Quaternary ammonium compounds to prevent oral biofilm formation

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Chapter 1

General introduction and aim of this thesis

INTRODUCTION

Bacterial contamination can lead to serious and costly problems for society in many different aspects. Reports on foodborne diseases point out 55,000 hospitalizations related to transmission and proliferation of foodborne pathogens in the USA each year [1]. Water contamination in hydraulic systems can also lead to dangerous gastrointestinal illnesses [2]. Other disruptive aspects of bacterial contamination are biofilm related. After aggregation and attachment to a surface bacteria start growing into sessile communities called biofilms. These biofilms are interactive congregates of single or heterogeneous bacterial species embedded in extracellular polymeric substances (EPS). EPS contains polysaccharides, lipids, proteins and extracellular DNA. These communities are interactive in the sense that bacteria communicate with each other through a mechanism called quorum-sensing (QS) [3–6].

Different descriptions of the continuous biofilm dynamics are presented in literature [4,7,8] most of them comprehends 5 stages: 1) Bacterial reversible attachment; 2) Adhesion – irreversible attachment; 3) EPS secretion, QS mechanism starts, 3D shaping of the biofilm; 4) Mature biofilm with water channels and QS; 5) Detachment of biofilm segments or planktonic cells and spreading to other locations. Once a biofilm is mature and enclosed in an EPS matrix, it becomes less susceptible to host immune defense, physical stresses, antibiotics or other antimicrobial substances [3,4,6]. Aside from sheltering bacteria, biofilm spreads resistant bacteria and worsen chances of eradicating the infection [5,9].

Dental plaque is a biofilm formed in the oral cavity on soft and hard tissues. This kind of biofilm, in the presence of dietary carbohydrates, can cause tooth decay by caries cavitation with further pulp injure and in some cases tooth loss. When this biofilm occurs in the subgingival region it can trigger gingivitis, periodontal disease and bone loss, which if not treated results in tooth loss. World Health Organization stated that dental caries and periodontal diseases are public health problems affecting developing and developed countries [3,6,10,11].

In oral biofilms initial adhesion occurs on the acquired pellicle. Pellicle adsorbed from saliva consists of mucins, glycoproteins, agglutinins, α-amylase, statherin and some other components. Initial colonizers are predominantly Gram-positive *Streptococci* and *Actinomyces*. Biofilm mass enlarges via bacterial growth and other species will adhere to the initial colonizers. Usually Gram-negative *Veillonella* and *Fusobacterium* [10,11] are bridging different species to each other in

dental biofilms. For orthodontic patients, biofilm accumulation is a problem, which is exacerbated due to brushing and flossing difficulties caused by fixed appliances. Not only the biofilm formation increases but a change among bacterial species occurs when the biofilm matures with an increase of the acidogenic ones (e.g. *Streptococcus mutans*) [12]. Besides oral health deterioration, oral biofilms are associated to atherosclerosis, rheumatoid arthritis [10], diabetes, adverse pregnancy outcomes and infective endocarditis [10,13,14].

Undoubtedly, bacteria organized into biofilms bring up expensive and alarming implications. From industrial point of view, machinery and pipelines can be corroded by biofilms byproducts, quality of food products and beverages endangered. From medical perspective biofilms can lead to prostheses losses, catheter-related blood stream infections, ventilator-associated pneumonia, dental and periodontal problems, urinary, pulmonary and cardiovascular infections leading to organs impairment, chronic sinusitis, tonsillitis or otitis, non-healing wounds and others [15] to which increased hospital stay, morbidity and mortality have been associated [8,16]. Together these bacterial infection issues cost billions of dollars every year [7,17,18].

Biofilms can shield bacteria against antimicrobial substances as a result of the deficient penetration and spread throughout its depth. Thus, levels of antibiotic are constantly below the minimum inhibitory concentration and triggering resistance development and spread. New studies already recorded bacteria resistant to the latest line of antibiotics for which no other antibiotic line would be available [17,19]. In most cases when a mature biofilm is formed on devices or in a wound, the most efficient measure remains the surgical replacement of the implant or mechanical debridement. Such treatments are not always feasible without risks for patients and increased healthcare costs [4].

Extensive and uncontrolled use of antibiotics has contributed to the emergence of multiple drug-resistant microorganisms. Available treatments for common infections and injuries are becoming ineffective which is evidenced by high rates of resistance registered for *Staphylococcus aureus* (MRSA), *Escherichia coli* and *Klebsiella pneumonia*. Infections that are not treatable with 3rd generation cephalosporins, rely only on carbapenems which is the last resource and resistant bacteria were already reported [17]. There is a constraint over the antibiotic demand once there are no new antibiotics entering the market on the same speed as resistance is being developed [20]. The urge for new counteracting strategies to

tackle bacterial infections is needed. The scientific community is in a collective endeavor either to improve mechanisms of action of available antibiotics or to develop new materials with inherent antimicrobial properties. To improve mechanisms of action of available antibiotics, actions against biofilm buildup are taken as e.g.: to interfere in the QS in order to prevent biofilm formation, degrade the EPS matrix, stimulate non-pathogenic bacteria as probiotics to compete with pathogenic bacteria, facilitate phagocytosis or targeting the bacterial membrane [5]. Actions not concerning microorganisms include: development of materials containing antimicrobials or chemically modified surfaces which will affect bacterial adhesion or bacterial growth [18,21].

Depending on the aimed clinical use polymers need to have certain physicochemical and mechanical characteristics. It is possible to incorporate into polymers mechanical reinforcement, shape memory, semi permeability, responsiveness to physical or chemical stimuli and antimicrobials. The most common polymers in use for biomedical application are displayed in Table 1 with their respective characteristics and applications. Due to the great variety of available polymers and also methods to insert antimicrobials or modify the surfaces of the polymers the final material can be tuned for a better performance for specific applications.

When polymers with an antimicrobial are not used as bulk materials, they can be used as coatings. This is especially appealing if the targeted substrate is metallic or if the aimed modification is exclusively related to the surface with bulk material properties maintenance. In the dental and medical field polymeric coatings appear as an option to bulk materials with antimicrobial properties. Coatings used in dentistry and medicine are fluoride and chitosan varnishes, surface sealants, carbopol coatings for preventing erosion [22], drug delivery coatings and anti-fouling coatings for catheters, valves, stents among others [18,23].

Table 1. Most common polymers used for biomedical purpose, their properties and applications.

Chemical Group	Polymer	Properties	Application
Silicon	poly(dimethyl siloxane) (PDMS)	Hydophobic, inert, low toxicity, optical transparency, elastomeric properties	Ophtalmologic devices, catheters and contact lenses, biosensors
Polyacrylates	poly(methyl methacrylate) (PMMA)	Rigid, inert	Intraocular lenses
	poly(hydroxyethyl methacrylate) (pHEMA)	Hydrophobic, non-degradable.	Anti-fouling hemocompatiblecoatings
Poly(tetrafluoroethylene)	Teflon®	Hydrophobic, non-degradable.	Vascular grafts
Polyolefins	poly(ethylene) (PE)	Hydrophobic, inert, can have different hardnesses depending on molecular weight	Containers, implantable devices
	poly(propylene) (PP)	Hydrophobic, inert, can have different hardnesses depending on molecular weight	Sutures, meshes
Polyester	poly(ethylene terephthalate) (PET)	Non-degradable polyester	Membranes, filaments, meshes, vascular grafts, ligament and tendon repair
	poly(glycolic acid) (PGA)	Degradable polyester	orthopedic fixation gadget, ligament and tendon repair, vascular stents
	poly-L-lactic acide (PLLA)	Degradable polyester	orthopedic fixation gadget, ligament and tendon repair, vascular stents

Table 1. Continued.

Chemical Group	Polymer	Properties	Application
Polyether	polyether ether ketone (PEEK)	High hardness	Orthopedic application
	Polyethylene glycol (PEG)	Hydrophilicity, low toxicity	
Polyamide	Nylon	High tensile strength	Suture, balloon of catheters for angioplasty
Polyurethane	Isocyanates/ polyols Urethanes	Excellent stability against oxidation and biodegradation, abrasive resistance	Foams, high-performance coatings, catheters, wound dressings
	Non-Isocyanate Urethanes (polyhydroxyurethanes)	Excellent stability against oxidation and biodegradation, abrasive resistance	Foams, high-performance coatings, catheters, wound dressings

Mechanisms that focus on microorganisms' behavior and characteristics as QS are specific and sometimes restricted to one specific strain or species. Materials grafted with antimicrobial compounds or modified surfaces have the advantage of expanding the antimicrobial effect to a broader spectrum of microorganisms [18,26]. Materials containing antimicrobials often refer to releasing systems also called leaching materials. Materials with a non-releasing modification on the surface are referred as anti-fouling or contact-active materials. These surfaces act via physical interference either by making the bacterial adhesion interactions weaker or by promoting bacterial membrane damage by contact, as for positively charged compounds.

Quaternary ammonium compounds (QAC) are antimicrobials used since 1930s as disinfectants [27] and their inclusion in oral care products date from the 1970s [28]. Their antimicrobial feature is exploited in many products such as soaps, contact lens solutions, shampoos, cosmetics and antiseptics in general. QAC molecules are characterized by a nitrogen atom (N) with four bonds (N^+) creating a positively charged molecule. Most common QAC molecules are: benzalkonium chloride, cetyltrimethylammonium bromide/chloride, cetylpyridinium chloride and quaternary ammonium methacrylates. There is extensive literature on the efficacy of these compounds against a wide variety of microorganisms including oral bacterial and fungal species (Table 2). The antimicrobial mechanism is based on electrostatic interactions with the negatively charged bacterial membrane causing its disruption and leakage of cytoplasmic material resulting in cell death [29,30].

Table 2. Frequently used QACs with corresponding molecular structure and spectrum of microorganisms to which there is antimicrobial activity.

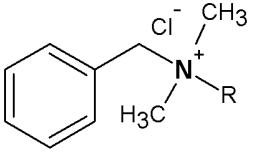
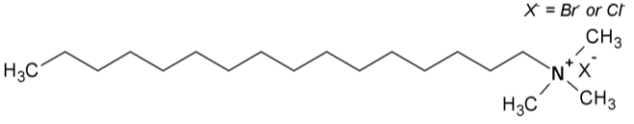
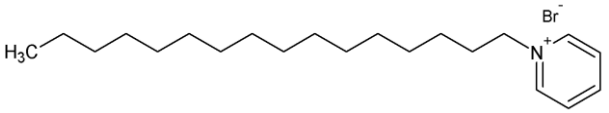
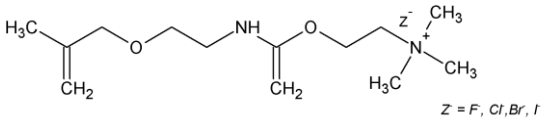
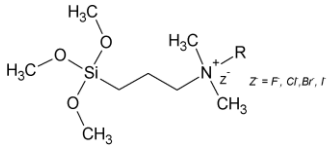
QAC	Molecule Structure Representation	Bacterial Strain
Benzalkonium Chloride		*Escherichia coli* [31] *Pseudomonas aeruginosa* [31] *Salmonella typhimurium* [31] *Legionella pneumophila* [31] *Enterococcus faecium* [31] *Staphylococcus aureus* [31] *Listeria monocytogenes* [31] *Campylobacter jejuni* [31]
Cetyltrimethylammonium bromide/chloride		*Streptococcus mutans* [33] *Staphylococcus aureus* [33] *Escherichia coli* [33] *Klebsiella pneumonia* [34]

Table 2. Continued

QAC	Molecule Structure Representation	Bacterial Strain
Pyridinium bromide compounds		*Streptococcus mutans* [30] *Streptococcus oralis* [30] *Streptococcus salivarius* [30] *Streptococcus gordonii* [30] *Streptococcus sobrinus* [30] *Actinomices naeslundii* [30] *Candida albicans* [30] *Candida glabrata* [30]
Quaternary ammonium methacrylates		*Streptococcus mutans*[30] *Enterococcus faecalis* [30] *Lactobacillus acidophilus* [30] *Staphylococcus aureus* [30]
Silicone quaternary ammonium salt		*Bacillus subtilis* [30] *Escherichia coli* [30] *Staphylococcus aureus* [30] *Aspergillus niger* [30]

AIM OF THIS THESIS

The aim of this thesis is to develop antimicrobial polymers containing quaternary ammonium compounds in dental composites or as coatings on metallic surfaces and investigate the killing efficiency against oral bacteria.

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