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Dental plaque as a biofilm

PD Marsh and DJ Bradshaw

Department of Microbial Pathogenicity, CAMR, Porton Down, Salisbury SP4 0JG, UK

Dental plaque is the diverse microbial community found on the tooth surface embedded in a matrix of polymers of bacterial and salivary origin. Once a tooth surface is cleaned, a conditioning film of proteins and glycoproteins is adsorbed rapidly to the tooth surface. Plaque formation involves the interaction between early bacterial colonisers and this film (the acquired enamel pellicle). To facilitate colonisation of the tooth surface, some receptors on salivary molecules are only exposed to bacteria once the molecule is adsorbed to a surface. Subsequently, secondary colonisers adhere to the already attached early colonisers (co-aggregation) through specific molecular interactions. These can involve protein–protein or carbohydrate–protein (lectin) interactions, and this process contributes to determining the pattern of bacterial succession. As the biofilm develops, gradients in biologically significant factors develop, and these permit the co-existence of species that would be incompatible with each other in a homogenous environment. Dental plaque develops naturally, but it is also associated with two of the most prevalent diseases affecting industrialised societies (caries and periodontal diseases). Future strategies to control dental plaque will be targeted to interfering with the formation, structure and pattern of development of this biofilm.

Keywords: dental plaque; biofilm; adhesion; co-aggregation

Introduction

The recognition that surface-associated bacteria can have novel properties compared to their planktonic counterparts was probably first made from studies of dental plaque. Antonie van Leeuwenhoek is reported to have failed to kill plaque bacteria on his teeth by prolonged rinsing with strong wine-vinegar, while plaque was 'killed' when the bacteria were first removed from his molars and mixed with vinegar *in vitro* [cited by 19].

The mouth is unique in the human body in that it provides non-shedding surfaces (teeth) for natural microbial colonisation. This can result in the accumulation of large masses of bacteria and their products at stagnant sites between teeth (approximal surfaces), in the pits and fissures on the biting (occlusal) surfaces of molars and premolars, and around the gums (gingival crevice). Plaque found above or below the gum margins is described as supra- or sub-gingival plaque, respectively. Elsewhere, desquamation ensures that the bacterial load is light on mucosal surfaces.

Dental plaque forms naturally on teeth and acts as part of the defences of the host by helping to prevent colonisation by exogenous, and often pathogenic microorganisms [28]. However, if plaque is allowed to accumulate beyond levels that are compatible with health, then disease can occur. Plaque is associated with two of the most prevalent diseases affecting industrialised societies, namely dental caries and periodontal diseases. The widespread nature of these diseases, together with their huge treatment costs, has provided great impetus for research into improved means of controlling plaque formation. The aim of this paper is to review recent advances in the microbiology of dental plaque, with particular emphasis on the mechanisms by which bacteria adhere to the tooth surface and produce a biofilm.

Definition of dental plaque

Dental plaque has been defined as the diverse microbial community found on the tooth surface embedded in a matrix of polymers of bacterial and salivary origin [31]. Plaque that becomes calcified is termed calculus or tartar. The resident plaque microflora consists of a wide range of Gram-positive and Gram-negative bacteria, including facultatively anaerobic and obligately anaerobic species (Table 1). The composition of plaque varies at different sites over the tooth surface due to differences in their local biological properties, and a detailed description of the microflora of

Table 1 Bacterial genera found in dental plaque

Gram-positive	Gram-negative
Cocci:	Cocci:
Streptococcus	*Neisseria*
Peptostreptococcus	*Veillonella*
Rods:	Rods:
Actinomyces	*'Bacteroides'*[a]
Bifidobacterium	*Campylobacter*
Corynebacterium	*Eikenella*
Eubacterium	*Fusobacterium*
Lactobacillus	*Haemophilus*
Propionibacterium	*Leptotrichia*
Rothia	*Prevotella*
	Porphyromonas
	Selenomonas
	Treponema

Most genera contain more than one species. Some genera are found rarely and only in low numbers at healthy sites

[a]The genus *'Bacteroides'* has been redefined. Eventually, oral bacteria included in this genus will be reclassified

Correspondence: Dr PD Marsh, Department of Microbial Pathogenicity, CAMR, Porton Down, Salisbury SP4 0JG, UK
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representative sites is provided in a later section. The main nutrients for plaque bacteria are those derived from the catabolism of endogenous nutrients (proteins, glycoproteins) in saliva. However, the ecology of the gingival crevice is influenced mainly by the properties of gingival crevicular fluid (GCF), a serum-like exudate that bathes the root of the tooth, the flow of which is increased during inflammation in periodontal disease [31]. This increased flow of GCF not only provides components of the host defences (eg IgG and neutrophils), but also a wider range of potential nutrients for bacterial growth (eg peptides, proteins and glycoproteins). These endogenous nutrients can be used by many asaccharolytic bacteria, and the gingival crevice has higher proportions of Gram-negative species, including obligately anaerobic bacteria, especially at sites with periodontal disease. The microbial composition of dental plaque in health and disease is also described in greater detail in a later section.

Approximately 80–90% of the weight of plaque is water; about 70% of the dry weight of plaque is bacteria, and the remainder is a matrix of polysaccharides and salivary proteins and glycoproteins. Extracellular polymers of glucose (glucans with 1, 6-α-linkages; mutan with 1, 3-α-linkages) and fructose (inulin, 2, 1-β linkages; fructan with 2, 6-β linkages) are formed from dietary sucrose by bacterial enzymes (glucosyltransferases, GTF, and fructosyltransferases, FTF, respectively). Some of these polymers are soluble and can be broken down and metabolised in plaque by bacteria that produce the appropriate hydrolases, while others are insoluble and are believed to play a significant role in determining the architecture of the biofilm. Some glucans can be involved in bacterial adhesion to the tooth surface. This process is described in more detail in the following section.

Mechanisms of plaque formation

The development of dental plaque can be sub-divided into several arbitrary phases, although these phases will overlap as plaque formation is a dynamic and continuous process. These phases include:

(a) the formation of a conditioning film (the acquired pellicle) on the tooth surface;
(b) a non-specific reversible phase involving physico-chemical interactions between salivary bacteria and the acquired enamel pellicle;
(c) short-range specific stereo-chemical molecular interactions between primary bacterial colonisers and host receptor molecules in the acquired pellicle;
(d) the attachment of secondary colonisers to already attached primary colonisers (co-aggregation);
(e) development of horizontal and vertical stratification within the developing biofilm, and increased bacterial succession; and
(f) growth and the formation of a climax community.

Bacteria rarely come into contact with a clean tooth surface. As soon as a tooth has been cleaned, host and bacterial molecules are selectively adsorbed on to the enamel surface to form a conditioning film. This film is usually <1 μm thick, and it takes 90–120 min for the adsorption of molecules to reach a plateau. Once formed, the composition and structure of the pellicle can be modified by bacterial metabolism.

The conditioning film contains proteins, glycoproteins, lipids and glycolipids derived from saliva, as well as extracellular molecules from bacteria. Some of the molecules in the pellicle have been identified by the use of, for example, immunological probes. Acidic proline-rich proteins, amylase, lysozyme, statherin and immunoglobulins are among the host molecules found in the acquired pellicle [1], while bacterial components such as glucosyltransferases and glucans can also be detected [50,51]. These adsorbed molecules can act as receptors or adhesins for oral bacteria, and therefore selectively influence the pattern of biofilm development.

Relatively few oral bacteria are motile; generally, microorganisms are transported passively to the tooth surface by the force of saliva flow. As a cell approaches pellicle-coated enamel, long range physico-chemical forces combine to provide a weak, non-specific area of attraction; this area is around 10–20 nm from the surface and is enhanced by the high ionic strength of saliva. The strength of this interaction is relatively weak, and this phase of attachment is reversible [7,46].

The strength of attachment can increase if the cell can get closer to the surface so that specific short-range stereochemical interactions can occur. For this to happen, water films must be removed from between the interacting surfaces. Hydrophobic bacterial cell surface components may play a role by means of their dehydrating effects, enabling the surfaces to get closer together. Also, many bacterial adhesins are located on surface structures, such as fibrils on streptococci, and fimbriae on other genera including *Actinomyces* spp [20].

Laboratory studies have identified a number of specific molecular interactions that may be involved in adhesion to the tooth surface. Fractions of saliva, including statherin and a high molecular weight acidic proline-rich protein (PRP-1), can bind to particular bacteria. For example, different regions of PRP-1 can bind to the early enamel colonisers, *Streptococcus gordonii* and *Actinomyces naeslundii* [16,18], α-amylase can bind to *S. gordonii* [12,48] while statherin can interact with *A. naeslundii* and *Fusobacterium nucleatum* [25,57]. Another adherence strategy involves lectin-like bacterial proteins interacting with carbohydrates or oligosaccharides on pellicle-associated glycoproteins. *Streptococcus sanguis* can bind to terminal sialic acid residues in adsorbed salivary glycoproteins [33], while *Streptococcus oralis* expresses either a galactose-binding lectin [52], or a lectin that interacts with a trisaccharide structure containing sialic acid, galactose and *N*-acetyl-galactosamine [39,40]. *Actinomyces* spp can also bind to salivary pellicles via a β-galactoside-sensitive mechanism [56].

Oral bacteria possess multiple adhesins and can use several strategies to attach. The large size of some cell wall proteins means that they may be involved in more than one function. A protein of *S. gordonii* can interact with salivary proteins in the pellicle as well as with a receptor on the

surface of cells of *A. naeslundii* [22]. *Actinomyces* spp have two antigenically and functionally distinct types of fimbriae [8,9]. Type 1 fimbriae recognise salivary proteins while type 2 are associated with a lectin-like interaction with streptococci.

This ability to interact with other bacteria, especially those that are already attached to the tooth surface, appears to be an important mechanism in the development of a complex biofilm community such as dental plaque. This process, termed co-aggregation, is the cell-to-cell recognition of genetically distinct partner cell types [25]. Initial plaque accumulation is enhanced by intra- and inter-generic co-aggregation between the primary colonisers, such as streptococci and actinomyces. Over 90% of more than 300 isolates of *A. naeslundii*, *S. gordonii*, *Streptococcus mitis*, *S. oralis* and *S. sanguis* tested in pair-wise inter-generic co-aggregations were found to co-aggregate [24]. These actinomyces–streptococcus interactions are not random but are highly specific, and can be reversed by treatment with sugars (eg lactose), or by protease (or heat) treatment of either or both cell surfaces. Some bacteria can act as co-aggregation bridges between otherwise non-coaggregating species [25]. Fusobacteria co-aggregate with the widest range of bacterial genera but, curiously, they do not co-aggregate with each other. Early colonisers of plaque co-aggregate extensively with *F. nucleatum*, while some later colonisers do not co-aggregate with early colonisers, but do usually co-aggregate with *F. nucleatum* [14,23]. It has been proposed that fusobacteria act as a bridge between early- and late-colonising bacteria [25]. In addition to enabling bacteria to anchor to a surface, co-aggregation may also offer metabolic benefits, perhaps by facilitating cross-feeding or co-operation in the breakdown of complex host molecules.

An important factor involved in plaque formation involves the need for specific inter-molecular interactions between bacteria and receptors to occur selectively on the enamel surface. These receptors lie on molecules that are not only adsorbed to the tooth surface but which are also freely in suspension in saliva. Some of these molecules are designed for aggregating bacteria, thereby facilitating their removal from the mouth by swallowing. Obviously, it is essential if plaque formation is to proceed that all bacteria are not aggregated before they reach the tooth surface. Recent work has shown that a highly selective mechanism may function to overcome this problem. Although *A. naeslundii* could bind to the acidic proline-rich peptides when the latter were adsorbed to a surface, it did not interact with these proteins in solution. It has been proposed that hidden molecular segments of these molecules become exposed as a result of conformational changes when they are adsorbed to a surface [15,17]. Such hidden receptors for bacterial adhesins have been termed 'cryptitopes'. In this way, a controlled mechanism for facilitating plaque formation has evolved by which the host can promote the attachment of specific bacteria without compromising this selective process in the planktonic phase. Another example of a cryptitope involved in plaque formation can arise following the enzymic modification of host molecules by oral bacteria. Neuraminidase-producing bacteria, such as *A. naeslundii*, *S. oralis* and *S. mitis*, can cleave the terminal sialic acid residue from oligosaccharide side chains of glycoproteins to expose the penultimate galactosyl sugar. Many oral bacteria possess galactosyl-binding lectins, including *A. naeslundii*, *Leptotrichia buccalis*, *F. nucleatum*, *Eikenella corrodens*, and *Prevotella intermedia*, and could therefore exploit such a cryptitope [15].

Another factor in the development of dental plaque is the synthesis of extracellular polysaccharides (EPS) from sucrose by adherent bacteria [61]. These polymers can include soluble glucans and fructans (which can be metabolised by other plaque bacteria), and insoluble glucans; these polymers can comprise about 10–20% of the dry weight of dental plaque. Originally, it was believed that polysaccharide production was important in the initial stages of attachment. Although pre-formed polymer may react with receptors in pellicle, these insoluble molecules are now considered to make a more important contribution to the structural integrity and pathogenic properties of plaque. Neither acid nor sugar diffusion is much affected by the EPS content of plaque [10,34]. However, EPS may lead to an increased cariogenic challenge at the tooth surface because, in a thick plaque, EPS will enable sugars to penetrate deeper into the biofilm, while the significant buffering effect of bacteria will be reduced, thereby producing a more pronounced pH fall at the plaque–enamel interface [11]. In support of this, studies comparing the effect of differing ratios of *S. mutans* and EPS found that demineralisation of experimental enamel slides was maximal when the artificial plaque consisted of 95% EPS and only 5% bacteria [62].

As plaque builds up, microbial metabolism produces gradients in biologically significant factors, and these will affect the spatial distribution of bacteria. Gradients in essential nutrients and end-products of metabolism will develop, as will others in pH, dissolved oxygen tension, and cations. This will lead to vertical and horizontal stratification of the plaque biofilm, and produce a mosaic of micro-environments [31]. Such stratification will enable organisms with widely differing requirements to grow, and ensure the co-existence of species that would be incompatible with one another in a homogeneous habitat. Thus, plaque contains many obligately anaerobic species, despite existing in an aerobic environment.

In a biofilm such as dental plaque, bacteria are in close proximity with one another and will interact as a consequence. Some of the interactions will be beneficial, and facilitate the development of food chains, or lead to the degradation of complex host molecules, such as salivary mucins, by the concerted action of several bacteria [6]. The best-described food chain involves the utilisation by *Veillonella* of lactate produced by streptococci or actinomyces. Such an interaction may modulate the demineralisation of enamel by bacterial acids. Other interactions may be antagonistic due to the production of inhibiting factors such as hydrogen peroxide or bacteriocins [31].

To date, there have been few studies to determine the effect on the phenotypic properties of oral bacteria being associated with a surface. Lactate production by nine strains of mutans streptococci, but by only two of five strains of

S. sanguis, displayed an enhanced glycolytic activity following the addition of hydroxyapatite beads to cell suspensions; acid production by the other strains of *S. sanguis* and a strain of *S. mitis* and *S. salivarius* were reduced in the presence of the beads [2]. The expression of genes that code for certain glucosyltransferases (*gtf*B/C) was increased in cells of *S. mutans* bound to a surface [21]. It was proposed that initial binding might signal *S. mutans* to produce insoluble glucan to enhance attachment. In contrast, fructosyltransferase activity was unaffected by surface binding.

Bacterial composition of dental plaque

After 2–4 h of plaque formation, single cells of mainly Gram-positive coccoid cells can be seen by microscopy on pellicle-coated surfaces, together with a few rod-shaped organisms. Culture studies have shown that these pioneer species are predominantly streptococci (61–78% of the total microflora) and actinomyces (4–30%), with some haemophili [43]. Perhaps significantly, the early streptococcal colonizers (*S. sanguis*, *S. oralis* and *S. mitis*) generally produce IgA_1 proteases [44], which presumably enables them to evade the adherence-inhibiting effects of s-IgA, the main immunoglobulin in saliva [45]. The attached cells then divide rapidly to form microcolonies in the first instance, which eventually coalesce to form a confluent film of varying thickness [42].

After 1–2 days, Gram-positive rods and filaments can be observed extending outwards from microcolonies of mainly coccoid cells. After several days of development, the morphological and cultural diversity of the microflora increases, and there is a shift from a streptococcal-dominated flora to higher numbers of *Actinomyces* spp. The depth of the biofilm increases, and there is believed to be a lowering of the oxygen tension and of the redox potential, resulting in a marked increase in the proportions of obligately anaerobic species [41]. The structure of dental plaque becomes more varied during this period. There is a layer of densely packed cells (3–20 cells deep) next to the tooth surface, many of which have thickened cell walls. Above this lies a layer with a more variable structure and a greater morphological diversity. The existence of this layering has been attributed to bacterial succession. The superficial layers of dental plaque often exhibit a high species diversity, with some unusual combinations of bacteria such as 'corn-cobs' (Gram-positive filaments covered by Gram-positive cocci), 'rosettes' (coccal bacteria covered by small Gram-positive curved rods), or 'bristle brushes' (large filaments surrounded by Gram-negative rods or short filaments) [26,41].

If plaque is left to develop undisturbed on exposed enamel surfaces for 2–3 weeks (as will occur at stagnant sites), a climax community will establish, and the bacterial composition will become relatively constant with time. The depth of the biofilm reaches approximately 50–100 μm, and is probably restricted on exposed surfaces by the shear forces imposed by saliva flow and mastication [41]. Plaque can develop to greater depths at stagnant or protected sites, such as in the fissures on occlusal surfaces, between the teeth at approximal sites, and in the gingival crevice. The climax community that develops at such anatomically distinct sites can vary quite markedly because of differences in the prevailing biological conditions (Table 2).

Fissure plaque

The microflora of fissures on occlusal surfaces of molars and premolars is mainly Gram-positive, and is dominated by streptococci [58]. With the exception of *Veillonella* spp, obligately anaerobic Gram-negative species are recovered only occasionally [59]. The microflora of fissures is less diverse than that found at other sites on the tooth surface. The median number of species found in a study of ten fissures was eight, and ranged from two to 11 [58].

Approximal plaque

The predominant bacteria at sites between teeth are streptococci and Gram-positive rods such as *Actinomyces*; when compared to fissures, there are much higher levels of obligately anaerobic Gram-negative species, including *Veillonella*, *Fusobacterium* and *Prevotella* spp [5,32]. This reflects an environment at this site with a lower redox potential. Generally, the microflora of approximal plaque is more diverse than that of fissures.

Gingival crevice plaque

As described earlier, the gingival crevice provides a distinct habitat, and the ecology of the site is influenced by the flow of, and novel nutrients provided by, GCF. Compared to fissures and approximal sites, obligately anaerobic species belonging to genera such as *Peptostreptococcus*, *Actinomyces*, *Propionibacterium*, *'Bacteroides'*, *Fusobacterium*, *Prevotella*, *Selenomonas* and *Veillonella* are found more commonly, and often at higher levels [53,60]. Some of these species are asaccharolytic but proteolytic, and derive their energy from the catabolism of host proteins and glycoproteins.

Dental plaque and disease

Although dental plaque forms naturally on teeth, in the absence of adequate oral hygiene it can accumulate at stagnant sites beyond levels compatible with oral health and, at a susceptible site, dental caries or periodontal disease can

Table 2 The predominant bacteria found at three distinct anatomical sites on the tooth surface

Bacterium	Percentage viable count (range)		
	Fissures	Approximal surfaces	Gingival crevice
Streptococcus	8–86	<1–70	2–73
Actinomyces	0–46	4–81	10–63
Other obligately anaerobic Gram-positive rods	0–21	0–6	0–37
Neisseria	0[a]	0–44	0–2
Veillonella	0–44	0–59	0–5
Obligately anaerobic Gram-negative rods	0[a]	0–66	8–20

[a]Detected occasionally

occur. As plaque mass increases, the beneficial buffering and antimicrobial properties of saliva are less able to penetrate and protect enamel, and there is a shift in the balance of the predominant bacteria away from those associated with health. In dental caries on enamel surfaces, there is a strong positive association between the presence of higher levels of mutans streptococci (especially *S. mutans* and *S. sobrinus*) and lactobacilli in plaque overlying lesions (Table 3) [4,27]. These bacteria metabolise sugars rapidly, and grow well in the acidic environment that is generated. Many of the species associated with sound enamel are sensitive to low pH, and their levels are reduced under such conditions. The regular exposure of enamel to low pH encourages demineralization of the tooth structure, and this can eventually lead to caries. In older age, recession of the gums can expose the root surface to caries attack. The microflora implicated with root surface caries is also associated with raised levels of mutans streptococci and lactobacilli, as well as with *Actinomyces* spp (Table 3) [3].

Periodontal diseases are a number of related conditions in which the supporting tissues of the teeth are attacked. The junctional epithelium at the base of the gingival crevice migrates down the root of the tooth to form a periodontal pocket. In advanced stages of disease, attachment fibres and alveolar bone are also lost; ultimately, teeth may become mobile in their sockets and require extraction. Generally, the predominant bacteria in periodontal pockets are obligately anaerobic or CO_2-requiring (capnophilic) Gram-negative rods, filaments or spiral-shaped bacteria, many of which are nutritionally fastidious and difficult (or, at present, impossible) to grow in the laboratory. Recent studies using sophisticated cultural techniques or gene probe technology have identified several new species that are unique to this habitat.

Gingivitis is an apparently non-specific inflammatory response to dental plaque growth around the gums (gingival margins). If good oral hygiene is restored, gingivitis is usually eradicated and the gingival tissue becomes clinically normal again. Probably the whole of the dentate population is affected by gingivitis at some stage in their life. Gingivitis is associated with an increase in plaque mass around the gingival margin. This leads to a shift in the composition of plaque away from a streptococci-dominated microflora [53] towards higher levels of *Actinomyces* spp and an increase in the isolation of capnophilic and obligately anaerobic Gram-negative bacteria [38,47]. The microflora increases in diversity during the development of gingivitis, but no particular group of bacteria is uniquely associated with disease. It is still not clear whether gingivitis is a prerequisite for the development of more advanced forms of periodontal disease but, perhaps significantly, it has been reported that some species that predominate in chronic periodontitis, but which are not detectable in the healthy gingival crevice, are also found as a small percentage of the flora in gingivitis [38].

Chronic adult periodontitis is the most common form of advanced periodontal disease affecting the general population. Rather than pocket formation progressing at a slow but consistent rate, it is now believed that attachment loss may occur during relatively short bursts of activity, which are followed by periods of quiescence or even repair [55]. The microflora in adult periodontitis is extremely diverse; in one comprehensive study, 136 distinct microbial taxa were isolated from 38 samples from 22 adults [37]. The predominant flora is highly variable both between subjects and even between sites in the same subject. There are also some acute or exaggerated forms of periodontal diseases due to a variety of predisposing conditions, eg those associated with HIV-infected patients. Some of the bacterial species that have been implicated in periodontal diseases in humans are listed in Table 3.

Bacteria associated with periodontal disease are often proteolytic, and produce enzymes that can damage tissue directly and/or interfere with the activity of the host defences [54]. They also produce metabolites that can be cytotoxic (acids, ammonia, and sulphur-containing compounds). As no single species produces all of these factors, periodontal diseases are probably an example of a polymicrobial infection, perhaps involving pathogenic synergism [31]. In this way, organisms that are individually unable to cause disease combine forces to do so. The host mounts an immunological response to the bacterial challenge, and the resulting inflammation can also lead to tissue damage due to the release of proteolytic enzymes from phagocytic and other host cells [13,35].

Table 3 Predominant plaque bacteria implicated in caries and periodontal diseases

Caries	Periodontal diseases
Streptococcus mutans	*Actinobacillus actinomycetemcomitans*
Streptococcus sobrinus	*Fusobacterium nucleatum*
Lactobacillus spp	*'Bacteroides forsythus'*
(*Actinomyces* spp)[a]	*Campylobacter rectus*
	Porphyromonas gingivalis
	Prevotella intermedia
	Eikenella corrodens
	Eubacterium spp
	Treponema spp

[a]Possibly implicated in root surface caries

Approaches to control dental plaque

The mechanical removal of plaque by efficient oral hygiene (brushing and flossing) can almost completely prevent plaque-mediated dental diseases, especially when this is combined with a reduced frequency of sugar intake. However, as it is difficult to alter established eating patterns and to maintain a high degree of motivation for effective oral hygiene, alternative preventive measures are being developed either to control dental plaque or to increase the resistance of the host (Table 4).

The addition of antiplaque or antimicrobial agents to toothpastes and mouthwashes is now being used widely as an adjunct to conventional mechanical oral hygiene procedures. These agents include plant extracts (sanguinarine), metal salts (stannous, zinc), enzymes (glucan hydrolases), quaternary ammonium compounds (cetylpyridinium chloride), bisbiguanides (chlorhexidine), and phenols (Triclosan) [29,49]. Delmopinol is a surface-active agent with low intrinsic antimicrobial activity, but it can reduce

Table 4 Strategies for preventing plaque-mediated disease

Disease	Strategy	Mechanism
Caries	Fissure sealant	Physical protection of enamel
	Fluoride	Encourages enamel remineralisation. Increases acid resistance of enamel. Reduces acid production by plaque.
	Antiplaque and antibacterial agents	Inhibits plaque formation and metabolism.
	Sugar substitutes	Non-fermentable sweeteners; reduces acid attack on enamel.
	Vaccination and passive immunisation	Oral antibodies suppress mutans streptococci in animal models. Passive immunisation can reduce *S. mutans* in human volunteers.
Periodontal diseases	Antiplaque and antibacterial agents	Inhibits plaque formation and metabolism. Selective inhibition of periodontal pathogens.
	Vaccination	Antibodies can suppress *P. gingivalis* in primate models of periodontitis.

plaque formation both *in vitro* and *in vivo*. Recent studies support data obtained with microorganisms from other ecosystems in showing that bacteria growing on a surface are more resistant to antimicrobial agents. This is of particular relevance to the use of such agents for plaque control. Mature biofilms of *S. sanguis* are more tolerant of chlorhexidine than cells in suspension [36], while mixed culture biofilms are also less sensitive to a range of inhibitors used in toothpastes than the planktonic cultures [30]. Future studies will lead to the development of improved strategies to control plaque, and reduce the burden of dental disease to public health programmes.

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