Antimicrobial Agent Evaluation Using Planktonic, Biofilm-Forming and Preformed Biofilm Cell States

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Statement of Purpose: Biofilms have been implicated in 80 % of all chronic human infections[1], yet little attention has been focused on studies of antimicrobial agent efficacy in bacterial biofilms. The planktonic state of bacteria is most commonly studied in the literature; however this model system does not accurately represent the complex biofilm response. Due to increasing antimicrobial resistance and the demand for new antimicrobial strategies and materials, robust protocols are urgently needed to assess antimicrobial agent effects on multiple bacterial stages, including planktonic growth, biofilm formation and preformed/mature biofilms. Quaternary pyridinium salts (QPSs) are unsaturated cationic heterocyclic compounds with antimicrobial properties. In this work, the effects of a novel QPS on the biofilm forming and cariogenic bacteria, *Streptococcus mutans* UA159 (*S. mutans*), were evaluated using planktonic, biofilm-forming, and preformed biofilm cells. This project provides insight into approaches to evaluate the effects of promising antimicrobial materials on multiple bacterial states, including preformed biofilms.

Methods: 4-acetyl-1-hexadecylpyridin-1-ium iodide (QPS-1) was synthesized by reacting 4-acetylpyridine with 1-iodohexadecane, and its purity was analyzed by ^{1}H nuclear magnetic resonance (NMR). *S. mutans* were grown in Todd Hewitt Broth (THB) for planktonic cultures or 25 % THYE (THB + yeast extract) + 30 mmol/L sucrose for biofilm cultures, all at 37 °C, 5 % (by volume) CO_2. Experiments were run in triplicate, repeated twice, and included controls without additive, with 4-acetylpyridine and with erythromycin, as well as an uninoculated control. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of three natural products and QPS-1 were determined in planktonic cultures using typical broth microdilution protocols. To evaluate effects on biofilm formation, *S. mutans* were incubated for 24 h with QPS-1 at concentrations of (0.09 to 25) μg mL^{-1}. THB agar inoculation was used to determine the QPS-1 concentration that resulted in no bacterial growth. Preformed biofilms were grown for 24 h, at which time the medium was replaced with fresh medium containing QPS-1 at concentrations ranging from (1.56 to 400) μg mL^{-1}. Biofilms were further incubated for 2 h and then assessed for metabolic activity with (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and viability (colony-forming units, CFUs).

Results: QPS-1 compound was synthesized, purified and characterized by Fourier transform infrared spectroscopy (FT-IR), ^{1}H and ^{13}C NMR. The natural products did not show significant antimicrobial activity against planktonic *S. mutans* except at very high concentrations. QPS-1 was the most potent agent with very low MIC and MBC values (Fig. 1). The QPS-1 precursor, 4-acetylpyridine, did not show antimicrobial activity in planktonic cells, as expected. MBC values for QPS-1 were similar in planktonic cultures and biofilm formation. In preformed biofilms, MIC and MBC values were less than one order of magnitude higher than for the planktonic cell state.

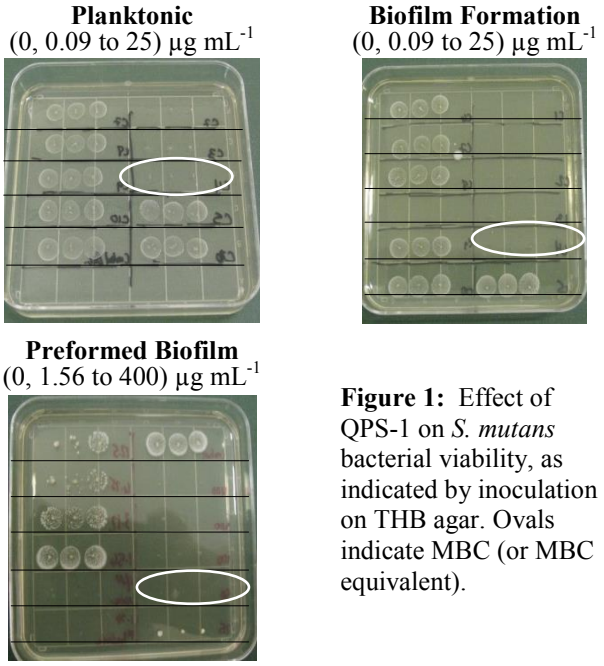

Figure 1: Effect of QPS-1 on *S. mutans* bacterial viability, as indicated by inoculation on THB agar. Ovals indicate MBC (or MBC equivalent).

Conclusions: The characterization of antimicrobial efficacy in three different stages of bacterial growth, including the often overlooked evaluation of preformed biofilms, provides a more comprehensive examination of antimicrobials and further sheds light on potential mechanisms of action. QPS-1 had similar effective concentrations in planktonic cultures and biofilm formation studies, suggesting that its mechanism of action occurs before biofilm formation. However, the small increase (< 10-fold) needed for full antimicrobial activity in preformed biofilms further indicates that the mechanism of action is not as affected in biofilms as compared to antibiotics, which typically require 100 to 1000 fold higher concentrations for anti-biofilm properties.[2] Due to its promising anti-biofilm activities at low concentrations, QPS-1 is a candidate molecule to impart antimicrobial activity to biomaterials.

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References:

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