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Do quaternary ammonium monomers induce drug resistance in cariogenic, endodontic and periodontal bacterial species?

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ABSTRACT

Objectives. Antibacterial monomers were developed to combat oral biofilm acids and caries; however, little is known on whether quaternary ammonium monomers (QAMs) would induce drug resistance in oral bacteria. The objective of this study was to investigate the effects of new antimicrobial monomers dimethylaminohexadecyl methacrylate (DMAHDM) and dimethylaminododecyl methacrylate (DMADDM) on the induction of drug resistance in eight species of cariogenic, endodontic and periodontal bacteria for the first time.

Methods. *Streptococcus mutans* (*S. mutans*), *Streptococcus sanguis*, *Streptococcus gordonii*, *Enterococcus faecalis* (*E. faecalis*), *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*), *Fusobacterium nucleatum* (*F. nucleatum*), *Porphyromonas gingivalis* (*P. gingivalis*), and *Prevotella intermedia* (*P. intermedia*) were tested. Minimum inhibitory concentration (MIC) was assessed using chlorhexidine (CHX) as control. Minimal bactericidal concentration (MBC), bacterial growth and membrane permeability properties were also investigated.

Results. CHX induced drug resistance in four species. DMAHDM did not induce any resistance. DMADDM induced drug resistance in only one benign species *S. gordonii*. The DMADDM-resistant and CHX-resistant *S. gordonii* had the same MIC and MBC values as *S. gordonii* parental strain against DMAHDM ($p > 0.1$), hence DMAHDM effectively inhibited the resistant strains. The resistant strains had slower growth metabolism than parental strain.

Significance. DMAHDM induced no drug resistance, and DMADDM had much less drug resistance than the commonly-used CHX in the eight common oral species. With its potent antimicrobial functions shown previously, the new DMAHDM is promising for applications

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in restorative, preventive, periodontal and endodontic treatments to combat cariogenic and pathological bacteria with no drug resistance in all tested species.

1. Introduction

Oral diseases such as caries, periapical disease and periodontal disease are directly associated with oral biofilm communities containing hundreds of species of bacteria [1]. Pathological changes in the biofilm composition can lead to infections which affect oral health as well as systemic diseases [2]. The emergence of certain bacterial strains becoming resistant to antibiotics could lead to severe and refractory infections. Indeed, antimicrobial resistance has become a global issue, which is not only a serious threat to global public health but also increases the cost of health care. Among various biofilms, oral biofilms are complex bacterial communities that tend to be highly resistant to antibiotics and the human immune defense [3]. To date, little has been reported on the investigation of possible antimicrobial drug resistance in oral bacteria with the use of quaternary ammonium monomers (QAMs) in dentistry.

Quaternary ammonium salts (QAS) have been widely used in water treatments, the food processing industry, textiles and surface coatings since the mid-1930s [4]. Due to their low toxicity and a broad spectrum of antimicrobial activity, QAS were first incorporated into mouth rinses to inhibit oral biofilms in the 1970s [5]. Antibacterial dental resins have attracted increasing attention; chlorhexidine (CHX) [6], silver [7] and several QAMs have been incorporated into resins to reduce biofilm growth, acid production and secondary caries [8–12]. Pioneering work by Imazato et al. developed 12-methacryloyloxydodecyl-pyridinium bromide (MDPB) and incorporated it into dental resins, showing effective suppression of biofilm growth [8]. Since then, several different antimicrobial compositions have been developed, including quaternary ammonium polyethylenimine (QPEI) [9], methacryloxylethyl cetyl dimethyl ammonium chloride (DMAE-CB) [10], dimethylamino dodecyl methacrylate (DMADDM) [11], and dimethylaminohexadecyl methacrylate (DMAHDM) [12]. The antibacterial mechanism of QAMs is suggested to be the action of causing bacterial cell membrane lysis via the binding to bacterial membranes [13].

Although the antimicrobial effects of QAMs have promising clinical benefits, their frequent use may lead to a selection of resistant strains or the emergence of acquired resistance to those sensitive strains [14]. Indeed, bacterial drug resistance is an increasingly worrying phenomenon [14]. For example, *Staphylococcus aureus* (a common cause of skin infections, respiratory infections and food poisoning), *Serratia marcescens* (involved in hospital-acquired infections such as urinary tract infections and wound infections) and *Escherichia coli* (occasionally responsible for product recalls due to food contamination) have been reported to have acquired resistance against QAS [15–17].

However, there are very few reports on the investigation of oral bacteria potentially developing resistance to QAMs used in antibacterial dental materials. A literature search revealed that, to date, there is only one publication on this important topic [18]. That study showed that after serial exposures to cationic biocides, *Streptococcus mutans* (*S. mutans*) (commonly found in human oral cavity), and *Enterococcus faecalis* (*E. faecalis*) (closely associated with apical periodontitis) did not exhibit resistance to MDPB after repeated exposures, while *E. faecalis* developed drug resistance to chlorhexidine (CHX) [18]. With the increasing efforts in developing a new generation of antibacterial dental resins, studies are urgently needed to investigate the possible antibacterial resistance of QAMs against oral bacteria.

Several oral streptococci strains are associated with dental caries. Acidogenic species, e.g., *S. mutans*, and aciduric species *Streptococcus sanguis* (*S. sanguis*) and *Streptococcus gordonii* (*S. gordonii*) play important roles in microecological balance regulations [18,19]. In addition, *E. faecalis* is a microorganism commonly detected in persistent endodontic infections, often leading to root canal treatment failures due to its various virulence factors [20,21]. Furthermore, several subgingival microbiota including *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*), *Fusobacterium nucleatum* (*F. nucleatum*), *Porphyromonas gingivalis* (*P. gingivalis*) and *Prevotella intermedia* (*P. intermedia*) are associated with aggressive chronic periodontitis or refractory periodontitis [22,23]. Therefore, the three common caries-related species (*S. mutans*, *S. sanguis* and *S. gordonii*), one common periapical periodontitis-related species (*E. faecalis*), and four common periodontitis-related species (*A. actinomycetemcomitans*, *F. nucleatum*, *P. gingivalis* and *P. intermedia*) were selected for the present study. We recently developed new QAMs, including DMADDM (with alkyl chain length of 12) and DMAHDM (with alkyl chain length of 16), whose chemical structures are shown in Fig. 1. They exhibited potent antimicrobial effects in suppressing biofilm growth and acid production [10,13]. However, there has no report on the investigation of potential bacterial drug resistance induced by DMADDM and DMAHDM.

Therefore, the objective of this study was to determine the potential drug resistance induced by DMADDM and DMAHDM against the aforementioned eight species of oral bacteria for the first time. The commonly-used agent CHX served as control. It was hypothesized that: (1) The new antibacterial monomers DMADDM and DMAHDM would not induce drug resistance against the eight species of oral bacteria; (2) CHX would induce resistance in several species of the tested oral bacteria; (3) The impermeability of the bacterial membranes would contribute to the development of drug resistance in the bacteria.

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