Controlling the oral biofilm with antimicrobials

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1. Introduction

The mouth provides an environment conducive to the colonisation and growth of a diverse range of microorganisms, of which bacteria are the most common and numerous. The largest accumulations of bacteria are found as biofilms on the tooth surface (dental plaque); desquamation ensures that the microbial load is lower on mucosal surfaces. The presence of these microbes on all accessible surfaces of the mouth is natural, and is essential for the normal development of the physiology of the oral cavity. The resident microflora contributes to the health of the host by preventing exogenous, and potentially pathogenic, micro-organisms, from becoming established in the mouth (“colonisation resistance”), and by regulating the inflammatory host response to oral commensal bacteria. Disruption of the resident oral microflora can result in overgrowth by previously minor components of the microflora (e.g. yeasts following antibiotic treatment), or colonisation by environmental organisms; such disruption can increase the risk of disease. In general, the mouth is colonised by a rich collection of beneficial micro-organisms that live in harmony with the host, providing benefit to both parties. Thus, oral healthcare products should attempt to control the levels of plaque rather than trying to eliminate it, so as to retain the beneficial properties of the resident oral microflora.

2. Dental plaque as a biofilm

Dental plaque forms via an ordered sequence of events. Early colonising bacteria attach to the acquired pellicle that adsorbs immediately to the tooth surface after cleaning. These early colonisers grow, modify the environment, and make conditions suitable for colonisation by later, more fastidious bacteria, many of which are obligately anaerobic. Attached organisms synthesise exopolymers such as glucans, which form the biofilm matrix that acts as a scaffold for the biofilm, and is biologically active and able to retain molecules within plaque. Eventually a thick biofilm develops made up of
a diverse community of interacting micro-organisms\textsuperscript{6}, and the composition becomes stable over time (microbial homeostasis). The properties of biofilms are distinct from those of the same species growing planktonically (i.e. in conventional liquid culture). Biofilms have a phenotype that is more tolerant of antimicrobial agents, stress, and the host defences than planktonic cultures, making them difficult to treat\textsuperscript{7}.

3. Plaque biofilms and disease

On occasion, microbial homeostasis breaks down in dental plaque, and disease occurs. The microflora from diseased sites is markedly different from that seen in health\textsuperscript{5}. These shifts in microflora occur as a response to changes in environmental conditions that alter the competitiveness of the bacteria, resulting in the selection of previously minor components within the microbial community (ecological plaque hypothesis)\textsuperscript{8}. In caries, the regular intake in the diet of fermentable carbohydrates leads to plaque spending more time at a low pH, eventually favouring acidogenic and acid-tolerating species, such as mutans streptococci, other acidogenic streptococci, lactobacilli and bifidobacteria, while inhibiting health-associated bacteria that prefer a neutral pH. In contrast, in periodontal disease, excessive plaque accumulation around the gingival margin causes an inflammatory response. The flow of gingival crevicular fluid (GCF) is increased if the host is unable to control this microbial insult, which not only introduces further components of the host response but also molecules such as haemoglobin, haptoglobin and transferrin which select for proteolytic bacteria. These proteolytic bacteria can also degrade host molecules that regulate inflammation, resulting in an exaggerated and inappropriate inflammatory response that can be severe enough to cause bystander damage to host tissues. Culture studies of biofilms from periodontal pockets have shown an increase in biomass and higher proportions of Gram-negative, obligately anaerobic, proteolytic bacteria\textsuperscript{9,10}. Molecular studies have detected an even greater diversity of bacteria from diseased sites\textsuperscript{11,12}, of which >50% are presently unculturable\textsuperscript{13}.

4. Control of oral biofilms

Dental diseases can be controlled by meticulous mechanical oral hygiene. However, most individuals have difficulty in maintaining the necessary standards of plaque control for prolonged periods. Additional approaches are being developed that are less dependent on the dexterity of the patient, and which augment conventional oral hygiene methods and keep plaque at levels compatible with oral health. Consequently, many oral care products are now formulated to contain proven antiplaque and antimicrobial agents to help achieve this goal\textsuperscript{14–16}.

Antiplaque agents are designed to (a) prevent the formation of the biofilm, and/or (b) remove established biofilm. In contrast, the mode of action of antimicrobial agents involves inhibiting the growth or killing the target bacteria, expressed in terms of their Minimum Inhibitory Concentration (MIC) or Minimum Bactericidal Concentration (MBC), respectively. Typically, the MIC/MBC of an agent is determined in the laboratory on liquid grown (planktonic) cells in tests where the agent is in contact with a pure culture of the organism for prolonged periods (24–48+ hours). However, bacteria growing on a surface as a biofilm show reduced sensitivity to killing by antimicrobial agents, especially in older (more mature) biofilms. The reasons for this vary among the inhibitors but include (a) reduced penetration of the agent, e.g. due to binding to the biofilm matrix or quenching of the agent at the surface of the biofilm, (b) the novel phenotype expressed by bacteria when growing on a surface, and (c) the slow growth rates of attached bacteria within biofilms\textsuperscript{17}. Moreover, the maximum length of time recommended for people to brush their teeth or rinse with a mouthwash is in the order of two minutes. A major requirement of the formulation, therefore, is to deliver sufficient concentration of the inhibitor in those two minutes to ensure retention on dental and mucosal surfaces in the mouth so that the active components can be released over time at levels that will still deliver biological activity; a representation of an antimicrobial flux curve in the mouth is shown in Figure 1. This property of product retention is termed substantivity, and

![Fig. 1 – Pharmacokinetics of antimicrobial agents delivered to the mouth. A schematic representation of the change in concentration over time following the delivery to the mouth on two occasions of an antimicrobial agent from an oral care product. The agent may be present above its MIC/MBC level for a relatively short period before it is lost from the mouth. The agent may be present for longer at sub-lethal concentrations; agents may still exert beneficial effects by inhibiting traits associated with bacterial pathogenicity. The dynamics of the curve will vary for each antimicrobial agent.](image-url)
varies markedly among antimicrobial agents. The most effective agents tend to show enhanced substantivity.

The pharmacokinetic profile (high concentration – short contact time; low concentration – long contact time) of orally-delivered antimicrobials has a significant influence on the mode of action of these agents. Studies with antibiotics have demonstrated that organisms with an apparent similar sensitivity to a drug (as determined in a standard MIC assay format – i.e. fixed concentration of agent/long contact time) can vary markedly in their susceptibility when exposed to the agent for only relatively short periods. Similarly, conventional MIC data did not predict the observed sensitivity of oral bacteria when triclosan was pulsed into a mixed culture laboratory model, giving a more realistic short contact time. Under these more relevant test conditions, triclosan was far more active against the Gram-negative anaerobes implicated in gingivitis and periodontal disease, whereas the Gram-positive species associated with oral health, and which had a similar or lower MIC than the periodontopathogens, were relatively unaffected.

5. Classes of inhibitors used as antiplaque/antimicrobial agents

A wide range of agents have been formulated into oral care products in order to enhance their plaque control potential (Table 1). Chlorhexidine displays good substantivity, and approximately, 30% of chlorhexidine dosed from a mouthwash is retained in the mouth. Chlorhexidine has a broad spectrum of activity against Gram-positive and Gram-negative bacteria, and yeasts, and can reduce plaque, caries and gingivitis. At high concentrations, chlorhexidine is bactericidal, and causes lethal damage to the bacterial membrane. At sub-lethal concentrations, chlorhexidine can interfere with the metabolism of oral bacteria by inhibiting (a) sugar transport and acid production in cariogenic streptococci, (b) various membrane functions in streptococci, including inhibiting enzymes responsible for maintaining an appropriate intracellular pH, and (c) a major protease (gingipain) in the periodontal pathogen, *Porphyromonas gingivalis*. Attempts have been made to deliver enzymes such as dextranases and glucanases in oral care products in order to disrupt the structure of the biofilm by destroying the plaque matrix. Essential oils such as menthol, thymol, eucalyptol and methyl salicylate have been incorporated into mouthrinses. These products can reduce the levels of dental plaque and gingivitis in clinical trials; these reductions were also accompanied by the inhibition of Gram-negative anaerobic species in supra- and subgingival plaque. Metal salts are highly substantive, and are active against Gram-positive and Gram-negative bacteria. They also have a valuable mode of action at sub-lethal levels; for example, zinc can inhibit sugar transport, acid production and protease activity. Plant extracts are being investigated as a source of potentially novel compounds; for example, apigenin and tt-farnesol can inhibit cariogenic traits of mutans streptococci and display anti-caries activity in rodent models. Triclosan is used in several oral care products, and has a purported broad spectrum of antimicrobial activity; it can also reduce inflammation. Sub-lethal levels of triclosan can also inhibit acid production by oral streptococci and protease activity by *P. gingivalis*. Additive anti-plaque and anti-gingivitis effects were reported when triclosan was combined with a complementary antimicrobial agent such as zinc. The half-life for clearance of bound triclosan is ca. 20 minutes, compared to ca. 45 minutes for zinc, although triclosan can be detected in plaque for at least eight hours after toothbrushing. Quaternary ammonium compounds such as cetyl pyridinium chloride (CPC) have been used widely in mouthrinses. CPC has good antimicrobial activity, but it loses activity when adsorbed to a surface, and is less substantive than some molecules. Detergents are present in most toothpastes, and surfactants such as sodium lauryl sulfate can disrupt biofilm structure, damage cell membranes and kill bacteria (when present at high concentrations) and inhibit enzymes (at lower concentrations).

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<tr>
<th>Class of inhibitor</th>
<th>Examples</th>
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<tr>
<td>Bisbiguanide</td>
<td>chlorhexidine</td>
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<tr>
<td>Enzymes</td>
<td>mutanase, glucanase; amyloglucosidase-glucose oxidase</td>
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<tr>
<td>‘Essential oils’</td>
<td>menthol, thymol, eucalyptol</td>
</tr>
<tr>
<td>Metal ions</td>
<td>copper, zinc, stannous</td>
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<tr>
<td>Natural molecules</td>
<td>plant extracts (apigenin, tt-farnesol)</td>
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<tr>
<td>Phenols</td>
<td>Triclosan</td>
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<td>Quaternary ammonium compounds</td>
<td>Cetyl pyridinium chloride</td>
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<tr>
<td>Surfactants</td>
<td>Sodium lauryl sulphate, delmopinol</td>
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6. Function of antiplaque/antimicrobial agents in oral care products
Oral health care products that contain antimicrobial agents to control plaque biofilms are required to deliver two apparently contradictory requirements in order to meet regulatory guidelines. These are to deliver a relevant and measurable clinical and microbiological benefit, while at the same time not disrupting the natural microbial ecology of the mouth, which might result in overgrowth by opportunistic pathogens (e.g. yeasts) or exogenous micro-organisms. An appreciation of some of the issues discussed earlier with respect to the properties of biofilms (i.e. reduced sensitivity) and to the pharmacokinetic issues surrounding the retention and penetration of agents into dental plaque can help reconcile these conflicting requirements. Although many antimicrobial agents used in oral care products are described as being broad spectrum, under the conditions of use in the mouth (variable concentration/short contact times) they probably have a favourably selective mode of action in which they mainly inhibit the growth and metabolism of organisms implicated in disease while leaving those associated with oral health relatively unaffected. In this way, they can function prophylactically to stabilise the normal microbial composition of plaque, even under conditions that may otherwise predispose a site to caries or gingivitis, thereby maintaining the benefits derived from the resident microflora.

7. Concluding remarks
From the arguments outlined above, it is clear that the function of antimicrobial agents delivered via over the counter products for regular, unsupervised use in the home is entirely different to that required from antibiotics deployed to treat an infection caused by a conventional medical pathogen. The aim of oral care products is to control dental plaque rather than eliminate it, whereas antibiotics are prescribed to kill the infectious agent. A significant challenge for the future will be to develop even more effective products that are able to improve clinical efficacy while still preserving the benefits of the normal, resident oral microflora.

8. Conflict of interest statement
The author is in receipt of a fee to write this article, and receives occasional consultancies from oral care companies.

References
