or absence of DNA, F-actin, LPS, LTA, at 125, 250, and 500 mg/l,

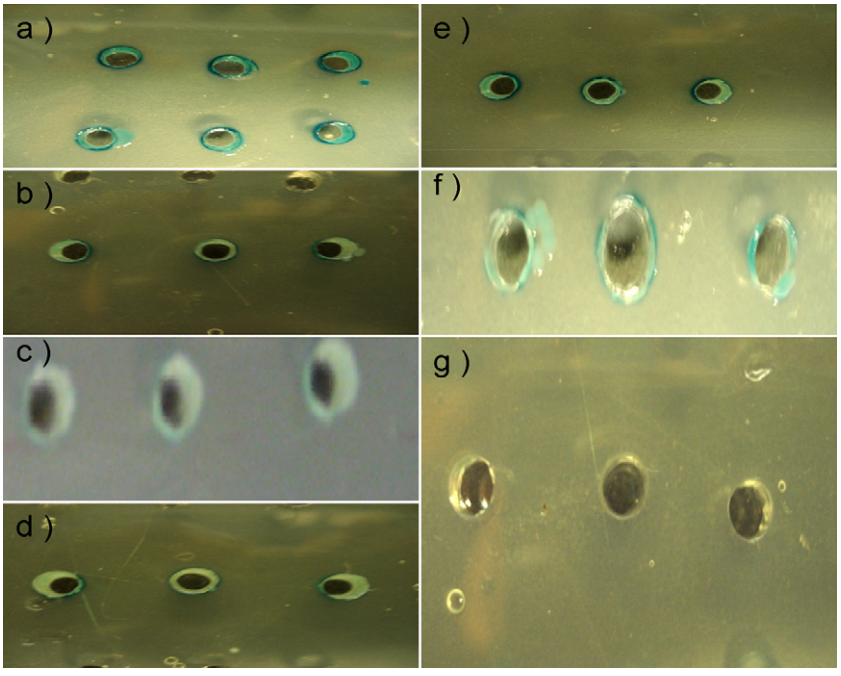

Fig. 3. QS molecules reduction assay: (a) untreated PA-48913 (positive control for AHL); (b) treated with BiEDT alone (2.5 μM); (c) free TOB (0.012 mg/l); (d) combination of BiEDT–TOB (1.25 μM–0.024 mg/l); (e) Lipo-BiEDT (2.5 μM); (f) Lipo-TOB (0.012 mg/l); (g) LipoBiEDT–TOB (0.0625 μM–0.012 mg/l).

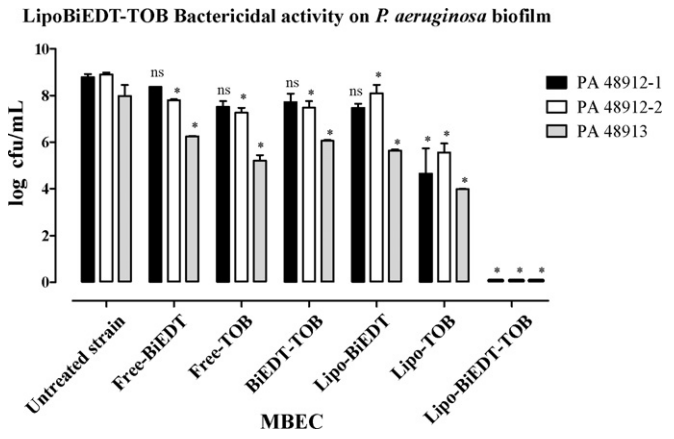

Fig. 4. Biofilm bacteria reduction assay: LipoBiEDT–TOB bactericidal activity on PA-48912-1 (black), PA-48912-2 (white), and PA-48913 (grey). LipoBiEDT–TOB (64 mg/l) was the only formulation that eradicated the bacterial growth completely of PA-48912-1 whereas TOB, BiEDT, BiEDT–TOB, lipo-BiEDT, and Lipo-TOB failed to do so at (64 mg/l). LipoBiEDT–TOB (256 mg/l) was the only formulation that eradicated the bacterial growth completely of PA-48912-2 whereas TOB, BiEDT, BiEDT–TOB, lipo-BiEDT, and Lipo-TOB failed to do so at (256 mg/l). LipoBiEDT–TOB (512 mg/l) was the only formulation that eradicated the bacterial growth completely of PA-48913 whereas TOB, BiEDT, BiEDT–TOB, lipo-BiEDT, and Lipo-TOB failed to do so at (512 mg/l). Data were obtained from three independent experiments and shown as the means ± S.E.M. $P \leq (0.001–0.01)$ significant.

respectively, alone or in combination for 6 h at 37 °C. In the absence of these molecules, BiEDT–TOB and LipoBiEDT–TOB at 1 mg/l and the free tobramycin at 2 mg/l were bactericidal. At 4 mg/l antibiotic, free tobramycin had no effect on the bacterial growth in the presence of polyanions (125 mg/l), while TOB (4 mg/l) reduced bacterial growth by 4 logs compared to untreated strain (4.41 ± 0.11 vs. 8.17 ± 0.12 log cfu/ml, $P < 0.001$). BiEDT–TOB and LipoBiEDT–TOB, on the other hand, completely eradicated the strain even at the highest concentrations of polyanions (500 mg/l), separately or in a combination. It is important to note that empty liposomes did not show any antibacterial activity either in the presence or absence of polyanions.

4. Discussion

Inhaled tobramycin subsides *P. aeruginosa* pulmonary infections in CF patients (Santos et al., 2007). Prolonged use of tobramycin, however, raises concerns for drug toxicity and emerging antibiotic resistance strains. Our previous reports and that of other investigators confirmed that the liposomal formulations of the drugs improve their efficacies *in vitro* (Alipour et al., 2008; Halwani et al., 2007; Mehta, 1996; Omri et al., 1994). Furthermore, incorporation of bismuth–ethanedithiol in our formulation (LipoBiEDT–TOB) enhances tobramycin antimicrobial activity against Gram-negative bacteria while reducing its cytotoxicity to a human lung epithelial cell line (A549) (Halwani et al., 2008). Herein, we report that the LipoBiEDT–TOB penetrates sputum, reduces QS signaling molecule production and inhibits the growth of biofilm-forming clinical strains of *P. aeruginosa*. In addition, LipoBiEDT–TOB was not affected by the presence of polyanions common to CF lungs.

The LipoBiEDT–TOB formulation is reasonably stable (<25% drug release in 48 h) in the presence of biofilm molecules that are known to hinder the activity of antibiotics in the lungs of CF patients. The stability data also indicate that the bacteria in the lungs would be exposed to the remaining of the drugs within the formulation for a long period of time. In order for an antibiotic to reach its target site in the CF lungs, barriers of thick sputum made up of F-actin, glycoproteins, DNA and alginate must be penetrated (Sanders et al., 2000). Based on our *in vitro* data, the sputum pool did not retard the movement of LipoBiEDT–TOB during a 3-h experiment at physiologic temperature. A study by Meers et al. (2008) also supports this notion and encourages us to infer that LipoBiEDT–TOB could penetrate the sputum barrier in CF lungs as well.

P. aeruginosa produces AHL molecules as signaling elements within their communities and with other species such as *Burkholderia*. QS molecules are involved in bacterial virulence and biofilm formation, which subsequently increase antibiotic resistances within the members of bacterial community (Hussain et al., 2008; Skindersoe et al., 2008). Among the formulations tested, only LipoBiEDT–TOB substantially suppressed AHL production at concentrations below its MIC. The molecular mechanism(s) by which the formulation inhibits AHL production is not yet clear and requires further investigations regarding gene and protein expression of the signaling molecules and corresponding receptors (De Kievit and Iglewski, 1999; Schuster and Greenberg, 2006).

In addition to alginate production and LPS structural changes, biofilm formation contributes to bacterial antibiotic resistance in the CF lungs. Upon bacterial colonization of the lungs, overproduction of exopolysaccharides can increase mucoidal phenotypes and biofilm formation as well (Alkawash et al., 2006; Bryan et al., 1984). The ability of bismuth–ethanedithiol to disturb bacterial membrane integrity and to prevent biofilm formation has already been reported (Huang and Stewart, 1999; Wu et al., 2002). Hence, we included bismuth–ethanedithiol in our liposomal tobramycin, the formulation that effectively inhibits the growth of antibiotic resistant *P. aeruginosa* clinical isolates *in vitro* (Halwani et al., 2008). Indeed, LipoBiEDT–TOB was the only formulation that eradicated the bacterial strains isolated from a *P. aeruginosa* biofilm community in MBEC™ plates (Fig. 4). This treatment effect was attributed to the ability of the liposomes to deliver both agents, BiEDT and tobramycin, concurrently.

Unlike the free tobramycin, LipoBiEDT–TOB bactericidal activity was not affected by high concentrations of DNA, F-actin, LPS, or LTA. Although BiEDT–TOB exhibited a similar bactericidal activity in this environment, cytotoxicity of the free BiEDT impedes its clinical application (Halwani et al., 2008; Wu et al., 2002).

In conclusion, introduction of a safe and multifunctional liposomal antibiotic LipoBiEDT–TOB containing an antibacterial metal (bismuth) could be the beginning of a new approach for optimizing the efficacy of the existing antibiotics.

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