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Philip D. Marsh and David J. Bradshaw
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PHYSIOLOGICAL APPROACHES TO THE CONTROL OF ORAL BIOFILMS

PHILIP D. MARSH1,2
DAVID J. BRADSHAW1

1Centre for Applied Microbiology & Research Research Division Salisbury, SP4 0JG, and
2Leeds Dental Institute Leeds LS2 9LU, UK


Abstract—Evidence that physiological strategies may be potential routes for oral biofilm control has come from (i) observations of the variations in the intra-oral distribution of members of the resident oral microflora, (ii) changes in plaque composition in health and disease, and (iii) data from laboratory model systems. Key physiological factors that were identified as significant in modulating the microflora included the local pH, redox potential ($E_h$), and nutrient availability. Increases in mutans streptococci and lactobacilli occur at sites with caries; growth of these species is included the local pH, redox potential ($E_h$), and nutrient availability. Increases in mutans streptococci and lactobacilli occur at sites with caries; growth of these species is selectively enhanced at low pH. In contrast, periodontal diseases are associated with plaque accumulation, followed by an inflammatory host response. The increases in Gram-negative, proteolytic, and obligately anaerobic bacteria reflect a low redox potential and a change in nutrient status due to the increased flow of gingival crevicular fluid (GCF). Consequently, physiological strategies for oral biofilm control should focus on reducing the frequency of low pH in plaque by (i) inhibiting acid production, (ii) using sugar substitutes, and (iii) promoting alkali generation from arginine or urea supplements. Similarly, strategies to make the pocket environment less favorable to periodonto-pathogens include (i) anti-inflammatory agents to reduce the flow of (and hence nutrient supply by) GCF, (ii) bacterial protease inhibitors, and (iii) redox agents to raise the $E_h$ locally. Most laboratory and clinical findings support the concept of physiological control. However, some data suggest that the ordered structure and metabolically interactive organization of mature dental plaque could generate a community with a high level of homeostasis that is relatively resistant to deliberate external manipulation.

Key words: biofilm, ecology, pH, antimicrobial agents, fluoride, urea, arginine, redox potential, homeostasis.

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Dental plaque is the biofilm that forms on the surfaces of teeth, and is comprised of a diverse community of bacteria, embedded in a matrix of polymers of microbial and host origin. Plaque develops naturally, and is generally considered of benefit to the host because of its ability to prevent colonization by exogenous (and often pathogenic) micro-organisms (Marsh, 1989). The formation of plaque involves a number of well-defined phases. Early colonizers, such as members of the genera Streptococcus, Actinomyces, Hemophilus, Neisseria, and Veillonella, adhere to a conditioning film (the acquired enamel pellicle) by specific and non-specific molecular interactions between adhesins on the bacterial cell and receptors on the substratum (Gibbons, 1989; Busscher et al., 1992). Subsequently, other bacteria adhere specifically to already-attached cells (co-aggregation; Kolenbrander and London, 1993), thereby increasing the diversity of the microbial community, and providing a mechanism to regulate bacterial succession during biofilm formation. In this way, late colonizers (many of which are obligately anaerobic and nutritionally fastidious species) would attach when the physiological conditions in the biofilm are more likely to support their growth.

ECOLOGY OF DENTAL PLAQUE IN HEALTH AND DISEASE

The plaque microflora at anatomically distinct surfaces on teeth varies quite markedly in its composition. The reasons for this can include the degree of protection from mastication and saliva flow, etc., to differences in key local environmental conditions. For example, the gingival crevice has a low redox potential and, during inflammation, is provided with gingival crevicular fluid, GCF, which is rich in potential nutrients for proteolytic bacteria. Not surprisingly, this site has higher numbers of asaccharolytic, Gram-negative anaerobes than plaque from fissures, the composition of which is influenced more by saliva.

Once established at a site, the microflora remains relatively stable over time, despite regular minor perturbations to the oral environment (Marsh, 1989). This stability (termed “microbial homeostasis”) stems not from any metabolic indifference among the components of the microflora, but rather results from a dynamic balance arising out of numerous, coupled microbial interactions (Sanders and Sanders, 1984). The precise mechanisms responsible for maintaining this homeostasis in plaque are not fully understood, but include both synergistic and antagonistic interactions. Microbial stability is enhanced through the development of nutritional inter-relationships, such as food chains, and the need for microbial collaboration in the catabolism of complex endogenous nutrients (ter Steeg et al.,...
The "ecological plaque hypothesis" provides a physiological approach to the control of biofilms associated with (a) caries and (b) periodontal disease. The postulated dynamic relationship between environmental change and ecological shifts within the biofilm implies that disease could be prevented not only by direct inhibition of the putative pathogens, but also by interfering with the key environmental/physiological factors driving the ecological shift (Marsh, 1994).

On occasions, homeostasis can break down at a site, and disease can occur. Disease is associated with major shifts in the balance of the resident plaque microflora and alterations...
in the metabolism at a site following changes to the habitat. Dental caries is associated with an increased frequency of consumption of fermentable sugars in the diet, which results in an increase in the isolation and proportions of acidogenic and aciduric species such as mutans streptococci and lactobacilli (Loesche, 1986). In contrast, periodontal diseases involve an inflammatory host response to the accumulation of plaque around the gingival margin. This response leads to an increased flow of GCF which, in addition to introducing components of the host response, also provides a novel source of potential nutrients for the microflora. In more advanced forms of periodontal disease, there are significant increases in the prevalence of obligately anaerobic Gram-negative bacilli, especially proteolytic species. Many of these bacteria are not detected in health, or are present at low (and clinically insignificant) levels.

The concept that (i) a direct relationship exists between the environment and the balance and behavior of the resident plaque microflora, (ii) changes to that environment can lead to the selection or enrichment of previously minor components of this oral biofilm, and (iii) such an enrichment can result in clinical changes to host tissues led to a modified hypothesis (the “Ecological Plaque Hypothesis”; Marsh, 1991, 1994) being proposed to explain the transition of the plaque microflora from having a commensal to a pathogenic relationship with the host (Fig. 1). The basic tenet of this hypothesis is that disease prevention can be achieved not only by inhibiting the causative bacteria directly, but also by identifying and controlling the ecological/physiological factors driving these transitions. Manipulation of these key physiological factors could lead to some degree of control over the composition of the plaque community, and lead to the identification of new physiological strategies to maintain the beneficial properties of the biofilm.

**PHYSIOLOGICAL FACTORS AFFECTING DENTAL PLAQUE**

**Fermentable sugars and low pH**

As stated previously, individuals who consume a diet rich in fermentable carbohydrates generally have higher levels of cariogenic bacteria and an increased incidence of dental caries. Whether such a response by these bacteria is due to their greater ability to (i) catabolize dietary sugars or (ii) tolerate the low pH generated from glycolysis has been debated for some time. Evidence suggesting that low pH was the major physiological force was provided when human volunteers rinsed with buffers of different pH (Svanberg, 1980). Buffer of neutral pH had no impact on the indigenous levels of mutans streptococci, whereas rinsing with a low pH buffer led to the enrichment of these bacteria. Definitive proof of the selective influence of low pH came from studies of microbial communities grown under defined conditions in chemostat models (Bowden and Hamilton, 1987, 1989; Bradshaw et al., 1989b). For example, pulses of glucose had no effect on the proportions of *S. mutans* and *L. casei* when the pH was maintained automatically at neutral values, whereas there was a gradual increase in the levels of these
two species, at the expense of organisms associated more with dental health, when the pH was allowed to fall after successive pulses (Bradshaw et al., 1989b). Indeed, when the degree of pH change was controlled, an inverse relationship was seen between the terminal pH and the viable counts of S. mutans and L. casei (Bradshaw et al., 1989a). A recent study has confirmed this observation in model biofilms, although the selection of potentially cariogenic bacteria and the inhibition of most acid-sensitive species were less marked than in planktonic cultures (Fig. 2) (Bradshaw et al., 1996b), demonstrating that susceptible bacteria could gain some protection within the biofilm.

**Redox potential**

Despite the mouth's ready access to air, with an oxygen concentration of approximately 20%, it is perhaps surprising that the plaque microflora is comprised of few, if any, truly aerobic species. Indeed, obligately anaerobic bacteria are isolated more commonly, although their distribution is not uniform, and proportions are highest at stagnant sites where plaque can accumulate. In periodontal disease, the diversity and number of such anaerobes increase still further, and many nutritionally fastidious bacteria can be recovered (see next section). Gradients generated within such biofilms can produce an environment sufficiently reduced to permit the growth of even strict anaerobes. Animal studies in diabetic rats indicated that progression of periodontal disease was associated with a reduced oxygen tension (McNamara et al., 1982), and studies of humans found a link between reduced oxygen tension, increased periodontal pocket depth, and bleeding, with the presence of spirochetes and other putative periodontopathogens (Loesche et al., 1983).

**Nutrients**

Populations within a microbial community are dependent solely on the habitat for the nutrients essential for their growth. Therefore, the association of an organism with a particular habitat is direct evidence that all of the necessary nutrients required for growth are being provided. The collective evidence from numerous *in vitro* and *in vivo* studies has demonstrated that the persistence and diversity of the resident oral microflora are due to the endogenous nutrients provided by the host (Littleton et al., 1967; Beckers and van der Hoeven, 1982a,b, 1984; Beighton et al., 1986; Beighton and Hayday, 1986). In particular, salivary mucins (glycoproteins) act as the main source of carbohydrates for plaque bacteria, and the degradation of the oligosaccharide side-chains depends on the concerted action of consortia of different species, each with complementary profiles of glycosidase activity (Beighton et al., 1986; ter Steeg et al., 1988; van der Hoeven and Camp, 1991; Bradshaw et al., 1994). Furthermore, the growth of some species can be dependent on the provision of nutrients by other oral bacteria. Thus, secondary feeders use the products of metabolism of other species (e.g., lactate or succinate), while others scavenge peptides or free amino acids generated during the breakdown of complex host molecules by other organisms. In this way, certain species occupy key niches (or metabolic roles; Alexander, 1971) within the consortium, and their levels or activity may dictate, in part, the final balance of the microbial community. Such species may provide valuable targets for physiological control strategies.

The isolation and the elevated levels of some of the major groups of periodontal pathogens may be related to the provision of increased levels of essential nutrients in the gingival crevice. For example, black-pigmented anaerobes require hemin for growth, and they probably obtain this key cofactor by degrading heme-containing host molecules (hemoglobin, hemopexin, haptoglobin) present in GCF, the flow of which is increased during inflammation. The detection and levels of periodontal pathogens were enhanced when enrichment cultures of subgingival plaque were carried out on human serum, which was used to simulate growth on GCF (ter Steeg et al., 1987, 1988). There are also (contradictory) data implying that the rise in subgingival levels of *Prevotella intermedia* seen sometimes during pregnancy is due to the metabolism of the hormones estradiol and progesterone (Kornman, 1982), which can be detected in GCF, while increases in *Prevotella* spp. have also been reported to occur in the plaque of women taking oral contraceptives (Jensen et al., 1981).

### Table 1

**Physiological Strategies for the Control of Oral Biofilms**

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control of plaque pH</td>
<td>Inhibition of acid production (fluoride, sugar substitutes, antimicrobial agents)</td>
</tr>
<tr>
<td>Control of redox potential</td>
<td>Redox agents, oxygenating agents</td>
</tr>
<tr>
<td>Control of nutrients</td>
<td>Addition of base-generating nutrients (arginine), reduction of GCF flow, anti-inflammatory agents, inhibition of key microbial enzymes</td>
</tr>
</tbody>
</table>

The main impetus behind the desire to control the bacterial composition of dental plaque is to prevent or reduce the
The rate of acid production by the microbial community was during pH-fall conditions (Bradshaw et al., 1990). Inhibitors of acid production. The principal mode of action of fluoride is to increase the resistance of enamel to demineralization and to promote remineralization. However, low (sub-MIC) levels of fluoride can also reduce glycolysis, and this inhibitory activity is enhanced as the environmental pH falls. Studies of microbial communities grown in a chemostat have shown that sub-MIC levels of fluoride have no effect on the proportions of individual species during growth at neutral pH, but could prevent the selection of S. mutans during pH-fall conditions (Bradshaw et al., 1990). The rate of acid production by the microbial community was reduced, enabling acid-sensitive species to persist at higher levels than in the absence of fluoride. Some researchers have questioned whether fluoride bound to enamel could be released during glycolysis in vivo in sufficient concentrations to modulate the rate of acid production. However, recent data have shown that biofilms of mono-cultures of several oral bacteria, when grown on fluoride-bound hydroxyapatite, could release sufficient fluoride from the substratum to reduce the growth and accumulation of cells (Li and Bowden, 1994), while sufficient fluoride was released from fluorhydroxyapatite by bacterial fermentation to inhibit lactic acid production by S. mutans (Guha-Chowdhury et al., 1995). Thus, the anti-caries properties of fluoride may also include the stabilization of the plaque microflora by slowing glycolysis and thereby removing the transient advantage given to mutans streptococci during periods of low pH within the biofilm (Hamilton and Bowden, 1982; Bradshaw et al., 1990).

Antimicrobial agents are being used as an adjunct to mechanical cleaning for plaque control (Addy, 1990). Such agents delivered from dental products are present in the mouth for considerable periods at sub-MIC levels. At such concentrations, agents such as chlorhexidine and Triocol can interfere with sugar transport and glycolysis (Marsh et al., 1983; Scheie, 1989), and may still be of therapeutic value, therefore, by helping to stabilize the biofilm community.

Sugar substitutes. Sugar substitutes could play a role in reducing the impact of low pH on microbial homeostasis in plaque in a number of ways. Their consumption in snack foods would reduce the frequency of acidic conditions in the biofilm, while their ability to stimulate saliva flow will provide increased buffering capacity, substrate clearance, and remineralization potential to enamel. Saliva also contains antimicrobial agents, as well as urea and peptides from which base (alkali) can be generated. Again, this combination of physiological factors would tend to stabilize the resident plaque community and reduce the competitive advantages normally given to cariogenic species during regular sugar consumption.

Some sugar substitutes, such as aspartame, saccharin, and xylitol, also have the potential to inhibit bacterial growth (Grenby and Saldanha, 1986), with saccharin and xylitol being particularly effective against mutans streptococci (Best and Brown, 1987; Mäkinen, 1989; Scheie, 1989). Delivery of xylitol with glucose to a consortium of oral bacteria resulted in a reduced rate of acid production and a selective suppression of S. mutans under conditions where this species would otherwise flourish (Bradshaw and Marsh, 1994). Clinical studies using xylitol-containing products, ranging from chewing gum to dentifrices, have also reported a selective inhibition of mutans streptococci (Isokangas et al., 1991; Petersson et al., 1991; Svanberg and Birkhed, 1991).

Alkali generation in dental plaque. Several endogenous and exogenous compounds can be metabolized by plaque bacteria to yield base (principally ammonia). Arginine-

### TABLE 2

<table>
<thead>
<tr>
<th>Diet</th>
<th>CFU.10^5 [mean ± SE] on 3 molars</th>
<th>A</th>
<th>Total CFU</th>
<th>B</th>
<th>Total CFU</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S. mutans</td>
<td></td>
<td>S. mutans</td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td></td>
<td>53 ± 7</td>
<td>S. milleri</td>
<td>259 ± 31</td>
<td>311 ± 30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>294 ± 46**</td>
<td></td>
<td>81 ± 10**</td>
<td>375 ± 50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17 ± 8**</td>
<td></td>
<td>498 ± 79**</td>
<td>516 ± 82*</td>
</tr>
<tr>
<td>Sucrose</td>
<td></td>
<td>76 ± 10</td>
<td>S. sanguis</td>
<td>130 ± 14</td>
<td>206 ± 15</td>
</tr>
<tr>
<td>Arginine</td>
<td></td>
<td>274 ± 38**</td>
<td></td>
<td>90 ± 16</td>
<td>365 ± 49*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70 ± 15</td>
<td></td>
<td>188 ± 15**</td>
<td>258 ± 20*</td>
</tr>
</tbody>
</table>

Significant differences in *0.01 < p < 0.05, **0.01 > p, compared with basal diet.

From van der Hoeven et al. (1985).

incidence of caries or periodontal diseases. As discussed in the previous sections, the main physiological targets for these diseases would include a reduction in conditions of low pH in supragingival plaque and an alteration to the environment (redox potential, nutrient availability) in a periodontal pocket (Table 1). Some potential strategies to achieve these aims will now be discussed.

Prevention of low pH

Bacterial homeostasis in plaque biofilms would be less likely to be disrupted if the frequency and depth of acidic conditions following sugar intake could be reduced. This could be achieved physiologically by (a) inhibitors of acid production, (b) consumption of food or drinks containing non-fermentable sweeteners, and (c) the local generation of base (alkali) in plaque. This strategy would not only remove the major environmental pressure selecting for cariogenic (aciduric) species, but also prevent the inhibition of the species predominating in health, which are generally acid-sensitive. Strategies to stimulate saliva flow would also help control plaque pH at favorable values, and increase clearance of fermentable substrates.

### Inhibitors of acid production.

The principal mode of action of fluoride is to increase the resistance of enamel to demineralization and to promote remineralization. However, low (sub-MIC) levels of fluoride can also reduce glycolysis, and this inhibitory activity is enhanced as the environmental pH falls. Studies of microbial communities grown in a chemostat have shown that sub-MIC levels of fluoride have no effect on the proportions of individual species during growth at neutral pH, but could prevent the selection of S. mutans during pH-fall conditions (Bradshaw et al., 1990).
Fig. 3—Schematic representation of the development in biofilms of gradients in substrates and products over relatively short distances due to bacterial metabolism. This would result in a spatial organization within the biofilm of consortia of bacteria with specific metabolic preferences. This could result in metabolic “activity domains” which, depending on the architecture of the biofilm, may restrict penetration of substrates for physiological control strategies. Similar “domains” may exist in neighboring areas of the biofilms. Such “domains” may give rise to a mosaic of micro-environments.

containing peptides and urea in saliva can be hydrolyzed to ammonia, and this activity may account, in part, for the relatively high pH in “resting” or “sugar-starved” plaque (Theilade, 1990). In addition, ornithine is produced which can be decarboxylated, particularly at low pH, leading to a rise in pH. Supplementation of carbohydrates with arginine when pulsed to arginolytic bacteria (e.g., S. sanguis) resulted in a higher terminal pH (Ferro et al., 1983), while arginine as either the free amino acid or as part of a short peptide can stimulate the growth of streptococci associated with sound enamel, such as S. sanguis and members of the “S. milleri” group (Rogers et al., 1987a; Rogers, 1990).

These findings have prompted attempts to manipulate the composition of the plaque microflora by augmentation of endogenous levels of base-generating compounds with pulses of arginine or urea. Several studies have been conducted in an artificial mouth model inoculated with human plaque. Supplementation of a basal medium (simulating the composition of saliva) with low concentrations of urea or arginine led to a rise in the resting pH of the biofilms, and an increase in urease activity (Sissons et al., 1991). This change in the biofilm metabolism was associated with only minor shifts in the microbial composition of the community, although the flora was not analyzed at the species level, so that any shifts in the principal ureolytic organism in plaque (S. salivarius) would not have been detected in this study. The pH response of these biofilms to urea was dose-dependent; the metabolism of arginine, however, produced only half the pH-rise response as the same concentration of urea. Subsequent studies on deep biofilms (maximum thickness, 5-8 mm) showed that urea-induced pH gradients can develop and last for several hours (Sissons et al., 1994b).
The initial pH gradients were alkaline at the biofilm surface, and only eventually did the inner layers become alkaline. Such slow changes in pH gradient were probably due to the slow penetration of urea to the inner layers of the biofilm, its rapid metabolism by the outer layers of the biofilm, and a rate-limiting clearance of ammonia (Sissons et al., 1994a).

The microbial organization of a biofilm will be of significance in attempts to alter the physiology of the community by the addition of nutrients. Recent studies have shown that model oral biofilms have an ordered structure—for example, with oxygen-consuming species located on the surfaces of the biofilm—thereby facilitating and protecting the growth of obligately anaerobic bacteria in the depths of the biofilm (Kinniment et al., 1996). The success of a physiological strategy may depend on overcoming highly coupled microbial interactions within highly structured biofilms, especially in mature biofilms.

Studies of simple mixed cultures of plaque bacteria in chemostats and gnotobiotic rats have confirmed that the potential does exist for shifting the composition of a simplified microbial community by manipulating the concentrations of key substrates. Steady-state levels of S. mutans, S. sanguis or “S. milleri” in mixed continuous culture could be regulated by the controlled addition of critical concentrations of glucose or arginine (Rogers et al., 1987a). Likewise, viable counts of S. sanguis or “S. milleri” could be increased, and those of S. mutans decreased, on the diet of rats fed an arginine-supplemented diet, whereas the converse occurred when the diet was switched to one containing sucrose (Table 2; van der Hoeven et al., 1985).

**Alteration of subgingival environment**

**Redox potential.** The Gram-negative anaerobes associated with periodontal disease require a low redox potential for growth. Therefore, a physiological route to the reduction of disease could involve the modification of the pocket Eₚₒ by the delivery of either oxygen or oxygen radicals to a site, or of compounds which increase the local redox potential. Addition of a redox agent, methylene blue, to reduced bacterial cultures raised the Eₚₒ of the medium to -125 mV and prevented the growth of the periodontopathogen Porphyromonas gingivalis (Fletcher and Wilson, 1993). In a clinical trial, a seven-day treatment regime involving methylene blue led to a decrease in the proportions of Gram-negative anaerobes (including spirochetes) and motile bacteria and a reduction in the flow of GCF, while bacteria associated with gingival health increased (Table 3; Wilson et al., 1992), suggesting that this approach has genuine potential.

Similar approaches to manipulation of the community composition of a laboratory microbial community via Eₚₒ were made with the use of aeration, with unexpected results (Bradshaw et al., 1996a). A two-stage chemostat model system was used to grow (i) a 10-species consortium anaerobically in the first stage, which was fed into (ii) an aerated second stage, which contained hydroxyapatite discs for biofilm formation. It was anticipated that anaerobes would be unable to grow in the second stage, except in the biofilms, and only when gradients developed and conditions within the biofilm became reduced. However, all obligate anaerobes persisted, both in the biofilms and in the planktonic phase, albeit initially at lower levels, but the predominant organism was an aerobe, Neisseria subflava. When the experiment was repeated without the aerobe, the anaerobes still grew, but there was an initial increase in facultative species, especially streptococci. The consortium was able to protect anaerobes, even in planktonic culture, for example, by consuming oxygen, emphasizing the significance of interactions among members of microbial communities in the maintenance of homeostasis.

**pH and nutrients.** Other physiological targets within the pocket environment could include altering the local pH or reducing the availability of endogenous nutrients from GCF. A small rise in pH (from pH 7.0 to 7.5) can occur within the gingival crevice during inflammation (Eggert et al., 1991), presumably due to proteolysis by the asaecharolytic microflora. A similar shift in pH dramatically altered the balance of a community of three black-pigmented anaerobes, with the proportions of P. gingivalis increasing from < 1% at pH 7.0 to > 99% of the cultivable microflora at pH 7.5 (McDermid et al., 1990). Similarly, growth of subgingival plaque on human serum (used as a substrate to mimic GCF) resulted in the enrichment of periodontal pathogens (ter Steeg et al., 1987, 1988). GCF and serum would provide peptides and heme-containing molecules which are essential for the growth of many periodontopathogens. These data suggest that strategies to reduce inflammation could also indirectly modify the biofilm community by restricting the availability of key nutrients.

The use of antimicrobial agents could also indirectly affect the physiology of the subgingival community. Chlorhexidine and Triclosan, which are widely used in anti-plaque products, can inhibit bacterial proteases when present at sub-MIC levels (Rogers et al., 1987b; Scheie, 1989; Cummins, 1991; Grenier, 1993). Such an inhibition may affect the ability of proteolytic anaerobes to acquire nutrients for growth, and

<table>
<thead>
<tr>
<th>TABLE 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EFFECTS OF A REDOX AGENT (METHYLENE BLUE) ON THE MICROFLORA OF PERIODONTAL POCKETS</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Decrease</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total anaerobes</td>
<td>Facultative anaerobes</td>
</tr>
<tr>
<td>Gram-negative anaerobes</td>
<td>Coccal cells</td>
</tr>
<tr>
<td>Spirochetes</td>
<td>Motile bacteria</td>
</tr>
<tr>
<td>GCF flow</td>
<td></td>
</tr>
</tbody>
</table>

Methylene blue (0.1% w/v) was applied subgingivally, daily for 7 days, to 25 sites in seven patients (ages, 31 to 57 yrs) (Wilson et al., 1992).
CONCLUDING REMARKS

It is clear from data on the site distribution of the oral microflora, and on the shifts in the balance of the predominant bacteria in plaque that occur in disease, that physiological processes can modify the composition and metabolism of oral biofilms. Therefore, it may be possible to design strategies to modify the composition of plaque deliberately more toward that found in health than in disease, and there is a limited amount of laboratory and clinical data to support this contention. However, some laboratory data also suggest that some of the biological features of plaque may make it less easy to predict the outcome of some strategies for control. Plaque is a biofilm with a high species diversity and an ordered structure. This diversity is maintained, in part, through highly coupled metabolic interactions, one consequence of which may be to increase the strength of homeostasis within the biofilm, making it less susceptible to manipulation by some physiological approaches.

The use of substrates that can be metabolized appears to generate gradients over relatively short distances (several microns) within a biofilm (Costerton et al., 1994). Therefore, the influence of such molecules on relatively deep biofilms (several 100 microns) requires further study, especially on their distribution within and penetration of the biofilm. Direct observations of the structures of living biofilms from aquatic environments have demonstrated a loose, open habitus, such as the mouth, have a similar architecture remains to be determined, but this aspect may be of critical importance in determining the outcome of some of the physiological approaches to control described in this review. Such studies should also be complemented by research into the fundamental properties of oral bacterial consortia, since it is clear that the metabolic potential of communities is greater than the sum of the individual populations. Further work on the influence of surface growth on the behavior of plaque communities will also be needed before the full potential of physiological approaches to biofilm control will be realized.

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