Are dental diseases examples of ecological catastrophes?

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Dental diseases are among the most prevalent and costly diseases affecting industrialized societies, and yet are highly preventable. The microflora of dental plaque biofilms from diseased sites is distinct from that found in health, although the putative pathogens can often be detected in low numbers at normal sites. In dental caries, there is a shift towards community dominance by acidogenic and acid-tolerant Gram-positive bacteria (e.g. mutans streptococci and lactobacilli) at the expense of the acid-sensitive species associated with sound enamel. In contrast, the numbers and proportions of obligately anaerobic bacteria, including Gram-negative proteolytic species, increase in periodontal diseases. Modelling studies using defined consortia of oral bacteria grown in planktonic and biofilm systems have been undertaken to identify environmental factors responsible for driving these deleterious shifts in the plaque microflora. Repeated conditions of low pH (rather than sugar availability per se) selected for mutans streptococci and lactobacilli, while the introduction of novel host proteins and glycoproteins (as occurs during the inflammatory response to plaque), and the concomitant rise in local pH, enriched for Gram-negative anaerobic and asaccharolytic species. These studies emphasized (a) significant properties of dental plaque as both a biofilm and a microbial community, and (b) the dynamic relationship existing between the environment and the composition of the oral microflora. This research resulted in a novel hypothesis (the ‘ecological plaque hypothesis’) to better describe the relationship between plaque bacteria and the host in health and disease. Implicit in this hypothesis is the concept that disease can be prevented not only by directly inhibiting the putative pathogens, but also by interfering with the environmental factors driving the selection and enrichment of these bacteria. Thus, a more holistic approach can be taken in disease control and management strategies.

Overview

Dental diseases pose distinct challenges when it comes to determining their microbial aetiology. Disease occurs at sites with a pre-existing natural and diverse microflora (dental plaque), while even more complex but distinct consortia of microorganisms are implicated with pathology. The aetiology is particularly challenging because it depends on determining which species are implicated directly in active disease, which are present as a result of disease and which are merely innocent by-standers. Although rarely life threatening, dental diseases are a major problem for health service providers in developed countries because of their prevalence and high treatment costs. For example, in the UK, the National Health Service spends over £1.6 billion per annum on dental treatment, and this figure increases to £2.6 billion if the burgeoning private sector costs are included. An improved understanding of the role of microorganisms in dental diseases is essential if their prevalence is to be reduced. It will be argued in this review that the key to a more complete understanding of the role of microorganisms in dental diseases depends on a paradigm shift away from concepts that have evolved from studies of other diseases with a simple and specific (e.g. single species) aetiology to an appreciation of ecological principles. Acceptance of such principles can more readily explain the transition of the oral microflora from having a commensal to a pathogenic relationship with the host, and also open up new opportunities for the control of dental plaque.

Ecological perspective

It has been estimated that the human body is made up of over $10^{14}$ cells, of which only around 10% are mammalian (Sanders & Sanders, 1984). The remainder are the microorganisms that comprise the resident microflora of the host. This resident microflora does not have a merely passive relationship with the host, but contributes directly and indirectly to the normal development of the physiology, nutrition and defence systems of that host (Marsh, 2000a; McFarland, 2000; Rosebury, 1962). For example, disruption of this microflora by antibiotics can result in deficiencies in absorption or metabolism of vitamins, in overgrowth by resistant bacteria or colonization by exogenous (and often...
The composition of the resident microflora is characteristic for distinct habitats such as the mouth, skin, gut, etc., despite the continual transfer of organisms between these sites (Tannock, 1995). Once established, the composition of the resident microflora of each site remains relatively stable over time. This stability (which has been termed microbial homeostasis) stems not from any biological indifference between the host and the microflora, but results from a dynamic balance arising out of numerous, coupled inter-microbial and host–microbial interactions (Alexander, 1971; Marsh, 1989). A change in the habitat or local environment can perturb this balance.

In order to explain the maintenance of microbial communities with a distinctive composition around the body, it has to be assumed that each of these habitats differs in terms of key ecological factors that enable certain populations to dominate at one site while rendering them non-competitive at others. Such factors include the provision of appropriate receptors for attachment, and essential nutrients and cofactors for growth, as well as an appropriate pH, redox potential and gaseous environment. Indeed, following extensive studies comparing the predominant microflora of dental plaque and that of the gastro-intestinal tract, only 29 out of over 500 taxa found in the mouth were recovered from faecal samples, despite the continuous passage of these bacteria into the gut via saliva (Moore & Moore, 1994). Within the mouth there are a number of distinct surfaces for microbial colonization, and again the consortia that establish on each vary in composition, reflecting intrinsic differences in the biology of these sites (Marsh & Martin, 1999). Surfaces that provide obviously distinct ecological conditions include mucosal surfaces (e.g. lips, cheek, palate and tongue) and teeth. There are even differences in the microflora that colonize distinct surfaces on teeth (see later) (Marsh, 2000b; Marsh & Martin, 1999). Thus, the properties of the habitat dictate the quantitative and qualitative composition of the resident microflora. This confirms that there is a direct and dynamic relationship between environment and microflora, even at the microhabitat scale.

A substantial change to a habitat can cause a breakdown of microbial homeostatic mechanisms, altering the balance among the resident organisms at a site. For example, occlusion of the forearm leads to an increase in the numbers of aerobic bacteria from 10^3 to 10^7 cells cm^{-2}, and a shift from a staphylococcal-dominated microflora to one with enhanced numbers of coryneforms (Aly & Maibach, 1981). This suggested that moisture is a major environmental determinant for microbes on the skin. Likewise, the balance of the oral microflora can shift due to changes in the diet (e.g. increased frequency of consumption of sugar-containing foods/beverages), the dentition (e.g. following eruption, extractions or insertion of dentures) or a reduction in saliva flow as a side-effect of medication or radiation therapy (Marsh, 2000b).

On occasions, disease can occur as a consequence of the breakdown of microbial homeostasis at a site, and dental plaque is associated with two of the most prevalent diseases affecting industrialized societies – caries and periodontal diseases. As will be detailed in a later section, the microbial composition of dental plaque from diseased surfaces differs from that found in health. It is the opinion of the author that these changes in microflora can be explained by the application of basic ecological principles, an understanding of which can open up new strategies for plaque control. The evidence generated by the author’s group that has led to this view will be outlined in subsequent sections.

The mouth as a microbial habitat

In order to identify the key ecological determinants that influence patterns of colonization, it is necessary to understand the properties of the mouth that influence microbial colonization. The mouth is continuously bathed with saliva, which keeps conditions warm (35–36°C) and moist at a pH of between 6.75 and 7.25, which is optimal for the growth of many micro-organisms. Saliva has a profound influence on the ecology of the mouth (Edgar & O’Mullane, 1996; Scannapieco, 1994); for example, its ionic composition promotes its buffering properties and its ability to remineralize (i.e. repair) enamel. In addition, the organic components (glycoproteins and proteins) can (a) influence the establishment and selection of the oral microflora by either coating oral surfaces, thereby promoting the adhesion of certain organisms by acting as a selective conditioning film, or by aggregating other species and facilitating their clearance by swallowing, and (b) act as endogenous nutrients. Saliva also contains components of innate (e.g. lysozyme, lactoferrin, sialoperoxidase, antimicrobial peptides, etc.) and adaptive immunity (e.g. slgA) and so can directly inhibit some exogenous micro-organisms (Edgar & O’Mullane, 1996; Scannapieco, 1994).

Adherence is a key ecological determinant for oral bacteria to survive and persist (Jenkinson & Lamont, 1997; Lamont & Jenkinson, 2000). The mouth is unique in the human body in possessing non-shedding surfaces (teeth) for microbial growth, leading to extensive biofilm formation (dental plaque). In contrast, desquamation ensures that the bacterial load is relatively light on mucosal surfaces. Teeth do not provide a uniform habitat for microbial growth (Table 1), but possess several distinct surfaces, each of which is optimal for colonization and growth by different populations of micro-organism due to the physical nature of the particular surface and the resulting biological properties of the site (Marsh, 2000b; Marsh & Martin, 1999). The areas between adjacent teeth (approximal) and in the gingival crevice afford protection from normal removal forces in the mouth, such as those generated by mastication, salivary flow and oral hygiene. Both sites also have a low redox potential and in addition, the gingival crevice region
is bathed in the nutritionally rich gingival crevicular fluid (GCF – a serum-like exudate), the flow of which is increased during inflammation and periodontal disease, so these areas support a more diverse community, including higher proportions of obligately anaerobic bacteria. GCF not only contains components of the host defences (antibodies and phagocytic cells) but also many proteins and glycoproteins that act as a novel source of nutrients for the resident bacteria of the gingival crevice. Many of these organisms are proteolytic and interact in a concerted and sequential manner as true consortia to degrade these complex molecules through to methane, H2S, H2 and CO2 (Marsh & Bowden, 2000). Essential co-factors (such as haemin for black-pigmented anaerobes) can be obtained from the degradation of host haem-containing molecules such as haemopexin, haemoglobin and haptoglobin. Smooth surfaces are more exposed to the environment and can be colonized only by a limited number of bacterial species adapted to such extreme conditions. Pits and fissures on the biting (occlusal) surfaces of teeth also afford some protection from the environment and in addition, are susceptible to food impaction. Few anaerobes grow at this site; the microflora is dominated by facultatively anaerobic Gram-positive bacteria, especially streptococci. Such protected areas are associated with the largest microbial communities and in general, the most disease.

Superimposed on the endogenously supplied substrates in saliva and GCF are the exogenous nutrients provided on an intermittent basis via the diet. Fermentable carbohydrates are the class of nutrients that most affect the microbial ecology of the mouth. They are catabolized to acids (e.g. lactic acid) which acidify plaque biofilms, inhibiting most of the species associated with enamel health while promoting the growth of acid-tolerating (aciduric) organisms such as mutans streptococci and lactobacilli, before saliva returns the pH to normal values. Frequent exposure to such conditions of low pH can disrupt microbial homeostasis and lead to the enrichment of such acidogenic and aciduric species, thereby predisposing surfaces to dental caries. Sucrose can also be metabolized to extracellular glucans that contribute substantially to the plaque biofilm matrix.

The relationship between the environment and the microbial community is not unidirectional. Although the properties of the environment dictate which micro-organisms can occupy a given site, the metabolism of the microbial community can modify the physical and chemical properties of their surroundings (Alexander, 1971). Thus, the environmental conditions change during the development of dental plaque with the metabolism of the facultatively anaerobic early colonizers depleting oxygen and producing carbon dioxide and hydrogen. This lowers the redox potential and creates an environment more suitable to the growth of later colonizers, many of which are strict anaerobes. Similarly, the environment on the tooth will also vary in health and disease. As caries progresses, the advancing front of the lesion penetrates the dentine. The nutritional sources will change and local conditions may become acidic and more anaerobic due to the accumulation of products of bacterial metabolism. Similarly, in disease, the junctional epithelium at the base of the gingival crevice migrates down the root of the tooth to form a periodontal pocket, and the production of GCF is increased in response

Table 1. The predominant groups of bacteria recovered from distinct surfaces on sound teeth

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Percentage viable count (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fissures</td>
</tr>
<tr>
<td>Strepotococcus</td>
<td>8–86</td>
</tr>
<tr>
<td>Actinomyces</td>
<td>0–46</td>
</tr>
<tr>
<td>An GPR*</td>
<td>0–21</td>
</tr>
<tr>
<td>Neisseria</td>
<td>+†</td>
</tr>
<tr>
<td>Veillonella</td>
<td>0–44</td>
</tr>
<tr>
<td>An GNR*</td>
<td>+†</td>
</tr>
<tr>
<td>Treponema</td>
<td>–</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Environment</th>
<th>Nutrient source</th>
<th>pH</th>
<th>Redox potential</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saliva and diet</td>
<td>Neutral–acid</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Saliva, diet and GCF</td>
<td>Neutral–acid</td>
<td>Slight negative</td>
</tr>
<tr>
<td></td>
<td>GCF</td>
<td>Neutral–alkaline</td>
<td>Negative</td>
</tr>
</tbody>
</table>

*An GPR, An GNR: obligately anaerobic Gram-positive and anaerobic Gram-negative rods, respectively.
†+, detected occasionally.
to the increase in plaque mass. These new environments will select for the microbial community most suitably adapted to the prevailing conditions.

**Dental plaque in health and disease**

**Health**

The formation of dental plaque involves an ordered pattern of colonization (microbial succession) by a range of bacteria. As soon as teeth erupt, or are cleaned, the enamel surfaces are coated with a conditioning film containing molecules derived from both the host (primarily saliva) and bacteria (e.g. some secreted products) (Busscher & van der Mei, 2000). The early colonizers can be retained near the tooth surface by non-specific, long range physicochemical interactions between charged molecules on the cell and host surface (Busscher & van der Mei, 1997). This may facilitate the establishment of specific, short-range and stronger intermolecular interactions between bacterial adhesins and complementary receptors in the conditioning film, resulting in irreversible attachment (Jenkinson & Lamont, 1997; Lamont & Jenkinson, 2000; Whittaker et al., 1996). These early colonizers then grow and modify local environmental conditions, making the site suitable for colonization by more fastidious species (e.g. obligate anaerobes). These later colonizers bind to the already attached species via similar adhesin-receptor mechanisms (a process termed co-aggregation or coadhesion) (Kolenbrander et al., 2000; Kolenbrander & London, 1993). In this way complex, structured, multi-species biofilms are formed (Table 1). Dental plaque is an example of both a biofilm and a microbial community (Marsh & Bradshaw, 1999), and studies of plaque are making a significant contribution to our understanding of these increasingly topical areas (Marsh & Bowden, 2000).

**Disease**

Numerous studies have been undertaken of the composition of the plaque microflora from diseased sites in order to try and identify those species directly implicated in the disease process. Interpretation of the data from such studies is difficult because plaque-mediated diseases occur at sites with a pre-existing diverse resident microflora, unlike most classical medical infections in which a single pathogen may be isolated from a site that is (a) normally sterile or (b) not usually colonized by that organism. Nevertheless, such studies have shown that caries is associated with increases in the proportions of acidogenic and aciduric (acid-tolerating) bacteria, especially mutants streptococci (such as *Streptococcus mutans* and *Streptococcus sobrinus*) and lactobacilli, which demineralize enamel (Bowden, 1990; Loesche, 1986; Marsh, 1999). These bacteria are able to rapidly metabolize dietary sugars to acid, creating locally a low pH. These organisms grow and metabolize optimally at low pH; under such conditions they become more competitive whereas most species associated with enamel health are sensitive to acidic environmental conditions. In contrast, gingivitis is associated with a general increase in plaque mass around the gingival margin, which elicits an inflammatory host response (including an increased flow of GCF), while increased levels of obligately anaerobic bacteria, including Gram-negative proteolytic species (especially bacteria belonging to the genera *Prevotella*, *Porphyromonas*, *Fusobacterium* and *Treponema*), are recovered from periodontal pockets (Moore & Moore, 1994; Socransky et al., 1998). Studies using appropriate culture media have suggested that these sites may also have much higher levels of *Eubacterium* spp. (Uematsu & Hoshino, 1992). Furthermore, perhaps 50 % of the microflora from periodontal pockets is currently unculturable (Kroes et al., 1999; Paster et al., 2001), and 16S rRNA studies have implicated novel taxa in disease (Dewhirst et al., 2000). Although some of these bacteria may cause tissue disruption directly by the production of proteases such as collagenase or hyaluronidase, much of the damage to the host is probably a result of the effects of the inflammatory response. Indeed, the role of proteolytic bacteria in periodontal disease includes the inactivation of host proteins that regulate the host response (Birkedal-Hansen, 1998; Curtis et al., 2001; Darveau et al., 1997).

Such findings led to two main hypotheses relating the plaque microflora to disease. The 'specific plaque hypothesis' proposed that out of the diverse collection of species present in plaque, only a relatively small number were directly involved in causing disease (Loesche, 1976). This proposal had the benefit of focussing studies on the control of specific microbial targets. However, although mutants streptococci are strongly implicated with caries, the association is not unique; caries can occur in the apparent absence of these species, while mutants streptococci can persist without evidence of detectable demineralization (Bowden et al., 1976; Marsh et al., 1989). Indeed, in such circumstances, some acidogenic, non-mutans streptococci are implicated with disease (Brailsford et al., 2001; Marsh et al., 1989; Sansone et al., 1993). An alternative view was expressed in the 'non-specific plaque' hypothesis (Theilade, 1986). This proposed that disease is the result of the overall interaction of all the groups of bacteria within plaque, and recognized the concept that plaque is a microbial community. However, if the aetiology of dental diseases is not entirely specific, they do show evidence of specificity in that a limited subset of bacteria are consistently recovered in higher numbers from diseased sites. These issues will be returned to later in the review.

What is clear and undisputed, however, is that the predominant species recovered from diseased sites are different from those found in health. The origin and role of these pathogens has been the subject of much debate. Indeed, the answer to this question is pivotal to the development of effective plaque control strategies. Conventional culture techniques often fail to recover the putative pathogens from healthy sites and when present, they comprise only a small proportion of the microflora,
suggesting that some of these 'pathogens' may be acquired exogenously. Certainly, molecular typing schemes have shown that identical strains of putative pathogens can be found in the plaque of mother and infants, and between spouses, implying that transmission of such bacteria can occur. However, the recent application of more sensitive immunological (e.g. ELISA; Di Murro et al., 1997; Gmüür & Guggenheim, 1994) and molecular (e.g. oligonucleotide probe or PCR; Asikainen & Chen, 1999; Greenstein & Lamster, 1997; Kisby et al., 1989; Socransky et al., 1999; Tanner et al., 2002) techniques has led to the frequent detection of low levels of several pathogens at a wide range of sites. This strongly suggests that plaque-mediated diseases result from imbalances in the resident microflora resulting from an enrichment within the microbial community of the pathogens due to the imposition of strong selective pressures. In either situation (i.e. natural low levels of 'pathogens' or low levels of exogenously acquired 'pathogens'), these species would have to outcompete the already established residents of the microflora to achieve an appropriate degree of numerical dominance to cause disease. As argued above, for this to happen, the normal homeostatic mechanisms would need to be disrupted and this is only likely to occur if there is a major disturbance to the local habitat (Fig. 1).

Factors responsible for the disruption of microbial homeostasis

Studies of a range of habitats have given 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, for example, following the introduction of a novel substrate. Thus, nitrogenous fertilizers can be washed off farm land and into surface water such as lakes and ponds, resulting in overgrowth by algae (Codd, 1995). 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). Other effects can result from a chemical change to the habitat, for example, following acidification of soil and lakes due to environmental pollution (acid rain) or following a physical disturbance, such as occurs in the body with implants such as catheters.

The local environment does change in plaque during disease. Caries is associated with more frequent exposure to fermentable carbohydrates (i.e. periods of carbohydrate excess), and hence a lower pH in plaque (Jensen & Schachtele, 1983). In contrast, during the inflammatory response to subgingival plaque, the pH rises to become slightly alkaline (Eggert et al., 1991), and flow of GCF is increased, the latter introducing potentially novel substrates for proteolytic anaerobes. The effect of such environmental changes on gene expression and virulence of oral bacteria predominating in either health or disease was studied initially in conventional pure cultures. This led to the design of laboratory modelling studies involving complex and defined communities of oral bacteria 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 hypothesis relating the role of oral bacteria to dental disease.

**Pure culture studies**

One approach to study the response of oral bacteria to relevant oral environmental stimuli has been to compare the properties of selected strains (representative of those predominating in health and disease) when grown under controlled conditions in continuous culture. The chemostat enables the physiological response of an organism to selected and defined environmental cues to be monitored accurately, since single parameters can be varied independently, enabling true cause-and-effect relationships to be established. Initially, the response of an organism was monitored by measuring the activity of specific enzymes or other read-outs of metabolism. More recently, whole genome approaches have been applied to oral pathogens, such as differential display (Bonass et al., 2000) and proteomics (Svensäter et al., 2001; Wilkins et al., 2002); in the future, microarrays will be available for some of these species.

Collectively, early studies compared the responses of *S. mutans* (implicated in dental caries) and *Streptococcus sanguinis* (formerly *S. sanguis*; associated with sound enamel) to sugar and pH stresses (Ellwood & Hunter, 1976; Ellwood et al., 1979; Hamilton, 1987; Hamilton et al., 1979; Marsh et al., 1985). These studies showed that *S. mutans* was able to grow over a wider pH range, that its growth was optimal at acidic pH (~pH 5-5), that rates of sugar uptake and glycolysis were greater, and the terminal
pH reached from sugar metabolism was lower than for *S. sanguinis*.

Analogous studies were conducted on periodontal pathogens. *Porphyromonas gingivalis* has an obligate requirement for growth for haemin, and probably derives this co-factor from the metabolism of host haem-containing proteins. *P. gingivalis* had increased proteolysis activity when growing under haemin-excess rather than haemin-limited conditions; this effect was enhanced when growing at alkaline pH (McDermid *et al.*, 1988; McKee *et al.*, 1986). Indeed, the optimum pH for growth of *P. gingivalis* was pH 7·5, which is higher than that determined for other Gram-negative oral anaerobes (see Marsh *et al.*, 1993). Such conditions emulate those predicted to occur in the inflamed periodontal pocket. When inoculated in a mouse model of virulence, cells grown under haemin excess were more virulent than the same cells grown under haemin limitation (McKee *et al.*, 1986). This was confirmed when cells were subsequently grown at the optimum pH for growth of *P. gingivalis* from the metabolism of host haem-containing proteins. These trends were supported by the work of other groups. Collectively, these data suggested that the changes in environment seen in disease are likely to alter the pattern of gene expression of oral bacteria such that the competitiveness of the species associated with disease is increased, while that of organisms that normally prevail in health is reduced. This also suggested that the transition seen in the composition of plaque between health and disease is driven by a response of the members of the microbial community to environmental change, resulting in the selection of previously minor components of the microflora. To test this hypothesis and to further explore these concepts, it was necessary to develop a more complex laboratory system in order to model the impact of changes in environmental conditions on the overall balance of the plaque microbial community.

**Mixed culture studies**

We again chose the chemostat as the basis of the laboratory system to study the response of mixed cultures of oral bacteria to environmental perturbation, because of the need to carefully control the environment and vary single parameters independently to determine cause-and-effect relationships (Bradshaw & Marsh, 1999; Marsh, 1995). However, chemostat theory predicts that the most adapted species within the community to the prevailing conditions should dominate, and out-compete the remaining organisms, resulting in a consortium with a simple composition. Therefore, a proof-of-principle experiment was carried out using human dental plaque as an inoculum to determine whether complex microbial communities could be established stably for prolonged periods in the chemostat (Marsh *et al.*, 1983a). It was demonstrated that (a) highly diverse oral consortia containing organisms with fastidious growth requirements could be established and maintained for prolonged periods, (b) the composition of such consortia could be modulated by changing the environment (e.g. nutrient status), and (c) sensible analyses could be undertaken (e.g. enzyme assays, substrate transport rates, etc.) by using the total microbial community as the unit of activity. Several drawbacks to this approach were also identified, however, including (a) the composition of the consortia was complex, and consequently nearly as difficult and time-consuming to identify as clinical material, and (b) the composition of the inoculum could not be manipulated for experimental purposes (e.g. some species were not present in the inoculum, or were present but were not appropriate for the question under study), and could not be controlled in replicate experiments.

Consequently, a decision was made to construct complex but defined inocula, consisting of species found in both health and disease, but which were of relevance to the particular study. Over the years, a range of such inocula (routinely comprising nine or ten species; Table 2) have been developed for specific applications; each organism is grown separately and then pooled with other community members (McKee *et al.*, 1985). This pooled inoculum can be divided into aliquots and stored over liquid nitrogen until required to inoculate the chemostat (Bradshaw *et al.*, 1989a). Major advantages of this approach are (i) relevant species/strains can be incorporated, (ii) the strains can have particular characteristics to facilitate their rapid identification, (iii) strains can be added/removed to answer biological questions/test hypotheses, (iv) the inocula can be stored indefinitely and give reproducible consortia in replicate experiments; the consortia can also be quality controlled before use to reduce the risk of contamination. These inocula have been used subsequently by other workers around the world. Initially, chemostats were inoculated on three occasions, to give slower-growing and anaerobic species an opportunity to establish (McKee *et al.*, 1985); however, it was found subsequently that all species could establish even after a single inoculation.

The choice of growth medium can be critical to the development of a relevant model. The early studies used standard bacteriological media with simple sugars as the carbon source, but following the work of others (Glenister *et al.*, 1988), a habitat-simulating medium based on hog gastric mucin (a commercially available glycoprotein with a structure similar to salivary mucins) as the main carbon source was adopted. This has permitted the superimposition of pulses of mono- or disaccharides (or sugar alcohols) to simulate the intermittent nutritional stresses caused by exogenous substrates introduced via the diet. For studies of relevance to the development of periodontal disease, the medium has also been supplemented with serum to mimic the increased flow of GCF during inflammation. Use of such
media has resulted in the establishment of diverse but stable communities because component species have to function co-operatively in order to catabolize these complex molecules.

The original studies were conducted in conventional, single-stage chemostats, in which all the interactions occur in one vessel. For some applications, however, multi-stage systems have been assembled; for example, two-stage systems have been used in which the second stage is subjected to different conditions. The original studies were conducted in conventional, single-stage chemostats, in which all the interactions occur in one vessel. For some applications, however, multi-stage systems have been assembled; for example, two-stage systems have been used in which the second stage is subjected to different conditions. The original studies were conducted in conventional, single-stage chemostats, in which all the interactions occur in one vessel. For some applications, however, multi-stage systems have been assembled; for example, two-stage systems have been used in which the second stage is subjected to different conditions. The original studies were conducted in conventional, single-stage chemostats, in which all the interactions occur in one vessel. For some applications, however, multi-stage systems have been assembled; for example, two-stage systems have been used in which the second stage is subjected to different conditions. The original studies were conducted in conventional, single-stage chemostats, in which all the interactions occur in one vessel. For some applications, however, multi-stage systems have been assembled; for example, two-stage systems have been used in which the second stage is subjected to different conditions.

The proportions of bacteria within a defined, 10-membered microbial community pulsed daily with glucose (28 mM; 10 consecutive days) with and without different inhibitors, with and without pH control, in a chemostat.

Data taken from Bradshaw et al., 1989b, 1990, 2002; Bradshaw & Marsh, 1994. ñ, strain not included in inoculum; ND, not detected; +, detected but at very low levels.

**Table 2.** The proportions of bacteria within a defined, 10-membered microbial community pulsed daily with glucose (28 mM; 10 consecutive days) with and without different inhibitors, with and without pH control, in a chemostat.

<table>
<thead>
<tr>
<th>Bacterium+</th>
<th>pH 7.0</th>
<th>Percentage viable count</th>
<th>Without pH control</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glucose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glucose + fluoride</td>
</tr>
<tr>
<td>Streptococcus <em>mutans</em></td>
<td>0.2</td>
<td>1.0</td>
<td>18.9</td>
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<tr>
<td>Streptococcus <em>sanguinis</em></td>
<td>29.9</td>
<td>25.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Streptococcus <em>oralis</em></td>
<td>15.0</td>
<td>16.9</td>
<td>1.3</td>
</tr>
<tr>
<td>Actinomyces <em>naeslundii</em></td>
<td>0.4</td>
<td>13.1</td>
<td>2.3</td>
</tr>
<tr>
<td>Lactobacillus <em>rhamnosus</em></td>
<td>0.1</td>
<td>0.2</td>
<td>3.6-1</td>
</tr>
<tr>
<td>Neisseria <em>subflava</em></td>
<td>0.1</td>
<td>&lt;0.1</td>
<td>ND</td>
</tr>
<tr>
<td>Veillonella <em>dispar</em></td>
<td>8.2</td>
<td>9.8</td>
<td>41.4</td>
</tr>
<tr>
<td>Fusobacterium <em>nucleatum</em></td>
<td>13.3</td>
<td>15.2</td>
<td>+</td>
</tr>
<tr>
<td>Prevotella <em>nigrescens</em></td>
<td>32.8</td>
<td>31.0</td>
<td>+</td>
</tr>
<tr>
<td>Porphyromonas <em>gingivalis</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>pH 7.0</td>
<td>7.0</td>
<td>7.0</td>
<td>3.83</td>
</tr>
</tbody>
</table>

*The nomenclature of strains changed during the period of study and has been standardized for clarity.

and also to fundamental issues of relevance to the recent resurgence of interest in microbial communities and biofilms (Marsh & Bowden, 2000). Micro-organisms recovered from a diverse collection of habitats grow under macro-environmental conditions that appear to be overly hostile. In the oral context, the mouth is exposed to air and yet the majority of bacteria are obligate anaerobes; many species in plaque are sensitive to low pH and yet survive repeated exposure to acidic conditions following the intake of fermentable sugars in the diet. Selected studies that have contributed to understanding how organisms cope with these stresses at the community level will be described briefly here.

**Nutrition**

In Nature, bacteria generally have to catabolize complex macromolecules. In the mouth, endogenous proteins and glycoproteins (mucins) are the main sources of carbon and nitrogen for the resident oral microflora. Pure cultures of oral bacteria can metabolize such molecules only poorly or not at all (Homer & Beighton, 1992). The importance of community interactions became apparent when the ‘defined microbial community’ concept was exploited to determine the role played by individual species (Bradshaw et al., 1994).

A five-membered community of oral bacteria was established in the chemostat with mucin as the main carbon source; these five species had minimal glycosidase or protease activity. It proved possible to introduce additional species into this community but only if they possessed novel and relevant catabolic capabilities. For example, _Streptococcus oralis_ and _Actinomyces naeslundii_ provided...
sialidase activity, and could remove terminal sialic acid residues, exposing new substrates, which could then be exploited by subsequent microbial additions, such as *Lactobacillus rhamnosus* (β-fucosidase activity) and *P. gingivalis* (protease activity). Addition of these species resulted in an increase in total biomass that was not due merely to the new strain, but was made up mainly of an increase in the extant species, presumably because of the exposure of additional substrates on the oligosaccharide side chains. Thus, catabolism of these complex host molecules involves the synergistic and concerted action of interacting species, each with complementary enzyme profiles, and explains how the members of such consortia can exist in a dynamic balance with each other and with the environment.

**Oxygen**

Anaerobic organisms can cope with the toxic effects of oxygen by interacting with oxygen-consuming species that can reduce the environmental levels of oxygen sufficiently to enable them to detoxify the residual low levels with a range of protective enzyme systems (Marquis, 1995b). Direct evidence that specific physical associations among members of oral microbial communities can provide protection for obligate anaerobes from the toxic effects of oxygen was obtained from studies using a two stage, mixed culture, biofilm model (Bradshaw *et al.*, 1996a, 1997, 1998). A stable microbial community was established in the anaerobic first stage fermenter, and this was then passed continuously into an actively aerated vessel containing surfaces for biofilm formation. Surprisingly, the obligate anaerobes grew both in the biofilm and in the planktonic culture in the aerated second stage. The predominant species, however, was an oxygen-consuming species, *Neisseria subflava* (>80% of the total microflora in early communities), which had been present at barely detectable levels (<0.01%) in the community grown anaerobically. No dissolved oxygen could be detected in the planktonic phase and this was attributed to the enhanced growth and metabolism of the aerobe *N. subflava*; the redox potential also remained reduced (~−250 mV) (Bradshaw *et al.*, 1996a). In a subsequent experiment, therefore, *N. subflava* was deliberately omitted from the inoculum and the effect of oxygen reassessed. Again the anaerobes persisted at high levels, but in this community, the loss of the aerobe was compensated for by an increase in the levels of facultatively anaerobic species, especially the streptococci. These data, together with direct microscopic observation of the community, suggested that close cell-to-cell contact between oxygen-consuming and oxygen-sensitive species must be occurring, enabling the obligate anaerobes to survive, especially in the planktonic phase. Co-aggregation (or co-adhesion) is a key process during the formation of biofilms such as dental plaque, facilitating intra- and inter-generic attachment (Kolenbrander *et al.*, 2000; Kolenbrander & London, 1993). Assays demonstrated that although the oxygen-consumer (*N. subflava*) co-aggregated only poorly with the obligate anaerobes, this interaction could be markedly enhanced in the presence of *Fusobacterium nucleatum*, which could act as a bridging organism between otherwise weakly coaggregating pairs of strains (Bradshaw *et al.*, 1998). The key role for this co-aggregation was confirmed when consortia lacking *F. nucleatum* were reintroduced into the aerated biofilm vessel. Viable counts of the black-pigmenting anaerobes within the community (*Prevotella nigrescens*, *Por. gingivalis*) fell by three orders of magnitude (Bradshaw *et al.*, 1998). Similarly, when the complete consortium was established in an aerated CDFF, anaerobes did establish in the depths of the biofilms; however, the air–biofilm interface was composed almost exclusively of a ‘plug’ of the aerobe *N. subflava* (Fig. 2) (Kinniment *et al.*, 1996a). These findings suggest that the role of co-aggregation is not necessarily restricted to anchoring cells during the establishment of a microbial community, but may also facilitate metabolism among strains that depend for their survival on close physical cell–cell contact. It is probable that similar physical interactions will occur to ensure that organisms needing to interact for nutritional or other environment-modifying purposes are appropriately spatially organized.

**pH**

Many bacterial species, including members of our oral bacterial consortia, have a relatively narrow pH range for growth, and yet survive repeated exposure to pH values beyond this range when present as part of a community (for examples, see Bradshaw *et al.*, 1989b; McDermid *et al.*, 1986). Their survival is probably due to several of the properties of biofilms when they function as surface-associated microbial communities. Individual bacteria possess specific molecular strategies which enable them to adapt rapidly to sudden changes in pH (Bowden & Hamilton, 1998; Foster, 1995; Hall *et al.*, 1995). In addition, bacteria are able to modulate their local pH, especially in biofilms, by up-regulating genes encoding enzymes involved with acid or base production (e.g. urease and arginine deiminase). These enzymes can be active at pH values lower than those at which the bacteria can grow. Also, gradients develop in key parameters in biofilms (Costerton *et al.*, 1987, 1994, 1995), and this environmental and spatial heterogeneity can enable organisms to grow together that would be incompatible with one another in a homogeneous habitat. Studies of our mixed culture oral biofilms (generated in the CDFF) using two photon excitation microscopy coupled with fluorescent lifetime imaging of pH-sensitive dyes (TPEM-FLIM) have provided visual proof of the development of such environmental heterogeneity in complex oral biofilms in terms of pH over short distances (Vroom *et al.*, 1999). The gradients in pH were not linear either in the x–y or x–z axes; discrete pH zones were observed adjacent to areas of quite differing pH. Thus, microbial communities are able to defy the constraints imposed by the external macro-environment by creating, through their metabolism, a mosaic of micro-environments that enable the survival and growth of the component species.

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Role of oral microbial communities in disease

Selection of cariogenic bacteria: role of diet

The ability to replace simple sugars with a glycoprotein (mucin) in laboratory media has enabled more realistic simulations to be made of the influence of dietary carbohydrates on the balance of the resident oral microflora. As stated earlier, individuals who frequently consume sugar in their diet generally have elevated levels of cariogenic bacteria such as *S. mutans* and lactobacilli in their plaque, and are at greater risk of dental caries. In animal studies or epidemiological surveys of humans, it can never be determined 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 catabolism. Exploitation of the unique benefits of parameter control in the chemostat, coupled with the reproducibility of the defined mixed culture inoculum, enabled these linked effects to be separated for the first time. Two mixed culture chemostats were pulsed daily for ten consecutive days with a fermentable sugar (glucose). In one chemostat, the pH was maintained automatically throughout the study at neutral pH (as is found in the healthy mouth), while in the other the pH was allowed to fall by bacterial metabolism for 6 h after each pulse; the pH was then returned to neutrality for 18 h prior to the next pulse (Bradshaw et al., 1989b). Daily pulses of glucose for 10 consecutive days at a constant pH 7.0 had little impact on the balance of the microbial community, and the combined proportions of *S. mutans* and *L. rhamnosus* stayed at <1% of the total microflora (Table 2). In contrast, 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 (~45% 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 (Bradshaw & Marsh, 1998). 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, and an inverse relationship was seen with species associated with enamel health (Fig. 3).

Collectively, these studies showed conclusively for the first time that it was the low pH generated from sugar metabolism rather than sugar availability that leads to the breakdown of microbial homeostasis in dental plaque. This finding had important implications for disease control and prevention (see later).

Selection of periodontal pathogens

pH. During inflammation, the pH of the gingival crevice has been shown to rise from pH <7.0 to >7.5 (Eggert et al., 1991). pH has a profound effect on gene expression, and as discussed earlier, can enhance the growth and

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**Fig. 2.** Transmission electron micrograph of an oral biofilm generated in a constant depth film fermenter and derived from a defined inoculum of 10 species. Images taken from the surface (a), middle (b) and base (c) regions. The microflora is diverse in the lower sections, whereas the surface shows a predominance of an oxygen-consuming *N. subflava* (Kinniment et al., 1996a).
protease activity of some periodontal pathogens. In order to determine the influence of an increase in pH on bacterial competition, a three-membered consortium of black-pigmented oral anaerobes was established in the chemostat, initially at pH 6.70; the pH was then increased step-wise to pH 7.00, 7.25 and 7.50 (Marsh et al., 1993). The inoculum consisted of *Prevotella melaninogena*

(associated with healthy sites), *Prevotella intermedia* (found in higher numbers and more commonly in periodontal disease) and *Por. gingivalis* (isolated in high numbers from advanced disease). *Pre. melaninogena* predominated at and below neutral pH; however, a small shift in pH to 7.25 resulted in *Pre. intermedia* becoming most numerous, while growth at pH 7.50 caused the culture to be dominated by *Por. gingivalis*.

Change in nutrient status. Serum has been used as a surrogate for GCF in experiments modelling how the balance of the oral microflora is affected by changes in nutrient status that might occur in the gingival crevice during inflammation. Human serum was used for batch-wise enrichments of samples of subgingival plaque (ter Steeg et al., 1987). After 5–6 enrichment steps, the composition of the microflora showed few similarities with the original plaque sample, and consisted mainly of black-pigmented and non-pigmented Gram-negative anaerobes together with anaerobic streptococci. These consortia were able to extensively degrade the serum glycoproteins provided. In addition, it was demonstrated that *Pre. intermedia* could not be detected in some of the original plaque samples, but became a major component (~ 10%) of the microflora after only two or three enrichments (ter Steeg et al., 1987). This study suggested that such organisms must be present initially in plaque, but at levels below the detection limit of the methods adopted. A change in local nutrient status could alter the relative competitiveness of individual species, enabling *Pre. intermedia* to escape from homeostatic control and predominate at a site. Similar findings have been found using our defined community.

When serum was introduced into the chemostat, the redox potential fell to even lower levels (~2380 mV), the pH rose from 6.95 to >7.5 through bacterial metabolism, *Por. gingivalis* increased in proportion to dominate the community (~80% of total c.f.u.) and overall protease activity rose.

**Implications for the aetiology of caries and periodontal disease: an ‘ecological plaque hypothesis’**

As discussed earlier, there have been two main proposals put forward to explain the role of plaque bacteria in disease (the ‘specific’ and ‘non-specific’ plaque hypotheses), but that issues have emerged that affect their general applicability. The data from the studies described in this review provide an argument for plaque-mediated diseases being a consequence of imbalances in the resident microflora resulting from an enrichment within the microbial community of these ‘oral pathogens’ (Marsh, 1991, 1994; Marsh & Bradshaw, 1997; Newman, 1990). Collectively, these and other published findings allowed a dynamic model to be constructed to explain the changes in the ecology of dental plaque that lead to the development of caries or periodontal disease. In the context of caries, potentially cariogenic bacteria may be found naturally in dental plaque, but at neutral pH, these
organisms are weakly competitive 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 de- and remineralization are in equilibrium. If the frequency of fermentable carbohydrate intake increases, then plaque spends more time below the critical pH for enamel demineralization (~pH 5.5). The effect of this on the microbial ecology of plaque is twofold. Conditions of low pH favour the proliferation of aciduric (and acidogenic) bacteria (especially mutans streptococci and lactobacilli), while tipping the balance towards demineralization. Greater numbers of bacteria such as mutans streptococci and lactobacilli in plaque would result in more acid being produced at even faster rates, thereby enhancing demineralization still further. Other bacteria could also make acid under similar conditions, but at a slower rate, but would be responsible for some of the initial stages of demineralization, or could cause lesions in the absence of other (more overt) cariogenic species in a more susceptible host. If aciduric species were not present initially, then the repeated conditions of low pH coupled with the inhibition of competing organisms might increase the likelihood of colonization by mutans streptococci or lactobacilli. This sequence of events would account for the lack of total specificity in the microbial aetiology of caries and explain the pattern of bacterial succession observed in many clinical studies.

Similarly, as discussed earlier, molecular studies have shown that subgingival plaque from healthy sites can harbour putative periodontal pathogens, but at extremely low levels. These organisms are unable to outcompete 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 LPS), (b) a rise in pH and (c) a further reduction 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' (Fig. 4) (Marsh, 1991, 1994, 1998; Marsh & Bradshaw, 1997). 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, the significance of disease of newly discovered species can be predicted on the basis of their physiological characteristics. For example, bacteria associated with dental caries can display a continuum from those that are slightly acidogenic, and hence only make a minor contribution, to species that are both highly acidogenic and also aciduric. Mutans streptococci are the organisms that are best adapted to the cariogenic environment (high sugar/low pH), but such traits are not unique to these bacteria. Strains of other species, such as members of the Streptococcus mitis group, also share some of these properties and therefore will contribute to the rate of demineralization of enamel (Brailsford et al., 2001; Sansone et al., 1993). The role in disease of any subsequently discovered novel bacterium could be gauged by an assessment of its acidogenic/aciduric properties. Following on this line of argument, another key element of the ecological plaque hypothesis, and one that most distinguishes it from the earlier proposals, is the fact that disease could be prevented not only by direct inhibition of the putative pathogens, but also by interfering with the key environmental factors driving the ecological shifts. Eh, redox potential. Adapted with permission from Marsh (1994).
responsible for their enrichment (Marsh, 1991, 1994, 1998; Marsh & Bradshaw, 1997). Some of these strategies will be discussed below.

Prevention strategies and the ecological plaque hypothesis

In the case of dental caries, the regular conditions of sugar/low pH appear to be the primary mechanism that disrupts microbial homeostasis. Strategies that are consistent with the prevention of disease via the principles of the ecological plaque hypothesis include: (a) inhibition of plaque acid production, (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. Some of these approaches have been investigated using the mixed culture system.

Inhibition of acid production

The primary dental benefit of fluoride is normally regarded in terms of its role in improving enamel chemistry by enhancing remineralization and increasing the acid resistance of enamel. However, fluoride can also inhibit bacterial metabolism, but the impact of this effect on dental caries has generally been dismissed (ten Cate, 2001). The fluoride inhibition of metabolism is pH sensitive, with the greatest impact occurring under acidic conditions where fluoride exists as H$^+$F$^-$ (Marquis, 1995a). This ionized form is lipophilic and can readily penetrate bacterial membranes. The pH inside a cell is relatively alkaline, so the intracellular H$^+$F$^-$ dissociates; the F$^-$ inhibits various enzymes associated with sugar metabolism (including sugar transport systems and glycolysis) and the H$^+$ acidifies the cytoplasm, again reducing the activity of key enzymes.

The defined mixed culture system has been used to demonstrate that physiologically relevant concentrations of fluoride (10 and 19 p.p.m.; 0-5 and 1-0 mM NaF) can reduce both the pH challenge from sugar metabolism (Table 2, Fig. 5) and the impact of the aciduric behaviour of some oral organisms (Table 2) (Bradshaw et al., 1990, 1992). Even 10 p.p.m. (0-5 mM) NaF had a small but significant inhibitory effect on the rate and depth of the pH fall following glucose pulses. The inhibition was even more pronounced in mixed culture biofilms, where fluoride caused an eightfold reduction in H$^+$ concentration (pH 4-55 versus 5-55) (Bradshaw et al., 2002). The numbers and proportions of S. mutans were also reduced in the presence of fluoride, while pH-sensitive species persisted at higher levels (Table 2). Comparison of these data with earlier results (Fig. 3) showed that fluoride exerts inter-related direct actions on S. mutans (antimicrobial/anti-metabolism) and indirect effects by preventing the development of a favourable low pH environment. These studies have contributed to the emerging view that fluoride may have subtle antimicrobial effects that help stabilize the microbial community during the regular low pH perturbations in plaque that occur at meal times and during snacking.

Antimicrobial agents delivered in dental products are retained in the mouth for only short periods (minutes) at concentrations above the MIC of oral bacteria, but can persist for prolonged periods (hours) at sub-MIC levels. The mixed culture system has demonstrated that transient exposure to agents classed as being broad spectrum (e.g. chlorhexidine, Triclosan) can lead to unexpected favourably selective antimicrobial effects. Target species such as S. mutans and Gram-negative anaerobes remain sensitive while many species associated with dental health are relatively unaffected by these short contact times with inhibitors (Bradshaw et al., 1993; Kinniment et al., 1996b; McDermid et al., 1987). In addition, these agents at sub-MIC levels reduce metabolism by inhibiting glycolysis, sugar transport and proteases, which again will help to stabilize microbial communities and maintain homeostasis (Cummins, 1991; Marsh et al., 1983b).

Food and snack items can be prepared with compounds that are as sweet as sucrose but which cannot be metabolized rapidly to acid (Edgar & Dodds, 1985; Grenby & Saldanha, 1986). Bulk agents, such as sugar alcohols (sorbitol, xylitol), and intense sweeteners (e.g. aspartame, saccharin) will stimulate saliva in the absence of significant acid production; this can even lead to the remineralization of early lesions. Thus, consumption of these types of food does not generate conditions in plaque conducive to the growth and enrichment of cariogenic bacteria. Some of these compounds can also act as metabolic inhibitors (Grenby & Saldanha, 1986). Delivery of xylitol with glucose to our

Fig. 5. Terminal pH (expressed in terms of H$^+$ ion concentration) in a mixed culture chemostat (see Table 2) following 10 daily pulses of 28 mM glucose in the presence (black squares) or absence (black diamonds) of 1-0 mM (19 p.p.m.) NaF. The terminal pH was measured 6 h after each pulse of glucose. Adapted from Bradshaw et al. (1990).
Table 3. Viable counts of a mixed culture of oral bacteria grown either in a chemostat or as a biofilm in a constant depth film fermenter (CDFF) treated with either a redox agent (methylene blue; 0.1%, final concentration) or water

Data for selected bacteria are shown (Marsh et al., 2002); biofilms were 100 μm deep and exposed for 2 h; chemostat cultures were treated for 1 h.

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Viable counts</th>
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<tr>
<td></td>
<td>Chemostat (log_{10} c.f.u. ml^{-1})</td>
<td>Biofilm (log_{10} c.f.u.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>Redox agent</td>
<td>Water</td>
</tr>
<tr>
<td>Total viable count</td>
<td>7.88</td>
<td>7.12</td>
<td>7.58</td>
</tr>
<tr>
<td>S. oralis</td>
<td>6.32</td>
<td>6.70</td>
<td>6.16</td>
</tr>
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<td>S. sanguinis</td>
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<td>5.53</td>
</tr>
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<td>S. mutans</td>
<td>6.13</td>
<td>6.00</td>
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</tr>
<tr>
<td>Por. gingivalis</td>
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</tr>
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<td>Pre. nigrescens</td>
<td>6.65</td>
<td>4.00</td>
<td>0.72</td>
</tr>
<tr>
<td>F. nucleatum</td>
<td>7.66</td>
<td>4.90</td>
<td>7.07</td>
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A consortium of oral bacteria reduced the rate and extent of acid production, and inhibited the anticipated enrichment of S. mutans (Table 2) (Bradshaw & Marsh, 1994).

In periodontal diseases, conventional treatment approaches involve mechanical removal of plaque or physical disruption of biofilm structure. Refractory disease may require treatment with antibiotics. From an ecological perspective, attempts could also be made to alter the local environment by reducing the flow of GCF (e.g., by the use of anti-inflammatory agents; some antimicrobial agents in dental products also have anti-inflammatory properties) or the site could be made less anaerobic by the use of oxygenating or redox agents (Ower et al., 1995). The mixed culture system has been used to demonstrate that methylene blue can raise the redox potential from highly reduced conditions (approximately −330 mV) to +70 mV, and selectively inhibits the growth of obligate anaerobes both in planktonic and in biofilm cultures (Table 3) (Marsh et al., 2002).

Concluding remarks

Commonly, when a clinician is faced with plaque-mediated disease, only the symptoms are treated. The appearance of disease should alert the clinician to identify the causal factor(s) driving this local ‘ecological catastrophe’ in plaque, and deal with both the cause and the effect of the disease. Examples of potential causal factors include poor oral hygiene, an inappropriate diet, smoking and the long-term use of medications that, as a side-effect, reduce the flow of GCF (e.g., by the use of anti-inflammatory agents; some antimicrobial agents in dental products also have anti-inflammatory properties) or the site could be made less anaerobic by the use of oxygenating or redox agents (Ower et al., 1995). The mixed culture system has been used to demonstrate that methylene blue can raise the redox potential from highly reduced conditions (approximately −330 mV) to +70 mV, and selectively inhibits the growth of obligate anaerobes both in planktonic and in biofilm cultures (Table 3) (Marsh et al., 2002).

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Strategies to maintain homeostasis (and hence a favourable ecology) in plaque.

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