Mechanisms of antimicrobial actions of quaternary ammonium compounds

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General Introduction
Biomaterials are an integral component of modern healthcare in the Western world. They are used more and more in modern medicine after trauma, oncological surgery, and support or restore human body function through e.g. hip prostheses, prosthetic heart valves, catheters or voice prostheses. One of the main drawbacks for the extended use of biomaterial implants and devices is the occurrence of biomaterial-associated infections due to microbial adhesion and biofilm formation. The incidence of this type of infections varies from 1 to 4% for hip prostheses to 100% for urinary catheters after 3 weeks of use. In most cases an infected implant can cause serious problems, often requiring the replacement of the infected device at the expense of dramatic patient suffering and considerable costs.

**Biofilm formation**

Normally, implant-related infections caused by microbial biofilms start with the formation of a conditioning film followed by initial adhesion of microorganisms to the biomaterial surface, finally leading to the formation of a biofilm. In a biofilm, microorganisms embed themselves in a matrix of extracellular polymeric substances (EPS), also-called ‘glycocalyx’ or ‘slime’, offering protection against the host immune system and antimicrobial treatment, to which planktonic organisms are usually much more susceptible. EPS matrices have also been proposed to contribute to this recalcitrance by limiting the diffusion of antibiotic solutes in biofilms, either by size exclusion or by interaction/reaction with the solutes. For example, a restricted accessibility of bacteria in biofilms based on size exclusion has been demonstrated for molecules in the range of 200–10,000 Da and 3–900 kDa. In staphylococcal strains, EPS formation depends in part on the presence and expression of the *icaADBC* gene cluster, which is involved in the production of a polysaccharide intracellular adhesin (PIA).
PIA is known to mediate bacterial contact with each other and embeds adhering bacteria in a slimy PIA matrix during biofilm formation. The ica operon is widespread in staphylococcal multi-resistant isolates and represents one of the most important virulence factors causing biomaterials-associated-infection.

**Quaternary ammonium compounds and prevention of biofilm formation**

Biofilms exhibit high levels of resistance to antibiotics, disinfectants and detergents. Mechanisms of resistance may include cell dormancy caused by a nutrient depletion deep into biofilms, expression of specific (such as porins, β-lactamas, etc.) or non-specific (multidrug efflux pumps, thicker cell wall) resistance mechanisms. Control of microbial growth is required in all microbiologically-sensitive environments, particularly when wet surfaces provide favourable conditions for microbial proliferation.

The word biocide is a general term to describe a chemical agent with antiseptic, disinfectant or preservative activity, which inactivates microorganisms. Some are capable of destroying microorganisms (e.g. bactericidal and fungicidal), while others can only prevent or inhibit their growth (e.g. bacteriostatic and fungistatic). Biocides have a broad spectrum of usage and differ from antibiotics in their lack of selective toxicity. In fact, they have multiple biochemical targets and have been used over the years in a diversity of areas. In the healthcare environment, biocides are used extensively mainly for the disinfection of surfaces, water and equipment, and in antisepsis; they are also used for the sterilization of medical devices, and the preservation of pharmaceutical and medicinal products. Additionally,
biocides are used in industrial systems to prevent and control microbial growth and biofouling \(^{21}\).

In this respect, wide ranges of antimicrobial biocides are being explored. Among these are quaternary ammonium compounds (QAC) that have been applied in solutions for their antimicrobial properties \(^{20,28}\). They have a broad application range, from clinical to industrial purposes, and are particularly used for the disinfection of surfaces, equipment and medical devices, and the preservation of products \(^{11,17}\).

QACs possess a strong bactericidal behaviour \(^9\), but weak detergent properties \(^{17}\). Cationic antimicrobial QAC have been prominent amongst those used both in infection control and within many consumers products. They are known to affect the viability of Gram-positive and Gram-negative bacteria in planktonic cultures as well as in biofilms. Their properties depend on their structure and size, but especially on the length (hydrophobicity) of the long-chain alkyl group. For Gram-positive bacteria and yeast, such activity maximizes with chain lengths of \(n=12-14\), whilst for Gram-negative bacteria, optimal activity is achieved for compounds with a chain length of \(n=14-16\). Compounds with \(n\)-alkyl chain length of \(n < 4\) or \(n > 18\) are virtually inactive \(^{12}\). The efficacy of QACs increases with increasing temperature and pH \(^9\).

The biocide *Cocoalkylmethyl [polyoxyethylene (15)] ammonium chloride* used in this work is a QAC (AKZONobel, Amsterdam, The Netherlands). Ethoquad C/25 is manufactured from coconut oil and is an amphiphilic antimicrobial composed of two hydrophilic polyethylene glycol chains and a hydrophobic alkyl tail (see Figure 1).

Commercially available QACs that utilize natural oils as the source of the alkyl chain substituent are highly diversified not only in their fatty-acyl chain length distributions but also in the degree of C–C saturation \(^{12}\). Ethoquad C/25 comprises, in contrast to many other QACs, hydrophilic moieties (PEG)
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next to the hydrophobic units, which improves the water solubility. Improved water solubility secures reliable planktonic behaviour.

![Chemical structure of Ethoquad C/25](image)

**Figure 1.** Chemical structure of Ethoquad C/25 (Cocoalkyl methyl (polyoxyethylene) ammonium chloride).

QACs have often been assumed to possess generic mechanisms of action directed towards biological membranes. They are commonly known as "membrane active antimicrobials" or as "biological detergents" broadly recognized by their lack of specificity in mechanism of action. Since it is not exactly known what the influence of a QAC is on the bacterial cell surface, it is vital to understand the mechanisms of action of these QAC biocides. It has been reported that the QACs possess antibacterial properties in solution, presumably requires its binding to the bacterial cell surface, yielding membrane damage, followed by leakage of intercellular constituents and eventually cell death 7,15.

Due to the presence of anionic groups (e.g. carboxyl and phosphate) in their membranes, most microorganisms have a negative surface charge under physiological conditions. It is believed that attractive electrostatic interactions play a prime role in the association of positively charged QACs and negatively charged bacterial cell membranes. Bacteria have a phospholipid membrane inner layer surrounded by a polysaccharide (peptidoglycan) outer layer (Figure 2A). Phospholipids consist of two long fatty acids connected via glycerol to phosphoric acid. The negative rest charge of the phosphoric acid is neutralized by calcium or magnesium ions. Cationic antimicrobials as QAC
interact initially with the wall and membrane by displacing these divalent cations. The replacements of these divalent ions destabilize the intracellular matrix of a bacterium. The hydrophobic tail then interdigitates into the hydrophobic bacteria membrane. The leakage of metabolites (such as $K^+$-ions and inorganic phosphate), the lysis of the bacteria and the disappearance of membrane enzymes are among the reported impacts of QACs on bacteria.

The presence of $Ca^{2+}$-ions may impede killing by QACs, as integration of a QAC molecule in the cell membrane requires removal of $Ca^{2+}$-ions from the membrane in order to maintain a neutral membrane charge. Over the past, the influence of a QAC on the bacterial cell surface was determined to be an exchange of $Ca^{2+}$-ions or a surfactant way of action.
Figure 2. Cartoon showing the mechanism of a hypothetical action for quaternary ammonium compounds.
(A) Gram-positive bacterial cell structure, 
(B) Phospholipid-membrane, 
(C) Progressive adsorption of the quaternary head group to phospholipids in the cytoplasmic membrane, 
(D) Replacement of Ca\(^{2+}\) ions by Et C/25 molecules followed by membrane destabilization, 
(E) Membrane disintegration. 
(Illustration adapted from Gilbert et al. \(^{12}\))
In the last decade, a number of groups successfully prepared coatings comprising immobilized QAC's \textsuperscript{10}. These QAC’s were fixed in a static way, preventing them to search for the heterogeneously distributed charges on bacteria. Since the mode of action of QAC’s is disruption of the cell membrane, the matching of QAC’s with the negative charges on the cell membrane is crucial.

**Aims of this thesis:**

1. To determine the efficacy of a quaternary ammonium compound (Ethoquad C/25 Cocoalkyl methyl (polyoxyethylene) ammonium chloride) against a number of staphylococcal strains in planktonic cultures and in their biofilm mode of growth.
2. To explore and understand the bactericidal mechanism of Ethoquad C/25 on staphylococci implicated in biomaterial-associated infections.
3. To determine the efficacy of Ethoquad C/25 when immobilized on a surface as possible coatings for preventing biomaterial-associated-infections.
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Reference list


