

REVIEW ARTICLE

Prospects of oral disease control in the future – an opinion

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The mouth supports a diverse microbiota which provides major benefits to the host. On occasions, this symbiotic relationship breaks down (dysbiosis), and disease can be a consequence. We argue that progress in the control of oral diseases will depend on a paradigm shift away from approaches that have proved successful in medicine for many diseases with a specific microbial aetiology. Factors that drive dysbiosis in the mouth should be identified and, where possible, negated, reduced or removed, while antimicrobial agents delivered by oral care products may function effectively, even at sub-lethal concentrations, by modulating the activity and growth of potentially pathogenic bacteria. In this way, the beneficial activities of the resident oral microbiota will be retained and the risk of dysbiosis occurring will be reduced.

Keywords: *plaque control; biofilm; oral microbiome; antimicrobial agents; modelling*

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Currently, one of the principal objectives of many grant funding bodies is to drive the translation of research findings into tangible public health benefits. It is apparent during visits to general medical practitioners that there has been considerable progress made in this area in medicine. There are new diagnostic tests, many of which are non-invasive, complemented by advances in drugs, vaccines and other interventions. There has also been progress towards assessing future risks of disease in patients, so that treatment can be targeted to their individual needs (personalised medicine). In contrast, an individual attending a routine appointment at a dentist would recognise fewer equivalent developments, either in diagnosis or treatment. This is despite the fact that in recent years we have seen advances in state-of-the-art molecular approaches being applied to extend our knowledge of the oral microbiome, the architecture of dental biofilms and the complexity of the host defences, as well as in the biochemistry of saliva and gingival crevicular fluid.

Many of the concepts being adapted and applied to improve the diagnosis and treatment in oral biology have been derived from recent advances in medicine. Perhaps it is now time to accept that we need a paradigm shift away from these approaches and recognise that plaque-related diseases are a consequence of a dysbiosis in a normally beneficial oral microbiome, which also might invoke an

inappropriate and damaging host response. Approaches that are being applied to understand and treat dysbiotic conditions that arise in other sites in the body, for example, inflammatory bowel disease or Crohn's disease, may provide more relevant models for oral disease. If we accept this premise, oral disease control could involve more subtle approaches. For example, rather than screening for broad-spectrum antimicrobial agents (defined in a conventional way by MIC/MBC criteria) for use in oral care products to target particular 'pathogens', and which could inflict considerable by-stander damage to the oral microbiome, we should consider applying ecological principles to control rather than eliminate components of the microbiota, and identify and inhibit/remove the factors that are driving the dysbiosis in that patient (1). Some of the issues to consider when contemplating the control of the oral microbiota in this way will be described briefly in the following sections.

Key factors to consider when attempting to control the oral microbiota

1. **The oral microbiome is natural and beneficial.** In medicine, the physician is often faced with treating an infection caused by specific micro-organisms not normally found at that site, or by microbes that have

colonised a site that is normally sterile. In contrast, the mouth has a microbiome that is natural and is essential for the normal development of the physiology of the host (2). Some of the beneficial aspects of the oral microbiota include:

- Colonisation resistance, for example, exclusion of exogenous micro-organisms (3);
- Immunomodulatory activity, for example, down-regulation of excessive pro-inflammatory responses to the resident microbiota on mucosal surfaces (4); and
- Enhancement of host defences and host physiology, for example, the metabolism of dietary nitrate to nitrite leads to a reduction in blood pressure, a stimulation of mucous production, and the generation of antibacterial nitric oxide (5).

Therefore, the eradication of the oral microbiome should not be attempted as these organisms have evolved to have a synergistic relationship with the host. Rather, the beneficial members of the oral microbiome should be nurtured and their relative proportions maintained, although research is needed to more fully characterise the composition and metabolic functions of the resident oral microbiota.

2. The oral microbiome exists as biofilms and microbial communities.

Micro-organisms grow on oral surfaces in structurally and metabolically organised communities of interacting species termed biofilms (6). The properties of these microbial communities are more than the sum of the component organisms. The combination of the oral microbiome being both a biofilm and a microbial community makes it particularly difficult to treat with antimicrobial agents (7). There are a number of mechanisms whereby these complex structures are more tolerant of inhibitors, including restricted penetration of agents into the depths of the biofilm, the slow growth rate and an altered phenotype of surface-associated microbes, and cross-protection by neighbouring cells.

Traditionally, microbiologists have focussed on the 'names' of species present in a sample such as dental plaque. Selected representative species are then grown planktonically in pure culture to evaluate the spectrum of activity of antimicrobial agents using conventional MIC/MBC read-outs as applied routinely in medical microbiology. The application of contemporary molecular approaches to understand and define the diversity and metabolism of the oral microbiota is starting to challenge this approach. The Human Microbiome project has used metagenomics to characterise the microbiota at several sites around the body. When the microbial profiles of several hundred subjects were compared, there was a degree of heterogeneity in the individual taxa

that were recovered from any one body site, such as the mouth, but when the samples were analysed in terms of biochemical functions (e.g. ATP synthesis, central carbohydrate metabolism) then a huge degree of consistency was observed (8). The inference was that different species can perform identical roles in a microbial community. Therefore, a way forward could be to consider developing assays to screen for (a) antimicrobial agents that inhibit key metabolic 'functions' rather than targeting specific bacterial species, and/or (b) prebiotics that promote the growth of resident microbial populations with beneficial functions.

3. There is a shift in the composition and metabolism of the oral microbiome in disease.

Numerous studies, using either traditional culture or contemporary molecular approaches to compare the microbiota in biofilms from healthy surfaces with that from sites with dental caries and periodontal diseases, have shown that there are substantial differences in the composition of the microbiota in disease (2). Many of the bacteria associated with disease (often referred to as 'pathogens') can be found in biofilms from healthy sites, but they are present in clinically irrelevant numbers and at a far lower frequency (9). Therefore, disease is due to a shift in the composition of the biofilm (dysbiosis) rather than as a result of exogenous 'infection'. Ideally, when a patient presents with disease, a clinician should attempt to determine the factors responsible for driving dysbiosis (e.g. impaired saliva flow; poor oral hygiene; inappropriate lifestyle, including dietary habits; presence of other risk factors), while recognising that these could vary from patient to patient. Unless there is an attempt to interfere with the factor(s) driving the dysbiosis then the patient is likely to return to the surgery suffering from further episodes of disease (10). Thus, oral disease control requires a holistic approach.

4. Antimicrobial paradox applies to the mouth.

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, for example, by permitting overgrowth by opportunistic pathogens (e.g. yeasts) or exogenous micro-organisms (11). Most antimicrobial agents used in oral care products are described as being broad spectrum (see earlier), but under the conditions of use in the mouth (twice daily for brief periods against drug tolerant biofilms) they are present at MIC or MBC levels for a relatively short time, with some retained

for many hours at sub-lethal concentrations (e.g. metal salts, triclosan) (12). At these levels, these agents may have an important mode of action which is consistent with this new paradigm for oral care. At sub-lethal concentrations, these agents can target key virulence traits such as sugar transport/acid production and protease activity, while also generally slowing bacterial growth. In this way, they may have a more 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 (1). The use of any antimicrobial agent carries the potential for bacterial resistance to develop, especially if used at sub-lethal concentrations. However, there is no evidence for a change in MIC to agents used in oral care products following long-term clinical use (13–15). Unlike the principles behind antibiotic therapy, oral care products could function prophylactically to stabilise the normal oral microbiota under conditions that may otherwise predispose a site to caries or gingivitis, thereby maintaining the benefits derived from the resident microbiome.

In order to pursue these theories, we have initiated *in silico* modelling studies to model in a biofilm the behaviour of two distinct microbial populations that differ in their aciduricity. This model is based on a standard hybrid algorithm that has been widely employed in studies of environmental biofilms, and couples individual cells to continuous dispersed phases within a defined environment. Our computational studies demonstrate how small changes to the local oral environment can have a major impact on the competitiveness of oral bacteria. The findings can be extrapolated to show how sub-lethal interference with the fluctuations in pH in a biofilm following dietary sugar intake can alter the proportions of different groups of bacteria over time. As shown in Fig. 1a, by varying the buffering capacity of the plaque fluid, it was possible to modulate the biofilm composition between a weakly acidogenic state with a low fraction of aciduric bacteria, to a dysbiotic state where the aciduric population dominated, resulting in a lower pH and an increased risk of enamel demineralisation. A similar trend was observed when the frequency of sugar intake was increased, as shown in Fig. 1b. Both of these measured trends were a sub-lethal consequence of differential growth between the two bacterial groups, and demonstrated that environmental alterations, such as those achieved by a putative external agent that reduces the rate or frequency of fall in environmental pH, can beneficially modulate biofilm community dynamics without requiring any form of direct lethal antimicrobial action. Full details of the model,

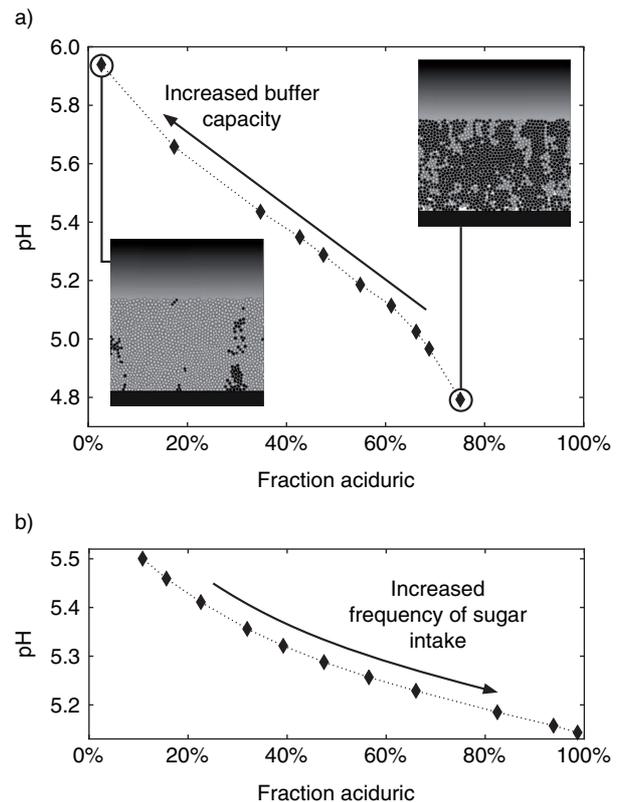


Fig. 1. The effect of varying (a) the buffering capacity of the plaque fluid, and (b) the frequency of dietary sugar intake, on the proportions of non-aciduric (light grey discs) and aciduric (black discs) populations in an *in silico* simulation of biofilm development. (a) Increased buffering (here achieved by lowering the effective dissociation constant from $K_a^{\text{eff}} = 10^{-8}$ to 10^{-10}) resulted in a higher pH during sugar intake (vertical axis), and reduced proportions of aciduric bacteria (horizontal axis). Image insets give representative examples of the composition of simulated biofilms at each end of the range sampled, where light grey (black) discs correspond to non-aciduric (aciduric) bacteria, respectively. (b) Increasing the frequency of sugar intake from once every 10 h to once every 2 h resulted in the opposite trend, i.e. an increase in the proportions of aciduric bacteria. Data and images were taken after 20 days of sugar pulsing, starting from an initial state with equal proportions of aciduric and non-aciduric bacteria. Error bars were smaller than the symbols.

including a range of other perturbations that similarly alter both biofilm composition and pH, can be found elsewhere (16).

Concluding remarks

It has been argued that advances in the control of oral diseases will require a move away from approaches that have proved successful in many areas of medicine. This may require a more holistic approach in which we monitor and manipulate the composition and metabolism of the oral microbiome in order to maintain the beneficial activities

we derive from their presence and activity, while minimising the impact of any environmental and lifestyle factors that might lead to dysbiosis in the future.

Conflict of interest and funding

There is no conflict of interest in the present study for any of the authors.

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