In Focus

Do dental diseases resemble ecological catastrophes?

Introduction

Ecological catastrophes can take many forms, and can come in all shapes and sizes! Nitrogenous fertilisers can be washed off farmland into lakes and ponds, resulting in overgrowth by algae. Such an overgrowth can lead to secondary effects to the ecosystem; the algae can consume dissolved oxygen in the water leading to the loss of aerobic microbial, plant and insect life (eutrophication). Similarly, atmospheric pollution with sulphur dioxide and nitrogen oxides can produce acid rain causing damage to plants and trees and loss of aquatic life. On a larger scale, it has been postulated that the extinction of the dinosaurs followed climate change resulting from the impact of a meteorite. At the other end of the spectrum, it will be argued in this article that the key to a more complete understanding of the role of micro-organisms in dental diseases depends on a paradigm shift away from concepts that have evolved from studies of diseases with a simple and specific (e.g. single species) aetiology to an appreciation of ecological principles similar to those outlined above, where a substantial change to a key parameter influencing the habitat can disrupt the natural balance of the resident oral microflora. Acceptance of such principles can more readily explain the transition of the oral microflora from having a benign to a pathogenic relationship with the host, while opening up new opportunities for the control of dental plaque-mediated diseases.

Dental plaque in health and disease

Dental plaque is the diverse microbial community found on the tooth surface embedded in a matrix of host and microbial polymers (Figure 1) [1,2]. Dental plaque displays the attributes of a typical biofilm; cells communicate and interact extensively, and there is evidence of spatial and functional organisation. Dental plaque is natural and confers benefit to the host by combining with the host defences to exclude exogenous (and often pathogenic) microbes (colonisation resistance). There is a shift in the balance of the plaque microflora at diseased sites. In caries, there are higher proportions of acidogenic and acid-tolerating species, such as mutans streptococci and lactobacilli [3], while plaque from sites with periodontal disease are dominated by obligately anaerobic, proteolytic, and often Gram negative species [4]. A key question, therefore, is where do the ‘pathogenic’ bacteria come from, and what drives these deleterious changes in the oral microbiota?

Source of cariogenic and periodontopathic bacteria

Conventional culture approaches using selective media have been the ‘gold-standard’ for determining the microbial composition of plaque. Early studies recovered putative pathogens commonly and in high numbers in disease, but these organisms were found infrequently in health. The recent application of more sensitive molecular (e.g. PCR) and immunological (e.g. ELISA) detection techniques, as well as the use of molecular microbial ecology approaches (e.g. amplification of 16S ribosomal RNA gene sequences) has radically changed our vision on plaque composition. Many unculturable taxa have been identified [5], and putative plaque pathogens have been detected more regularly in plaque overlying healthy teeth [6], albeit at very low levels. This implies that dental diseases are not conventional exogenous infections but are most likely due to...
deleterious perturbations of the resident plaque microflora; a key issue is what could drive these shifts in community composition (Figure 2).

**Perturbation of the resident microflora**

The resident microflora of any site is generally stable over time (microbial homeostasis), in spite of regular minor changes to the habitat. This stability is not due to any biological indifference among the component species, but results from a dynamic balance arising out of numerous, coupled inter-microbial and host-microbial interactions. A substantial change to the habitat or in local environmental conditions can perturb this balance. Studies of numerous habitats provide clues as to the type of factors capable of disrupting the intrinsic homeostasis that exists within microbial communities. A common feature is a change in the nutrient status at the site; as stated earlier, fertilisers washed off farmland into lakes and ponds enable algae to outcompete other organisms resulting in eutrophication. Other effects can result from a chemical (e.g. acidification of soil and water due to acid rain) or physical (insertion of implants and catheters) change to the habitat.

The local nutritional and chemical environment in plaque does change during disease. Caries is associated with individuals who have a more frequent intake of fermentable carbohydrates, which results in plaque spending longer periods at low pH. In contrast, the flow of a serum-like exudate, gingival crevicular fluid (GCF), is increased during the inflammatory response to plaque accumulation in periodontal disease. This not only introduces components of the host response (IgG, complement, neutrophils), but also potentially novel substrates (proteins and glycoproteins such as haptoglobin, haemoglobin, transferrin, etc) which are catabolised by proteolytic anaerobes, resulting in a rise in local pH.

These environmental changes differentially affect gene expression and growth of oral bacteria predominating in either health or disease. In brief, the metabolism of cariogenic bacteria such as *mutans streptococci* and *lactobacilli* is more suited to high sugar/low pH conditions (e.g. optimal growth at low pH; effective stress response strategies to combat acidic conditions; rapid sugar transport and glycolysis; etc) than organisms predominating in health. In contrast, the growth and metabolism of many Gram negative anaerobes is enhanced under alkaline conditions and in the presence of haemin provided by the catabolism of host haeme-containing proteins in GCF.

These findings led to the design of laboratory modelling studies involving complex but defined communities of oral bacteria (generally comprised of 9 or 10 species, representative of those predominating in health) in continuous culture and biofilm systems to answer specific questions concerning the consequence of such changes on the relative competitiveness of individual species and the impact on community stability. Analysis of these studies led to the formulation of an alternative (ecological) hypothesis relating the role of oral bacteria to dental disease.

**Selection of cariogenic and periodontopathic bacteria.**

Exploitation of the unique benefits of pH control in the chemostat, coupled with the reproducibility of a defined mixed culture inoculum, enabled the question to be answered for the first time as to whether the rise in cariogenic bacteria is due to the sudden availability of sugar per se (e.g. because of more efficient sugar transport systems), or is a response to the inevitable conditions of low pH following sugar consumption. Daily pulses of glucose for 10 consecutive days at a constant pH 7.0 in a mixed culture chemostat (9 or 10 species) had little impact on the balance of the microbial communities, suggesting that sugar availability per se was not critical for cariogenic growth. In contrast, a sustained or daily acidic challenge in the chemostat generated a select population of *mutans streptococci* due to their highly efficient stress response strategies to combat acidic conditions. The finding of a differential response of the cariogenic flora to a change in pH is consistent with other studies comparing the effects of an acidic (pH 5.5) or high sugar (5% glucose) challenge in a stirred, dynamic culture system for cariogenic bacteria and *periodontopathic* bacteria (e.g. *aeromonas hydrophila*).

Figure 2. Schematic representation of the relationship between the microbial composition of dental plaque in health and disease. Potential pathogens (grey) may be present in low numbers in plaque or transmitted in low numbers to plaque; both situations may be compatible with health. A major ecological pressure would be necessary for such pathogens to outcompete other members of the resident microflora (white) and achieve the levels needed for disease. Possible ecological pressures include a sugar-rich diet, low saliva flow, inflammation, etc. Disease could be prevented not only by targeting the pathogens directly (e.g. with antimicrobial agents) but also indirectly by interfering with the ecological pressures responsible for selection of the pathogen.
community, and the combined proportions of the cariogenic bacteria, *Streptococcus mutans* and *Lactobacillus rhamnosus*, stayed at ca.1% of the total microflora. However, when the pH was allowed to change after each pulse, there was a gradual but progressive selection of the cariogenic (and acid-tolerating) species at the expense of bacteria associated with dental health. After the final glucose pulse, the community was dominated by species implicated in caries (ca. 55% of the microflora). This study was repeated, but the pH fall was restricted after each glucose pulse to either pH 5.5, 5.0 or 4.5 in independent experiments. A similar enrichment of cariogenic species at the expense of healthy species was observed again, but their rise was directly proportional to the extent of the pH fall, while an inverse relationship was seen with species associated with enamel health. The addition of fluoride to inhibit acid production and restrict the fall in pH, also prevented the selection of *S. mutans*. Collectively, these studies showed that it was the low pH generated from sugar metabolism rather than sugar availability per se that leads to the breakdown of microbial homeostasis in dental plaque, and predisposes sites to caries.

Figure 3. The ecological plaque hypothesis and the prevention of (a) dental caries, and (b) periodontal diseases. The postulated dynamic relationship between the environment and deleterious shifts in the composition and metabolism of plaque biofilms implies that disease could be prevented not only by direct inhibition of the putative pathogens, but also by interfering with the key environmental factors driving their selection.

The pH of the healthy gingival crevice rises from <7.0 to >7.5 during the inflammatory response to excessive plaque accumulation. Even such small changes in pH have a profound affect on gene expression, enhancing the growth and protease activity of some periodontal pathogens and increasing their competitiveness. In a three-membered consortium of black-pigmented oral anaerobes in a chemostat, *Prevotella melaninogenica* predominated at and below neutral pH (conditions and a species associated with healthy sites); however, a small shift in pH to 7.25 resulted in *Prevotella intermedia* becoming most numerous, while growth at pH 7.50 caused the culture to be dominated by *Porphyromonas gingivalis* (isolated in high numbers from advanced disease). Serum has been used as a surrogate for GCF in experiments modelling the impact of the changes in nutrient status that might occur in the gingival crevice during inflammation on the balance of the oral microflora. Human serum was used for batch-wise enrichments of samples of sub-gingival plaque. After 5-6 enrichment steps, the composition of the microflora showed few similarities with the original plaque sample and consisted mainly of consortia able to extensively degrade the serum glycoproteins provided. In addition, *P. intermedia* could not be detected in some of the original plaque samples, but became a major component (ca. 10%) of the microflora after only 2 or 3 enrichments. These organisms must have been present initially in the plaque samples, but became a major component (ca. 10%) of the microflora after only 2 or 3 enrichments. These organisms must have been present initially in the plaque samples but at levels below the detection limit of the methods adopted. A change in local nutrient status altered the relative competitiveness of individual species, enabling *P. intermedia* to escape from homeostatic control and predominate. Similar findings have been found using defined microbial communities in chemostat experiments. When the growth medium was supplemented with...
serum, the Eh fell to even lower levels (-380 mV), the pH rose from 6.95 to >7.5 through bacterial metabolism, P. gingivalis increased in proportion to dominate the community (ca. 80% of total CFU), and overall protease activity rose.

**An ecological approach to explain plaque-mediated dental disease**

The data from the studies outlined above provide a basis for plaque-mediated diseases being viewed as a consequence of imbalances in the resident microflora resulting from an enrichment within the microbial community of ‘oral pathogens’. Potentially, cariogenic bacteria may be found naturally in dental plaque, but, at neutral pH, these organisms are weakly competitive with other resident species and are present only as a small proportion of the total plaque community. In this situation, with a conventional diet, the levels of such potentially cariogenic bacteria are clinically insignificant, and the processes of enamel de- and remineralisation are in equilibrium. However, if the frequency of fermentable carbohydrate intake increases, then plaque spends more time at low pH; this favours the proliferation of aciduric (and acidogenic) bacteria (especially mutans streptococci and lactobacilli), while tipping the balance towards demineralisation. Greater numbers of bacteria such as mutans streptococci and lactobacilli in plaque would result in more acid being produced at even faster rates, thereby further promoting both demineralisation and the disruption of the natural ecology of plaque.

Similarly, putative periodontal pathogens can be present in subgingival plaque at healthy sites, but at extremely low levels. These organisms are unable to out-compete the Gram-positive, saccharolytic bacteria that predominate in health. If plaque accumulates, however, to levels that are no longer compatible with health, then the resultant inflammatory response causes an increased flow of GCF, thereby altering the local nutrient status. As demonstrated in the modelling studies described above, this drives

a) an outgrowth in proteolytic and invariably Gram negative, bacteria (containing more inflammatory LPS);

b) a rise in pH; and

c) further reductions in redox potential.

The proteases produced also fuel this damaging cycle by deregulating the host control of the inflammatory response, which is aggravated still further by the increase in Gram negative biomass.

The concept that caries and periodontal diseases arise as a result of environmental perturbations to the habitat has been encapsulated in the ‘ecological plaque hypothesis’ (Figure 3a and b)\(^\text{15}\). Key features of this hypothesis are that

a) the selection of ‘pathogenic’ bacteria is directly coupled to changes in the environment and

b) diseases need not have a specific aetiology; any species with relevant traits can contribute to the disease process.

Thus, mutans streptococci are among the best adapted organisms to the cariogenic environment (high sugar/low pH), but such traits are not unique to these bacteria. Strains of other species also share some of these properties and, therefore, will contribute to enamel demineralisation. A key element of the ecological plaque hypothesis is that disease can be prevented, not only by targeting the putative pathogens directly, e.g. by antimicrobial or anti-adhesive strategies, but also by interfering with the selection pressures responsible for their enrichment. In dental caries, regular conditions of sugar/low pH or reductions in saliva flow appear to be primary mechanisms that disrupt microbial homeostasis. Strategies that are consistent with the prevention of caries via the principles of the ecological plaque hypothesis include:

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a) inhibition of plaque acid production (e.g. by fluoride-containing products),
b) avoidance between main meals of foods and drinks containing fermentable sugars
c) the consumption of foods/drinks that contain non-fermentable sugar substitutes and
d) the stimulation of saliva flow after main meals, e.g. by sugar-free gum.

In periodontal diseases, conventional treatment involves mechanical removal of plaque or physical disruption of biofilm structure. From an ecological perspective, attempts could also be made to alter the local environment by

a) reducing the flow of GCF (e.g. by anti-inflammatory agents) or
b) making the site less anaerobic using oxygenating or redox agents.

Concluding remarks

The development of oral disease at a site could be viewed as an example of a (micro-) ecological catastrophe in which some event has led to the breakdown of the homeostatic mechanisms that normally maintain a beneficial relationship between plaque and the host. When assessing treatment options, an appreciation of the ecology of the oral cavity will enable the enlightened clinician to take a more holistic approach and take into account the nutrition, physiology, host defences and general well-being of the patient, as these will affect the balance and activity of the resident oral microflora. Future episodes of disease will occur unless the cause of this breakdown in homeostasis is recognised and remedied. Future developments in oral care should use strategies to encourage the maintenance of homeostasis (and hence a favourable ecology) in plaque.

References