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Reduction of bacterial attachment on hydroxyapatite surfaces: Using hydrophobicity and chemical functionality to enhance surface retention and prevent attachment

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ABSTRACT

Water-soluble, linear polymers with high-acid functionality are commonly used in oral care formulations to provide benefits such as bioactive complexation and delivery, as well as inhibition of the bacteria deposition and colonization, commonly referred to as 'anti-attachment'. Unfortunately, structure-activity relationship (SAR) studies of these polymers are scarce, thus, a systematic approach to design polymers with a desired property (*e.g.* anti-attachment) is limited.

Multifunctional anti-attachment amphiphilic molecules (AMs) featuring a sugar backbone, hydrophobic arms, a poly(ethylene glycol) tail, and a chemical anchor effectively deposited on soft ceramic surfaces and reduced bacterial adhesion. The chemical compositions of the AMs were fine-tuned to better coordinate with dental enamel surfaces and prevent bacterial colonization. A graft-to approach was used to investigate the effect of the chemical anchor on AM deposition and retention. The chemical composition, absorption/desorption, and wettability properties of the bioactives and bioactive-coated surfaces were investigated using nuclear magnetic resonance, X-ray photon spectroscopy, quartz crystal microbalance, and contact angle. In addition, the ability of the AMs to provide anti-bacterial attachment on a simulated enamel surface was evaluated *in vitro* using bacterial repulsion assays. The SAR between surface retention and anti-attachment properties of the AMs demonstrates the feasibility and tunability of using these polymers as bioactive agents that provide anti-attachment benefits on dental enamel surfaces.

1. Introduction

Periodontitis and caries, both caused by oral bacterial biofilm formation, are the among most common oral diseases worldwide [1]. Although the mechanism of biofilm formation is complex, bacterial adhesion to teeth is considered the first step. This process is driven by both specific (*e.g.*, ligand-receptor) and non-specific interactions (*e.g.*, van der Waals, hydrogen bonding, acid-base, and electrostatic forces) [2,3]. Upon bacterial adhesion, the enamel is demineralized due to the bacteria's acidic metabolites, which leads to the formation of oral diseases, such as caries. Thus, inhibiting bacterial adhesion is a critical step to prevent the proliferation of these oral ailments.

Introducing an anti-attachment layer on the surface of teeth that repels bacteria can prevent biofilm formation. Prevention of bacterial colonization on oral-relevant surfaces has been investigated using hydrophilic polymers [4], anionic polymers [5–7], and branched polymer systems [8,9]. To varying extents, these polymers can inhibit bacterial attachment on oral-relevant surfaces. The level of attachment is likely due to the culmination of several factors, such as hydrophobicity, branching, and functional end groups of the polymer. However, determining the appropriate balance of these properties remains a challenge. A thorough understanding of the structure-activity relationship (SAR) is imperative to design the optimal polymer for anti-bacterial attachment.

In designing these polymers, hydrophobicity can play a role in preventing bacteria from depositing on the surface. Traditionally, hydrophilic polymers have been used as anti-attachment

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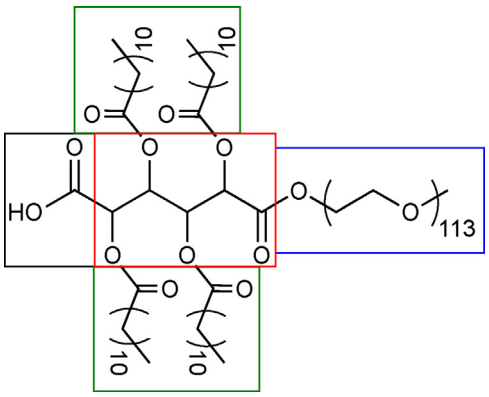

Fig. 1. Structural features of AMs are highlighted using colored boxes: red is the sugar backbone, green is the hydrophobic arms, black is the chemical anchor, and blue is the PEG tail. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

layer due to their ability to deter bacterial adhesion and protein adsoprtion [10,11]. For example, polyurethane surfaces modified with poly(ethylene glycol) (PEG) reduced bacterial adhesion more effectively than poly(propylene glycol) [11]. The electrostatic interactions between the polymer and the surface have also been exploited to enhance polymer deposition. For example, α,β-polyaspartate, an anionic polymer, was effective in reducing levels of attached microflora on hydroxyapatite (HAP; $Ca_{10}(PO_4)_6(OH)_2$) coated discs [5]. The carboxylate functional groups of the polymer modulates adhesion by competitively binding to the calcium on the HAP surface; thereby creating a negatively charged surface repelling bacteria.

HAP is the main component of dental enamel, and is largely composed of calcium ions that result in an inherent binding affinity toward negatively charged bacteria [12–14]. To modulate this effect, polymers composed of carboxylates and phosphates have been used to create electrostatic repulsion between the bacteria and the polymer-treated surface [15–20]. In general, negatively charged polymers efficiently adsorb onto HAP due to electrostatic interactions with calcium ions [5–7]. Phosphates have additionally been shown to efficiently chelate the calcium ions [21–23] and prevent demineralization of HAP [24,25]. Therefore, using polymers with phosphate or carboxylate groups can ensure efficient deposition onto HAP while simultaneously increasing the surface electronegativity to promote bacterial repulsion [26].

The aim of this study was to understand the design principles required to create an anti-attachment barrier to prevent biofilm formation on HAP. Studies show that pretreating enamel with polymers is a facile approach to prevent oral biofilm formation [19]. In this work, the preparation of amphiphilic macromolecules (AMs) featuring a sugar backbone with hydrophobic arms, a PEG tail, and a carboxylate or phosphate anchor (Fig. 1) is detailed. The PEG portion of the molecules was selected for its biocompatibility [27] and anti-attachment [28] properties. The carboxylate and phosphate groups were chosen for their ability to bind calcium ions. Finally, the degree of branching and hydrophobicity on surface retention were also investigated. Polymer compositions were confirmed using nuclear magnetic resonance (NMR) and gel permeation chromatography (GPC) techniques. The coating properties of the polymers including the composition, deposition, and retention of the polymer-modified surface were determined using X-ray photoelectron spectroscopy (XPS) and quartz crystal microbalance with dissipation (QCM-D). Lastly, the anti bacterial attachment properties of the polymer-modified surfaces were evaluated against early colonizing bacteria, *S. oralis* and *A. viscosus*, which facilitate pathogenic bacteria to bind thereby creating a biofilm [29,30].

2. Materials and methods

2.1. Materials

4-Dimethylaminopyridine (DMAP), dichloromethane (DCM), *N,N*-dimethylformamide (DMF), *N,N*-dicyclohexylcarbodiimide (DCC) solution (1 M in DCM), 1,1′-carbonyldiimidazole (CDI), lauroyl chloride, caproyl chloride, triethylamine (Et_3N), tetrahydrofuran (THF), sodium chloride, mucic acid, tartaric acid, 2-aminoethyl dihydrogen phosphate, magnesium sulfate, chloroform, pyridine, palladium on carbon, zinc chloride, sodium citrate, citric acid, sand, celite 512 medium, sodium carbonate, toluene, diethyl ether (Et_2O), and chloroform-*d* ($CDCl_3$) were used as received from Aldrich. Poly(ethylene glycol) methyl ether (M_n = 5000 Da) was azeotropically distilled with toluene prior to use. Ethyl acetate (EtOAc), hexanes (hex), methanol (MeOH), and hydrochloric acid (HCl) were used as received from Fisher. Dibenzyl L-tartrate was used as received from TCI chemicals. Phosphate buffered saline (PBS) was used as received from Gibco. *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI) was used as received from AK Scientific. Hydroxyapatite coated MBEC lids were used as received from Innovotech. BacTiter-Glo microbial cell viability assay was used as directed from Promega. Trypticase soy broth was used as received from BD. *Actinomyces viscosus* (ATCC#43146) and *streptococcus oralis* (ATCC#35037) were purchased from ATCC. HAP coated MBEC™ lids were purchased from Innovotech.

2.2. Techniques

^{1}H NMR (400 MHz, 500 MHz), ^{13}C NMR (100 MHz, 125 MHz), and ^{31}P NMR (162 MHz, 202 MHZ) spectra were recorded on a Varian NMR spectrometer. Peak multiplicities are denoted as follows: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, and m = multiplet. GPC in DCM (1 mL/min) was performed using a Waters 1515 liquid chromatography pump system equipped with a 2414 RID, 717 plus auto sampler, and a Grace Jordi GPC column of mixed-bed linear (5 μm).

2.3. AM Synthesis

2.3.1. Preparation of T6

2.3.1.1. Dibenzyl(2R,2S)-2,3-Bis(hexanoyloxy)butanedioate (DBT-T6). A solution of dibenzyl-tartaric acid (1.00 g, 3.03 mmol) in $CHCl_3$ (10 mL) with Et_3N (1.1 mL, 7.6 mmol) and a catalytic amount of DMAP (47.2 mg, 0.35 mmol) was gradually added to an ice-cooled solution of caproyl chloride (0.85 mL, 6.06 mmol) in $CHCl_3$ (10 mL). The reaction mixture was allowed to warm to room temperature and stirred for an additional hour under a N_2 atmosphere. The reaction mixture was diluted with $CHCl_3$ and washed with 1 N HCl, 10% (w/v) $NaHCO_3$ solution, brine and then dried with anhydrous $MgSO_4$. The solids were removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was precipitated into cold hexanes to yield **DBT-T6** as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.33 (m, 10H), 5.74 (s, 2H), 5.20 (dd, 4H), 2.24 (quint, 2H), 2.10 (quint, 2H), 1.53 (m, 4H), 1.27 (m, 8H), 0.87 (t, 3H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 172.4, 165.7, 134.8, 128.6, 128.5, 70.5, 67.7, 33.4, 31.1, 24.2, 22.2, 13.9.

2.3.1.2. (2R,3R)-2,3-Bis(hexanoyloxy)butanedioic acid (T6). A solution of compound **DBT-T6** (0.70 g, 1.32 mmol) in EtOAc (13 mL) was subjected to catalytic hydrogenolysis (Pd/C) for 19 h. The catalyst was removed by filtration using a Celite™ filter using DCM/MeOH