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Synthesis of Bacteriostatic Materials from Quaternary Ammonium Salts

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Abstract— **Dental implants are commonly used to address tooth loss. Since the implant's surface is exposed to the oral cavity, it becomes susceptible to colonization by microorganisms. Recent in vivo research demonstrated that bacterial colonization begins as early as 30 minutes after implant placement. The most common bacteria associated with dental implant infections include *Staphylococcus aureus*. Engineering the implant surface to provide antimicrobial activity is one line of defence that has been taken up in recent years. An antimicrobial coating was prepared by quaternising the copolymer of vinylbenzyl chloride (VBC) and [3-(Methacryloyloxy)propyl]trimethoxysilane (MPS). The copolymer MPS-VBC was synthesized by free radical polymerization. The obtained copolymer was then quaternized to form Quaternized Salts (QAS) which were characterized by Fourier Transform Infrared (FTIR). The baceriostatic property of these compounds was investigated in gram positive, *S. Aureus,* by serial dilution method. This work explores the development and application of a novel quaternary ammonium, attached to economical and clinically relevant material surfaces.**

Keywords— **Vinylbenzyl chloride (VBC), [3-(Methacryloyloxy)propyl]trimethoxysilane (MPS), Quaternary Salts, Antimicrobial activity**.

I. Introduction

Infection due to dental implants is a major challenge in dental surgeries. Implant related infections cases are very high in Canada with the majority being Gram-positive, *Staphylococcus aureus* mostly [1]. These microorganisms form colonies protected by a biofilm situated over the implant's surface where antibiotics cannot access. Dental infections occur due to bacteria grows to form a plaque that affects our gum and the underlying bones. If an implant is present, the plaque biofilm forms between the titanium implant and the prosthetic crown, progressively spreading to the gums. Thus, the proactive prevention of bacterial colonization by engineering the Ti alloy surface to provide some antimicrobial benefit is one line of development that has been taken up in recent years. More recently, studies explored ways to give antibacterial capabilities using coating procedures that include covalent bonding of biocides to the surface [2,3]. Commercially, antimicrobial agents available for coating dental implants include metal ions, quantum dots, triclosan, silanes, Chlorhexidine and many more [9]. These lack have long term antimicrobial efficacy data, promote bacterial resistant and may have cytotoxic effects [9].

II. Background

Quaternary Ammonium Salts (QAS), are biomaterials exhibiting a broad range of antibacterial activity while being low in cytotoxicity. Due to its nontoxic nature quaternary ammonium salts have been synthesised in a variety of concentrations to achieve the best antibacterial activity in dental care to eliminate the biofilm from dental tissues. Quaternary ammonium Compounds (QACs) and its salts feature positively charged groups [5], making them particularly favorable for adsorption when compared to other antimicrobial compounds. The cationic QAC head and interact electrostatically as shown in Fig. 1. The bactericidal mode of action for the QAS entails the disturbance and breakdown of membrane bilayers by the alkyl chains, along with the alteration of the membrane's charge distribution through the charged nitrogen groups as shown in Fig. 1[4]. QACs have demonstrated significant anti-biofouling and osseointegration qualities when linked with cell-adhesive sequences in contrast to those in the market, and have proven to be of great bacteriostatic outcomes in vitro [9].

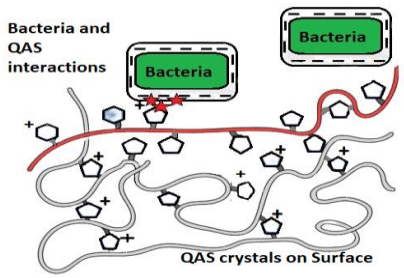

Fig. 1 Mechanism of Antimicrobial Activity of QACs with gram positive bacteria

III. Methods

A. Synthesis of the Quaternized Salts

The copolymerization of VBC and MPS was carried out by following a published report [7] and was conducted with a minor modification as shown in Fig. 2. The reaction was carried out using equal molar amounts of VBC and MPS in toluene. Azobisisobutyronitrile (AIBN) was used as an initiator and the reaction mixture was stirred at 70 °C for 24 hr. The resulting copolymer was purified using hexane precipitation. Lastly, the purified copolymer samples were subjected to vacuum drying at room temperature.

The quaternization of the vacuum dried copolymer was carried out by adding dimethyl octylamine (DMA) to the copolymer solution and stirring at 60˚ C for 8 hr. The white product obtained was repeatedly washed with pentane and filtered using vacuum filtration to remove any impurities.

The copolymer will be further attached covalently to the substrate surfaces *via* Si-O linkages by virtue of their reactive silanol groups [6]. The QAS was characterized by FTIR and the peaks were used to verify the synthesis.

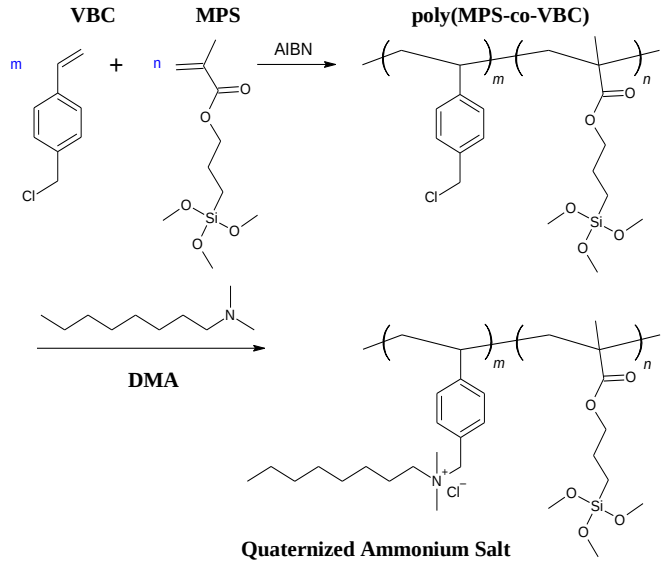

Fig. 2 Synthesis of QAS; ratios of m and n are 1:1.

B. Bacteriostatic Assays

The growth inhibition studies of the QAS compounds were screened by serial dilution method [8] against *Staphylococcus aureus* USA300 (LAC). The QAS compounds were weighed and dissolved in 1 ml of Tryptic Soy Broth (TSB) to create varying test concentrations. Test tubes with concentrations of 200 mg/ml, 100 mg/ml, 50 mg/mL and a solution of TSB without any QAS was used as control. 10 µl of bacteria at an Optical Density (O.D) of 1 at wavelength of 600 nm, was added to each test tube. After incubation and shaking for 24 hours, 100 µl of each sample was diluted further in 900 µl of saline for O.D measurements, and the respective growth was noted. The experiment was conducted in triplets, with the mean result being used.

IV. Results

A. Characterization of the Quaternized Salts

Characterization of polymer and quaternized (VBC and MPS) were conducted by FTIR spectrums of the copolymer and the QAS. From Fig. 3, both the copolymer and QAS spectrums had a peak at 675 cm^{-1} can be ascribed to the C–Cl stretching, whereas that at 1267 cm^{-1} is assigned to CH_2Cl wagging vibrations. However, the intensity ratio between peaks at 675 cm^{-1} (C–Cl) and 2924 cm^{-1} (C–H) of the copolymer was higher (2 times) than that of QAS. It was because

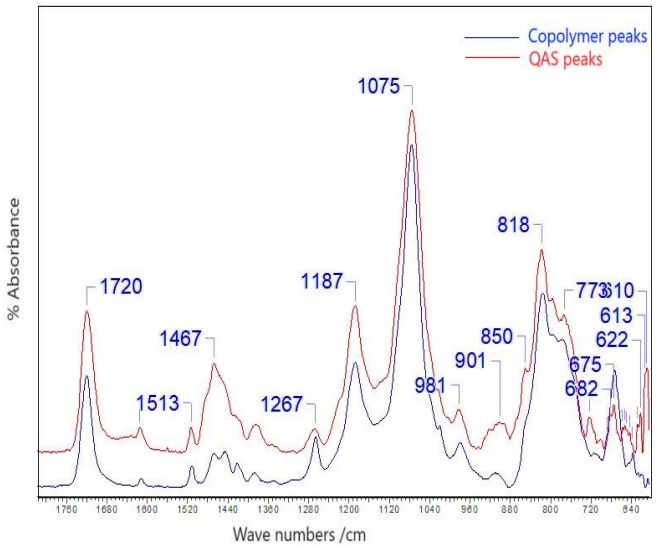

Fig. 3 FTIR Spectrum of Copolymer (blue) and QAS(red)

the quaternization consumed the C–Cl group in copolymer to result in QAS.

B. Bacteriostatic Activity

To evaluate the changes in the growth for the synthesized QAS of different concentrations, their growth was measured with respect to their O.Ds. The O.Ds for all three concentrations of QAS read approximately zero indicating bacteria did not grow in the suspensions. Visual observations could be made for the assays after incubation of 24 hr in Fig 4, as bacteria grew in the control (appearing cloudy) whereas there was no visible growth in the other tubes with the QAS (appearing clear). The findings indicate that 200 mg/mL, 100 mg/mL and 50 mg/mL, all successfully inhibited the growth of *S. aureus.*

Table 1. Growth Inhibition of different QAS concentrations on *S. aureus*

QAS Conc (mg/mL)	OD_{600} Readings
200	0.00
100	0.00
50	0.00
TSB (control)	>1

The mode of action of the synthesized QACs, primarily involves two key mechanisms. Firstly, the alkyl chains present in QACs interact with the lipid bilayer of cell membranes, causing perturbation and disruption. This disruption weakens the structural integrity of the membrane. Secondly, the charged nitrogen group in QACs interferes with the distribution of charges across the membrane. This disruption in charge distribution further destabilizes the membrane, contributing to its dysfunction. Together, these actions lead to compromised membrane integrity, which can result in cellular leakage, loss of essential cellular components, and ultimately, cell death.

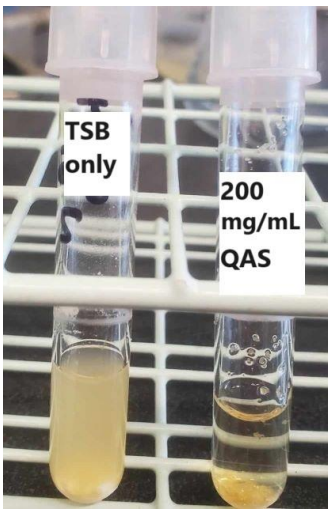

Fig. 4 Bacteriostatic assay tubes with TSB (left) and with QAS (right) after 24 hr incubation.

IV. Discussion

Our findings indicate that Quaternary Salts derived from the co-polymer MPS-VBC, when present at concentrations of 50 mg/mL and higher, exhibit a 100% inhibition of *Staphylococcus aureus* growth compared to controls. Further investigations are necessary to determine the minimum inhibitory concentration (MIC) of QAS needed to impede the growth of *S. aureus*. Moreover, alternative bacterial strains warrant exploration.

Study limitations include the need to be for less polymer degradation and more stability. Future work needs to be done on its QACs' efficacy against biofilms which are more applicable in dental plaques.

The inevitability of bacteria developing resistance to our fabricated surfaces underscores the need for proactive measures to prevent the emergence of resistance. Developing next-generation antimicrobial surfaces using QAS is crucial for effectively addressing these challenges.

Acknowledgment

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Conflict of Interest

The authors declare that they have no conflict of interest.

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