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Dental plaque as a biofilm and a microbial community – implications for treatment

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Abstract

Background. The mouth supports a diverse microbiota which exists as structurally-organised biofilms on mucosal and dental surfaces. The oral microbiota provides major benefits to the host including: (a) colonisation resistance, (b) down-regulation of potentially damaging host inflammatory responses, and (c) active contributions to the normal development of the physiology of the mouth and the host defences.

Highlight. Generally, these communities live in harmony (symbiosis) with the host but, on occasions, this symbiotic relationship breaks down and disease occurs (dysbiosis). Disease is associated with shifts in the balance of the oral microbiota driven by changes in the local environment. These changes include more regular conditions of low pH in the biofilm, as a result of an altered diet or reduced saliva flow, thereby favouring the growth and metabolism of acidogenic and acid-tolerating bacteria, at the expense of beneficial oral micro-organisms, and increasing the risk of dental caries. The host mounts an inflammatory response if biofilm accumulates around the gingivae beyond levels compatible with health. If this fails to reduce the biomass, the altered environment selects for increased proportions of obligately anaerobic and proteolytic species that can subvert the host response leading, ultimately, to pocket formation and loss of attachment.

Conclusion. An appreciation of ecological principles can lead to new strategies for treatment by identifying and removing the factors that drive dysbiosis, while actively supporting the growth of the natural oral microbiota. Also, the beneficial activities of the resident oral microbiota are retained and the risk of dysbiosis is reduced.

Key words: Plaque control; biofilm; oral microbiota; antimicrobial agents; mathematical modelling

1. Introduction

From birth, the infant is exposed to and colonised by a wide range of micro-organisms, derived mainly from the mother, although only a subset are able to establish successfully. The biological properties of each habitat determine which micro-organisms can colonise and grow, and dictate which will be major or minor components of the resident microbiota of a site. This results in different surfaces having distinct but characteristic microbiotas [1-5].

In their natural environment, micro-organisms revert to their so-called 'biofilm phenotype', and down-regulate certain activities, e.g. those related to motility, and up-regulate the production of polymeric substances that act as viscoelastic inter-cellular binding material and extra-cellular energy storage compounds, amongst other roles [6, 7]. This mode of existence offers protection against external stresses, e.g. by limiting the penetration of antimicrobial agents, and by providing mechanical resilience to shear generated by saliva flow, etc., and promotes interactions among neighbouring microbial cells [8], as well as between the biofilm and the host, resulting in a complex and dynamic interplay.

2. Benefits of the resident human microbiota

The human microbiota and the host have co-evolved to have a symbiotic or mutualistic relationship [9]. The resident micro-organisms gain a secure, warm, nutritious habitat from the host, and, in return, contribute to food digestion, nutrition, regulation of human metabolism, differentiation of the host mucosa, immune development and function, and prevention of colonisation by exogenous and often pathogenic microbes [10]. This relationship between the resident microbiome and the host is dynamic and, whilst the composition of resident populations in health is

remarkably stable [11], this can be perturbed by changes in lifestyle, immune status or by broad spectrum antibiotic therapy. Such perturbations have been associated with a number of clinical disorders such as obesity, allergy and a variety of inflammatory diseases [4, 12].

3. Dental biofilms and oral health

The mouth is similar to other habitats in the body in having a characteristic microbiota, with different surfaces in the oral cavity supporting distinct microbial communities [13], the composition and metabolism of which are dictated by the local environmental conditions. The microbiota grows on oral surfaces as structurally- and metabolically-organised communities of interacting species, termed biofilms [14]. These communities are in a dynamic equilibrium with their environment, and there can be significant re-assortment and rearrangement of the composition and metabolic activity of these microbial consortia in response to changes in the biology of the mouth (e.g. eruption of teeth; flow of saliva; subversion of the host defences) and in the lifestyle of the individual (e.g. in response to smoking, dietary alterations, or to the side effects of medication, etc) [1, 5].

The bacteria found in occlusal fissures are mainly Gram positive (especially streptococci), are facultatively anaerobic and primarily metabolise host and dietary sugars; these sites are particularly influenced by the properties and flow rate of saliva. In contrast, the biofilms from the healthy gingival crevice contain many more obligately anaerobic and proteolytic species, and the community is influenced predominantly by gingival crevicular fluid, GCF [15].

The composition of the oral microbiota at a site can remain stable over time (microbial homeostasis) [16]. This is not due to any biological indifference among the members of the biofilm community, but is a consequence of many highly regulated inter-dependencies among the resident microbes. These basic observations on site distribution of oral bacteria are highly significant. They provide direct evidence that the composition and metabolism of the oral microbiota at a site is sensitive and responsive to the oral environment, and that there is a dynamic relationship between them both. Biofilm composition can shift in response to changes in local environment and lifestyle.

4. **Significance of a biofilm and microbial community life-style to the oral microbiota.**

Oral micro-organisms gain substantial advantages by growing as a biofilm and by developing as a microbial community [17]. Micro-organisms are in close proximity to one another in biofilms, thereby providing many opportunities for synergistic interactions. For example, bacteria can co-operate with one another to metabolise complex host molecules, such as glycoproteins, that would be recalcitrant to the action of single species. The metabolism of these communities is also more efficient, with food chains and food webs developing to catabolise substrates to the simplest end products of metabolism. Also, oral microbial communities display a broader habitat range, for example, with obligate anaerobes persisting at sites that are overtly aerobic. Biofilms are inherently tolerant to environmental stresses, the host defences, and antimicrobial agents, for example, due to limited access or penetration of molecules, while cross-protection of sensitive species by neighbouring organisms that produce neutralising enzymes (e.g. β-lactamase, catalase, etc) can occur. In

this way, the properties of microbial communities are more than the sum of the component species [17].

5. **Benefits of the oral microbiota to the host**

As with other habitats in the body (see earlier), the general relationship between the oral microbiota and the host is mutualistic. The micro-organisms are maintained in an environment which is supplied with a diverse array of host molecules which serve as nutrients, and the resultant microbiota provides benefits to the host.

The resident oral microbiota prevents the establishment of the many exogenous micro-organisms that the host comes into contact with on a regular basis. This 'colonisation resistance' is because the natural oral microbiota is better adapted at attaching to oral surfaces, is more efficient at metabolising the available nutrients for growth, and can produce inhibitory factors and create hostile environments that restrict the growth of potential microbial invaders. Colonisation resistance can be 'lost' if the resident microbiota is disrupted, for example, by long-term exposure to broad spectrum antimicrobial agents, a consequence of which can be an overgrowth by yeasts [18].

There is active communication ("cross-talk") between the resident oral microbiota and host cells. Some resident bacteria, especially streptococci, down-regulate potentially damaging pro-inflammatory host responses to components of the normal oral microbiota, such as the Gram negative commensals, while the host retains the ability to respond to genuine microbial insults [19-22]. The precise biological mechanisms involved in this "cross-talk" are still being determined;

pathogenic and non-pathogenic bacteria may initiate different intracellular signalling pathways and innate immune responses.

Resident oral bacteria make a major contribution to the general health of their host by regulating gastrointestinal and cardiovascular systems via the metabolism of dietary nitrate [23]. Approximately 25% of ingested nitrate is secreted in saliva, from where it is reduced to nitrite by oral bacteria. Nitrite regulates blood flow, blood pressure, gastric integrity and tissue protection against ischemic injury. Nitrite is converted to nitric oxide in the acidified stomach, and this has antimicrobial properties, and contributes to defence against enteropathogens, and in the regulation of gastric mucosal blood flow and mucus formation. When the resident salivary microbiota is deliberately suppressed using antimicrobial agents, the reduction of nitrate to nitrite in saliva falls markedly in human volunteers [23-26] and laboratory animals [25]. In the animal model, the suppression of endogenous nitrate reduction resulted in a loss of the predicted biological benefits of nitrite, including reduced gastric mucus thickness, while the expected fall in blood pressure following a nitrate supplement was prevented [25]. These findings further emphasise that it is essential not to perturb or lose the beneficial functions of the resident oral microbiota, and this has implications for treatment strategies (see later).

6. Dental biofilms in disease

Numerous studies, using either traditional culture or contemporary molecular approaches, have compared the microbiota in biofilms from healthy surfaces to that from sites with dental caries and periodontal diseases. These studies have shown that there are substantial differences in the composition of the microbiota in disease

[27, 28]. The microbial analysis of biofilms overlying carious lesions has generally demonstrated higher proportions of bacteria such as mutans streptococci, bifidobacteria and lactobacilli, but not exclusively so. These organisms rapidly metabolise dietary sugars to acid, and also preferentially grow under the acidic conditions so generated. In contrast, in periodontal disease, the accumulation of biofilm around the gingival margins provokes an inflammatory response by the host. This involves an increased flow of GCF which not only introduces components of the host response but also many molecules that can act as potential nutrients for some of the minor components of the normal resident subgingival microbiota [27].

More recently, the application of sensitive, culture-independent molecular techniques has led to the detection from healthy sites of many of the bacteria associated with disease in biofilms. When detected, they are present in low numbers that are clinically irrelevant and are found at a far lower frequency [28, 29]. An interpretation of these findings is that disease is most probably due to shifts in the composition of the biofilm (dysbiosis) rather than as a result of exogenous 'infection', and is associated with markedly higher proportions of certain species that, if present in health, are normally non-competitive with the beneficial bacteria, and hence are only minor components in the biofilm.

An ecological hypothesis has been put forward to explain the relationship between the resident oral microbiota and dental disease [30]. In essence, it proposed that any major changes in local environmental conditions will alter the competitiveness of individual bacteria within the biofilm leading to the enrichment of organisms most suited to the new environment, and these might increase the risk of

dental disease. Thus, an increased frequency of sugar intake, or a reduction in saliva flow, results in supragingival biofilms spending more time at low pH. This selects for acid-producing and acid-tolerating species at the expense of health-associated bacteria that prefer pH values around neutrality. Some supragingival bacteria are also able to adapt to regular conditions of low pH, and this has been reflected in the Extended Ecological Plaque Hypothesis [31]. Over time, the gradual selection of these fermentative bacteria results in the biofilm spending even more time at low pH values, thereby favouring the growth of these bacteria still further, and increasing the extent of demineralisation of enamel or dentine.

We have recently initiated *in silico* modelling of the oral microbiota to demonstrate the effects of environmental conditions and perturbations on the structure (bacterial composition) and function (acid production) of supra-gingival plaque [32]. Our mathematical model is agent-based, i.e. cells (or cell aggregates) are represented as discrete entities whose carbohydrate metabolism and growth rates are determined by parameterised Monod expressions as discussed below. Agent-based modelling is attractive since it permits a variety of external and endogenous factors to be easily included, and the corresponding effects to be graphically demonstrated [33]; see Figure 1. This is to be contrasted with the continuum approach pioneered in this field [34], and more recently extended to include realistic chemical pathways [35]. Such approaches can be solved with less computational resources than agent-based schemes, but do not allow cell-based rules to be naturally implemented.

For our *in silico* study, the plaque was composed of two functionally different bacterial populations, both capable of metabolising sugar to acid but differing in their ability to function at low pH, i.e. in their aciduricity (acid tolerance). The total cellular growth rate increased (up to saturation) with the available glucose, which was taken as the limiting substrate, and decreased with pH using the functional forms shown in Figure 2. The parameters were chosen such that the growth of aciduric bacteria at low pH exceed that of non-aciduric bacteria. Systematically varying a range of all model parameters revealed a multifactorial contribution to the biofilm composition where small changes in most parameters had a measurable effect, although with a range of sensitivities. In all cases, the correlation between composition and acid production was the same – biofilms consisting of a high fraction of aciduric bacteria were driven to a dysbiotic, low pH state with the potential for enamel demineralisation. However, even small changes in key factors could have a profound impact on the outcome of these bacterial interactions. For example, when glucose is pulsed into the model, the biofilm becomes dominated by the acidogenic and acid-tolerating bacterial population over time (Figure 1b), but by inhibiting the metabolism of these organisms merely by 10% (such as by some putative antimicrobial agent at sub-lethal concentration) nullifies their advantage and allows the non-acid tolerating (beneficial) species to be competitive again (Figure 1c).

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Other key drivers identified from the model are highlighted in Figure 3. For instance, increasing the bacterial load (i.e. the biofilm thickness) promotes dysbiosis by increasing the number of acid-producing cells, lowering the pH and selecting for cariogenic bacteria, as does increasing the frequency of sugar intake and allowing

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the pH to fall further (see later) [32, 36].

A different set of environmental pressures operates in periodontal disease. If plaque is allowed to accumulate beyond levels that are compatible with health, for example, due to poor oral hygiene, then the host mounts an inflammatory response. This results in an increased flow of GCF, which not only delivers components of the host defences (immunoglobulins, complement, neutrophils, etc) but also introduces other host molecules to the site, such as haemoglobin and transferrin, that can act as nutrients and a source of essential cofactors for many anaerobic and proteolytic bacteria. The metabolism of these bacteria makes the site more anaerobic and the local pH will rise due to proteolysis, and these environmental changes select for microbial consortia that are more suited to these new conditions. These include many species implicated in periodontal diseases, such as *Porphyromonas gingivalis*, *Tannerella forsythia*, numerous spirochaetes and other currently unculturable taxa [30]. The proteolytic metabolism of these consortia can damage host tissues directly, but more significantly, they degrade many of the host proteins whose role is to regulate the inflammatory response. The consequence is an increased and inappropriate level of inflammation that causes extensive tissue damage, leading to an even stronger host response and a further increase in the flow of GCF.

7. Implications for treatment

A key concept implicit in the Ecological Plaque Hypothesis is that disease can be prevented not only by targeting the key bacteria directly (e.g. with antimicrobial agents) but also by interfering with the factors that drive the disruption of the

microbiota. The preceding paragraphs have argued the following:

(a) the oral microbiota is natural, and is beneficial to the host,

(b) disease is a consequence of a deleterious shift in the microbiota (dysbiosis),

(c) these shifts are driven by a change in the local environment,

(d) disease can be prevented by eliminating or reducing the pressures that drive dysbiosis, and

(e) active maintenance of microbial homeostasis would be a relevant stratagem to promote oral health.

Effective manual plaque control remains at the centre of oral care, but is not always feasible or sufficient for many patients. An acceptance of the principles embedded within the Ecological Plaque Hypotheses suggests that the following strategies could augment more traditional approaches.

7.1 Reduce microbial acid production. The production of acid from the microbial fermentation of dietary sugars and the lowering of the pH in dental biofilms has several consequences. Acidic conditions will cause demineralisation of the hard tissues of the teeth, while the conditions of low pH will select for acidogenic and acid-tolerating (aciduric) bacteria while inhibiting the growth of beneficial species. If the conditions of low pH are repeated on a regular basis, then the balance of the microbiota can be significantly disrupted, resulting in increased numbers and proportions of acid-producing bacteria, thereby increasing the risk of caries still further. Similar effects would result if saliva flow was impaired or its composition was altered, for example, as a result of medical interventions or as a side-effect of a number of medications.

There are several approaches that could reduce microbial acid production. Fluoride, in addition to its role in preventing demineralisation and promoting remineralisation, can interfere with several aspects of sugar metabolism by oral saccharolytic bacteria, including the inhibition of sugar transport and glycolysis [37, 38]. Other antimicrobial agents that are commonly formulated into oral care products persist in the mouth for long periods at sub-lethal concentrations. At these levels, these agents can also disrupt sugar metabolism and acid production [37, 38]. Patients can be encouraged to reduce the intake of sugar between main meals, either by dietary restriction or by consuming snack foods and beverages that contain sugar substitutes (e.g. sugar alcohols, such as xylitol, or intense sweeteners like aspartame or saccharin). These sugar substitutes cannot be fermented rapidly to acid by oral bacteria, thereby reducing damage to dental hard tissues and also removing the environmental conditions needed for acid-tolerating bacteria to outcompete beneficial species, while they also stimulate saliva flow, which delivers numerous important benefits to the oral ecosystem. Attempts have also been made to raise the pH in the biofilm, for example, by delivering supplements such as arginine or urea that can be metabolised by plaque bacteria to alkali [39, 40].

The potential efficacy of such strategies has been confirmed by the *in silico* model discussed earlier. We have demonstrated that controlling the pH by modulating the degree of acid buffering can select between healthy (strong buffering) and dysbiotic (weak buffering) states [36]. In addition, agents that directly modulate the metabolism of one or both populations, such as sub-lethal concentrations of antimicrobial agents (as discussed previously), can similarly

play a selective role [41]. This is in addition to reducing the frequency of sugar intake or limiting the bacterial load as discussed earlier [32]. Note that in all of these *in silico* studies, cellular death was fixed at a constant rate shared by both populations, so observed changes were not due to any bactericidal activity. This represents a key benefit of mathematical modelling: cell metabolism and viability can be independently controlled, allowing the underlying mechanisms driving any observed changes to be identified unambiguously.

7.2 Prevent the growth of proteolytic and anaerobic subgingival bacteria.

The majority of the bacteria associated with periodontal diseases are both obligately anaerobic and highly proteolytic. The growth of these bacteria, therefore, depends on the plentiful supply of essential nutrients (proteins, peptides) and cofactors such as haemin, and a low redox potential. Strategies have been investigated to alter the subgingival environment to make it unfavourable for the growth of putative periodontal pathogens. These include delivering (a) redox agents that raise the local Eh in the periodontal pocket [42], (b) novel anti-inflammatory agents such as resolvins or lipoxins that promote tissue healing while also reducing the flow of GCF [43, 44], which in turn, denies the microbiota access to factors essential for their growth, and (c) antimicrobial agents in oral care products that inhibit bacterial proteases at sub-lethal concentrations [41]. It has been noted that Triclosan, which has been widely formulated into dentifrices and mouthwashes, is both antimicrobial, anti-metabolic (inhibitory to bacterial sugar metabolism and protease activity), and anti-inflammatory [45, 46].

7.3 Promote beneficial bacteria. The strategies described above will not only restrict the opportunities for potentially detrimental bacteria to grow and damage host tissues, but will also help in maintaining conditions that are favourable for beneficial oral bacteria. Additional approaches are being developed that are designed to actively promote the growth of the resident microbiota [47]. These include 'prebiotics' to selectively promote the growth of beneficial oral bacteria, and the development of functional foods that would have a potentially positive effect on oral health beyond basic nutrition (these are also referred to as neutraceuticals). Other approaches that are being investigated include identifying appropriate oral probiotic bacteria and/or creating non-pathogenic strains that can prevent colonisation by wild-type organisms (replacement therapy, e.g. using molecular biology to produce strains of *S. mutans* that not only cannot produce lactic acid but which also secrete a bacteriocin to inhibit and exclude natural mutans streptococci) [48]. The use of probiotic bacteria for oral applications remains a contentious area, as most strains that have been evaluated to date are lactobacilli or bifidobacteria, which have also been implicated in the aetiology of dental caries, while there is no evidence that these organisms actually successfully colonise the mouth. The deliberate re-implantation of resident bacteria into surgically-treated periodontal pockets has also been evaluated as means of promoting colonisation resistance and tissue healing [49].

8. Conclusions

It has been emphasised in this review that the oral microbiota is natural and beneficial to the host, and that disease is associated with shifts in the balance of the

normal resident microbiota. In this way, dental diseases represent examples of (minor) ecological catastrophes, in which the oral microbiota is disrupted in response to a change in the local environment [30]. This has implications for treatment, and opens up potentially new avenues to control or prevent disease, that are distinct from those that are used in medicine. Therefore, when a patient presents with dental disease, a clinician should attempt to determine the factors responsible for driving dysbiosis, while recognising that these could vary from patient to patient. These factors could include impaired saliva flow, poor oral hygiene techniques, inappropriate lifestyle, poor dietary habits, an impaired immune system, or the presence of other risk factors. Unless there is an attempt to interfere with or alter the factor(s) driving the dysbiosis then the patient is likely to keep returning to the surgery suffering from further episodes of disease. An appreciation of ecological principles may lead to opportunities in the future to manipulate the composition and metabolism of the oral microbiota in order to maintain the benefit we derive from their presence and activity, while minimising the impact of any environmental and lifestyle factors that might lead to dysbiosis.

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Figure 1: Snapshot of the *in silico* model [32], showing a mixture of aciduric (light red) and non-aciduric (dark green) bacteria. The starting composition had equal proportions of aciduric and non-aciduric populations.

(a) Biofilm composition after 5 days, with glucose pulsed every 5 hours.

(b) Biofilm composition after 50 days, with glucose pulsed every 5 hours.
There is now a significant overgrowth by aciduric bacteria.

(c) Biofilm composition after 50 days, with glucose pulsed every 5 hours, but with a 10% inhibition of the metabolism of the aciduric bacteria.
Even such a small inhibition has resulted in a loss of competitiveness by the aciduric population such that the non-aciduric (beneficial) bacteria are able to compete successfully.

Figure 2: Schematic representation of the effect of nutrient availability (a) and local acidity (b) on the metabolic growth rates as included in the mathematical model. For both graphs, the left (green) line corresponds to the non-aciduric bacteria, and the right (red) line to the aciduric bacteria. The vertical solid lines show typical values of glucose and pH during and between glucose pulses. The final rate is the maximum growth rate multiplied by each of these factors.

Figure 3: Examples of environmental and endogenous factors leading towards or away from a dysbiotic supragingival microbiota, as identified by the *in silico* model [32]. Increasing or decreasing the metabolism of pathogenic and commensal

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organisms was achieved by varying the Monod parameters (i.e. the maximum rate of metabolism and/or half-concentration of nutrients).