Dental Plaque Biofilm in Oral Health and Disease

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Dental plaque is an archetypical biofilm composed of a complex microbial community. It is the aetiological agent for major dental diseases such as dental caries and periodontal disease. The clinical picture of these dental diseases is a net result of the cross-talk between the pathogenic dental plaque biofilm and the host tissue response. In the healthy state, both plaque biofilm and adjacent tissues maintain a delicate balance, establishing a harmonious relationship between the two. However, changes occur during the disease process that transform this 'healthy' dental plaque into a 'pathogenic' biofilm. Recent advances in molecular microbiology have improved the understanding of dental plaque biofilm and produced numerous clinical benefits. Therefore, it is imperative that clinicians keep abreast with these new developments in the field of dentistry. Better understanding of the molecular mechanisms behind dental diseases will facilitate the development of novel therapeutic strategies to establish a 'healthy dental plaque biofilm' by modulating both host and microbial factors. In this review, the present authors aim to summarise the current knowledge on dental plaque as a microbial biofilm and its properties in oral health and disease.

Key words: *dental plaque biofilm, health and disease, properties*

Dental plaque biofilm – historical aspects

The relationship between microorganisms and dentistry dates back to the earliest observations of microorganisms. In a letter to the Royal Society in September 1683, Antoni van Leeuwenhoek described his observation of 'white little matter between his teeth' as 'an unbelievable great company of living animalcules, a-swimming more nimbly than any I had ever seen up to this time, the biggest sort bent … their body into curves in going forwards'[1,2]. Later studies revealed that the 'biggest sort' he referred in his letter could be *Selenomonas* species residing in the dental plaque[3,4]. Hence, dental plaque has been known to be a reservoir of microorganisms since the dawn of microbiology. However, until the 1980s it was assumed that microbes predominantly live in a suspended phase. Therefore, most studies on microbial diseases and drug-resistance mechanisms were based on this free-floating or 'planktonic' mode of growth. This concept would have influenced the genesis of Koch's postulates, which assumed that a specific pathogenic agent is accountable for a specific infectious disease. In its early days, dentistry embraced Koch's postulates and attempted to link specific pathogenic agents with particular dental diseases, such as *Streptococcus mutans* – which was discovered by Clarke as early as 1924[5] – with dental caries.

It is only as late as the 1970s that pioneering studies by Costerton and colleagues led to an understanding of the community lifestyle of microorganisms in nature[6]. Incidentally, dental plaque was one of the first few samples used in these ground-breaking studies on microbial biofilms. Costerton and colleagues showed that microbial cells adhere to the tooth surface and form

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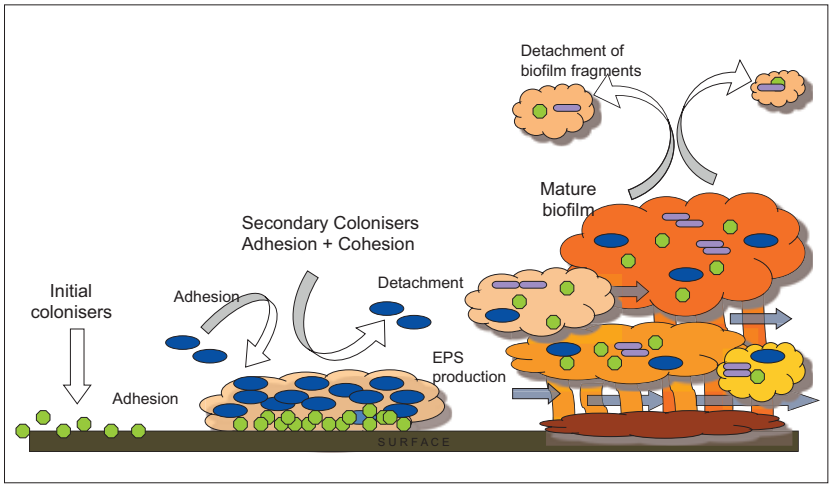

Fig 1 Sequence of biofilm development. A biofilm typically develops in four sequential steps: first, adhesion of a microorganism to a surface; second, individual colonisation and organisation of cells; third, secretion of EPSs and maturation into a three-dimensional structure; and finally, dissemination of progeny biofilm cells. (Reprinted with the permission of Oral Diseases, Blackwell Munksgaard).

a microbial community, as opposed to the common notion at that time that microbes live as freely floating organisms in suspensions. It is now widely accepted that most, if not all, microorganisms in nature preferably live as surface adherent microbial communities or 'biofilms'[7]. More importantly, it has been revealed that at least 65% of all infectious diseases are linked to the biofilm mode of growth of microbes, including otitis media and cystic fibrosis, and dental diseases such as dental caries and periodontal disease[8]. Biofilm microbes display phenotypic characters that are dramatically different from their planktonic counterparts[9]. Higher drug resistance is one of the notable features of the biofilm mode of growth[10]. Biofilms are spatially arranged, well-organised microbial communities that display properties as a unit. Hence biofilm microorganisms exhibit 'social' features, as opposed to the sum of individual features in the community. Therefore, an understanding of microbial biofilms provides us with a clearer view of their role in oral health and disease.

Formation and structure of dental plaque biofilm

All surfaces of the human body that are exposed to the exterior, such as the oral cavity, skin and gastrointestinal tract, are colonised with resident microbiota[11]. However, each of these habitats harbours a unique group of microbes, with properties that are different from those of the other habitats. Oral microbiota is distributed in stratified squamous oral mucosal surfaces, tooth surfaces and muco-gingival margins.

Dental plaque is an archetypical biofilm composed of a complex microbial community[12,13]. The National Institutes of Health initiated the Human Oral Microbiome Database, based on 16S rDNA gene sequencing techniques, to obtain a holistic view of the dental plaque biofilm as conventional culture-based techniques had only limited success in recovering plaque microbiota[14]. Use of the metagenomics approach has indicated that the number of bacterial species in the mature dental plaque biofilm could be as high as 19,000[15,16]. It is also noteworthy that the composition of the dental plaque biofilm is highly diverse between individuals and these subtle differences may generate a unique fingerprint for each individual[17,18]. However, under certain conditions, shifts in the composition and properties of the dental plaque biofilm could lead to dental diseases, such as dental caries and periodontal diseases. It is important that clinicians are aware of advances in the field of dental plaque biofilm which could be used in the development of new treatment options in the future. Therefore, in this brief review the present authors examine the structure and properties of dental plaque biofilm and critically evaluate the dynamic relationship between biofilm and host in terms of oral health and disease.

The adherence of microbes to an oral surface is a prerequisite for the formation of dental plaque biofilm. However, simple surface contact or sedimentation of microbes does not lead to the formation of a biofilm. Instead, a highly organised sequence of events must occur[12,19] (Fig 1). First, planktonic microorganisms adhere to the surface. Then, multiplication of bacteria leads to the formation of discrete colonies. These microcolonies secrete extracellular polymeric substance (EPS) in which they become embedded, resulting in the development of biofilm. EPS is a distinctive feature seen in microbial biofilm and provides a physical scaffold for the biofilm community. Moreover, EPS also contains biologically active components, such as antimicrobial enzymes that protect the biofilm community against noxious environmental stimuli. In a later stage of development, microcolonies embedded in EPS

become linked together in an organised manner, leading to formation of a three-dimensional, spatially arranged mature biofilm community[20].

A similar sequence of events can be observed in the formation of dental plaque biofilm. A cleaned tooth surface immediately comes into contact with bacterial and host products in saliva and gingival crevicular fluid. These products are absorbed into the negatively charged hydroxyapatite tooth surface, making a thin layer of conditioning film called 'acquired pellicle'. This layer is covered in the supra-gingival areas by positively charged molecules, such as salivary glycoproteins, statherin, histatin, proline-rich proteins and alpha-amylase, and by products from gingival crevicular fluid in the sub-gingival areas[21]. Some bacterial components, such as glucosyltransferases (GTFs) and glucan, have also been found in the acquired pellicle. Interestingly, the principal composition of acquired pellicle in different areas of the oral cavity and between individuals seems to be remarkably consistent. Gram-positive streptococci such as *Streptococcus sanguinis*, *Streptococcus oralis*, *Streptococcus mitis* and *Neisseria* species are the primary colonisers of the tooth surface. The negatively charged cell wall surface of the bacteria facilitates their binding to the positively charged receptor molecules on the pellicle. These primary colonisers initially make non-specific, reversible long-range (>50 nm) van der Waals bonds with the molecules in acquired pellicle[11,12]. Subsequently they develop stronger, irreversible short-range (10–20 nm) adhesion with receptors in the acquired pellicle using their cell surface adhesion molecules. Streptococcal group oral bacteria have various adhesion mechanisms, such as GTFs, glucan-binding proteins and pili, whereas other bacteria such as *Actinomyces* uses their fimbriae to adhere to the surface[22].

Following adhesion of the first layer of primary colonisers, dental plaque biofilm continues to build up by multiplication of the primary colonisers, and by coaggregation and coadhesion of secondary colonising bacteria. A specific set of secondary colonisers with the propensity to attach to the receptors of primary colonisers are favoured over the organisms that do not possess those properties. Development of dental plaque biofilm reflects the natural succession of niche-specific microorganisms. Primary colonisers of the dental plaque are either aerobic or facultative aerobes, such as the streptococcus and fusobacterium groups of bacteria. These reduce the oxygen, allowing anaerobic bacteria to enter the biofilm community as secondary colonisers. Secondary colonisers are mainly Gram-negative species such as *Actinomyces* species, *Fusobacterium nucleatum*, *Prevotella intermedia* and *Capnocytophaga* species. It has been shown that coaggregation may occur between Gram-positive species, such as *S. sanguinis* and *Actinomyces*; between Gram-negative species, such as *Prevotella melaninogenica* and *F. nucleatum*; and between Gram-positive and Gram-negative species, such as *Streptococcus* and *Fusobacterium*, respectively. Some specific structural features in dental plaque biofilm, such as 'corn cob' and 'test-tube brush' appearance, can be observed due to adherence of cocci to filamentous bacteria. Recent studies have further confirmed the corn-cob appearance using the species-specific fluorescent *in situ* hybridisation (FISH) technique[23]. At this stage of development, plaque bacteria secrete EPS, which forms the scaffold for the dental plaque biofilm.

If dental plaque biofilm is left undisturbed for approximately 7 days the local environment rapidly changes, favouring colonisation by some Gram-negative anaerobic bacterial species known as 'tertiary colonisers'. These are mainly strict anaerobes which opportunistically exploit the environment provided by other bacteria. Tertiary colonisers include pathogenic bacteria such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* and spirochetes such as *Treponema denticola*. *P. gingivalis* has been shown to coaggregate with facultative aerobic bacteria such as *Neisseria* species as a survival strategy. However, as mentioned earlier, advanced sequencing techniques have redefined the traditional understanding of the dental plaque as a microbial biofilm. One example is the finding of TM7, an uncultivable bacterial phylum which could be associated with periodontal disease, in the dental plaque biofilm[24,25].

Maturation is an important property of a biofilm. It is believed that, within a given environment, the biofilm community acquires the greatest stability with respect to both time and space during the maturation stage. *In vitro* studies on both single species and multiple biofilms have shown that maturation could occur by 24 to 72 hours, depending on the species and environmental conditions. However, it is rather difficult to determine the maturation of *in vivo* biofilms due to the highly dynamic environment with complex interactions between host and biofilm. It is generally assumed that dental plaque biofilm 'matures' by 72 hours, although this timing could be altered by factors such as dietary intake or immunity of the host.

Although development of dental plaque generally follows the aforementioned sequence of events, there is huge variability in this process between individuals, even under healthy conditions. In a recent study

by the present authors, differences in the ultrastructure and morphology of the dental plaque biofilm were found between 'slow plaque formers' and 'fast plaque formers'[26]. Other studies have also demonstrated that considerable variation in dental plaque thickness can be seen within individuals. One study reported similar plaque thicknesses in the buccal region of the maxilla and mandible but thinner biofilm on the palatal side[27].

Dynamic processes of synergism and antagonism occur during development but, once established, dental plaque biofilm is considered to be in a state of homeostasis. Therefore, mature dental plaque biofilm acts as a community or a unit, rather than as a sum of the properties of individual bacterial members. Microbial homeostasis of the dental plaque is only disturbed if drastic changes occur in the immediate environment of the host, such as changes in dietary intake or changes in immunity leading to invasion of host tissues by biofilm bacteria. This initiates a pathological sequelae of infection and concurrent host inflammatory response, representing a shift from healthy plaque biofilm to a 'pathogenic biofilm'. Pathogenic dental plaque biofilms not only result in dental diseases, such as dental caries and periodontal disease, but could also be involved in diseases in the cardiovascular, respiratory, renal and other systems.

Properties of dental plaque biofilm

One of the intriguing features of microbial biofilm, including dental plaque biofilm, is its self-sustainability. A microbial biofilm community would survive under highly challenging environmental conditions that its planktonic counterparts would not. For instance, it has been shown that the biofilm mode of growth allows microbes to survive under nutrition-limited conditions for a long period of time. Similarly, microbial constituents of the dental plaque biofilm do not rely on the nutrition taken by the host; they are sustained by establishing 'food-chains' involving other members of the community. Growth of the dental plaque biofilm depends on the nutrients derived from endogenous sources, such as saliva and gingival crevicular fluid, rather than the exogenous food intake of the host. Therefore, a metabolic product of one organism may be an essential primary source of nutrition of another. For instance, acidogenic bacteria such as *Streptococcus*, *Lactobacillus* and *Actinomyces* produce lactate as a by-product of their carbohydrate metabolism. Other bacterial species, such as *Veillonella* and *Propionibacterium*, utilise lactate as a carbon source, and by doing so convert lactate into weak propionic acid, reducing the risk of dental caries[28]. Similarly, the menaquinone produced by *Veillonella* and *Propionibacterium* is vital for the synthesis of vitamin K, which promotes the growth of bacteria such as *Porphyromonas*, *Prevotella* and *Bifidobacterium*[28]. Another example is the use of thiamine and isobutyrate by spirochetes, which is produced by *Fusobacterium* species in the dental plaque biofilm. We are only beginning to gain an understanding of the fascinating and intriguing relationships among the microbial members of dental plaque biofilm, which could certainly help us to devise better strategies for modulating the plaque biofilm towards a healthy state.

A unique feature related to microbial biofilm is higher antimicrobial resistance, and this can be seen in dental plaque biofilm. There are studies that show microbes in the dental plaque biofilm community are more resistant to commonly used antimicrobial agents than are corresponding planktonic counterparts. Several hypotheses have been put forward to explain the higher antibiotic resistance of microbial biofilms, such as altered metabolic state, contribution of extracellular matrix, higher antioxidative capacities, differential transcriptomic and proteomics expression, and presence of 'sleeping cells' or persister cells[29-31]. However, the exact mechanism by which biofilm microorganisms acquire higher antimicrobial resistance remains to be elucidated.

In vivo studies of dental plaque have corroborated these *in vitro* observations. A study on subgingival biofilm showed that the concentration of antibiotics required to inhibit bacteria in steady-state biofilm could be up to 250 times greater than would be required to inhibit their planktonic mode of growth. Furthermore, the antibiotics tetracycline, doxycycline, minocycline, amoxicillin, metronidazole or combinations such as amoxicillin/clavulanate and amoxicillin/metronidazole were ineffective in eradicating a 7-day-old mature dental plaque biofilm[32]. Another study showed that, in biofilm, periodontal pathogen *P. gingivalis* could be 60 and 160 times more resistant to doxycycline and metronidazole, respectively, than its planktonic counterpart[33]. Misuse of tetracycline may enrich the bacteria in the dental plaque with broad-range antibiotic resistance[34]. These studies demonstrate the relative lack of efficacy of systemic antibiotic therapy for periodontal diseases and emphasise the need for alternative methods for control for dental plaque-related diseases.

Quorum sensing (QS) or 'communication between bacteria' is an intriguing property of microbial biofilms. QS works as a gatekeeper, controlling the growth of the microbial community by signalling to bacteria to leave the biofilm to find new habitats[35]. QS is mediated by small molecules, such as competence stimulating

peptide (CSP) and autoinducer-2 (AI-2), which are involved in both intra- and interspecies communication among members of biofilm consortia. CSP produced by many streptococcal species is involved in a diverse set of biological activities, including biofilm formation, antimicrobial resistance, horizontal gene transfer and acid tolerance of dental plaque biofilm[36,37]. AI-2 encoded LuxS gene is produced during bacterial amino acid metabolism and plays a key role in both interspecies and intraspecies communication and expression of virulence factors[38]. For instance, *S. oralis* and *Actinomyces naselundii* form profuse biofilms only when the two organisms grow in co-culture, and this phenomenon depends on luxS-encoded AI-2[39].

When living as a microbial community in the biofilm, individuals tend to share their virulence traits by gene transfer, particularly the antibiotic-resistance genes located in conjugative plasmids and conjugative transposons. Exchange of genetic material by means of horizontal gene transfer and the presence of pathogenic islands provide strong evidence that biofilm communities have co-evolved and share their strategies to survive as a community. Not only do the bacteria share the survival advantage, but co-adhesion also allows them to elicit the maximum effect of their pathogenicity. A recent study showed that a member of the dental plaque biofilm *Veillonella dispar* could transfer Tn916, a conjugative transposon, to *Streptococcus* species in oral biofilms[40]. Purified genomic DNA of *V. dispar* could also transform *S. mitis* to tetracycline resistance. Another mutual relationship in dental plaque biofilm could be seen between *P. gingivalis* and *F. nucleatum*[41,42]. The minimum dose required for *P. gingivalis* to elicit pathogenicity was reduced by 1000-fold when the bacterium coexisted in the biofilm with *F. nucleatum* compared with the individual pathogenicity[43]. These studies clearly show the synergistic advantages provided by the biofilm mode of growth for establishment and survival of bacterial species within the dental plaque biofilm.

Although the current knowledge on dental plaque biofilm has come a long way, from the initial observations of van Leeuwenhoek to the current investigations using molecular microbiology, still the complex biological interactions in the biofilm community are not fully demystified. Therefore it is imperative to revisit the role played by plaque biofilm in oral health and disease. The ecological plaque hypothesis proposed by Marsh elegantly explained that dental diseases result from ecological catastrophes in the local environment and the resulting changes in the microbiota of the dental plaque biofilm[12].

Dental plaque biofilm in health

Healthy dental plaque biofilm predominantly comprises commensal, non-pathogenic microbial members. These commensal members, however, are not mute counterparts. There is a continuous cross-talk between commensal bacteria in the dental plaque and with host tissues, such as gingiva, even in the healthy state. However, it is a harmonious, mutually beneficial relationship. The host provides a colonisation surface for the commensals and the bacteria provide 'colonisation resistance' against pathogenic and more harmful organisms[44]. The benefits of this relationship become evident under the circumstances seen in an antibiotic sore mouth, a state in which suppression of normal flora leads to overgrowth of opportunistic pathogens. Studies have shown that some commensal bacterial species, such as *Veillonella* species, *Streptococcus salivarius*, *S. sanguinis* and *Atopobium parvulum*, could indicate a healthy state of the biofilm, although more studies are needed to verify this claim. Interestingly, *S. salivarius* has been shown to inhibit quorum sensing and biofilm formation of *S. mutans*, which may provide some evidence of its protective role against dental caries[45].

Commensal bacteria in the dental plaque contribute to the development of a normal immune system by constantly providing a versatile set of bacterial antigens for the host's innate immune system. Commensals initiate signal cascades that converge the messages of tolerance, whereas pathogenic bacteria induce a strong inflammatory response of the host. Therefore, there is a continuous production of pro-inflammatory cytokines in the oral epithelial cells at a low level, which cause expression of E-selectin in the vascular endothelial tissues and establishment of an interleukin-8 chemokine gradient[46]. Hence, commensal bacteria elicit a host innate immune response that places neutrophils strategically alongside the subgingival plaque bacteria and junctional epithelium.

Role of dental plaque in dental caries

The ecological plaque hypothesis suggests that changes in the environment in the vicinity of the dental plaque biofilm could lead to dental diseases, such as dental caries and periodontal diseases[44,47,48]. Frequent intake of dietary sugars provides an opportunity for acidogenic and aciduric bacteria in the dental plaque biofilm, such as *S. mutans* and *Lactobacillus acidophilus*, to create a persistent acidic environment, which results in a shift in balance towards the demineralisation of the tooth surface[49]. Recent studies of *S. mutans* biofilms have shown

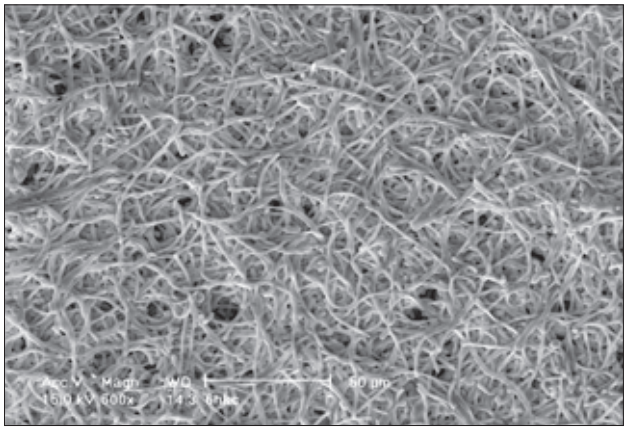

Fig 2 Scanning electron micrograph showing mature *Candida albicans* biofilm.

that the biofilm mode of growth has greater tolerance of acidic stress, which could be as high as six orders of magnitude higher compared with the planktonic form of bacteria[50]. Mature *S. mutans* biofilm down-regulates the main energy generating glycolytic pathway in order to be acid tolerant. There is other evidence to suggest that members of the dental plaque biofilm community undergo phenotypic changes during health and disease. Recent studies showed that *S. mutans* strains recovered from caries-active and caries-free individuals differ in sensitivity to host antimicrobial peptides. Genes associated with glucan (Gtf) and fructan (ftf) have been shown to be differentially expressed between the planktonic and biofilm bacteria. The ecological changes that occur in the dental plaque biofilm may therefore contribute to the disease process seen in dental caries.

Conversely, some studies have suggested that the presence of high numbers of *S. mutans* in the dental plaque is not sufficient for the development of dental caries. Therefore, it is assumed that the presence of a single species alone is not the initiating factor, but that multiple cariogenic species, such as *S. mutans*, *S. mitis*, *Rothia*, *Actinomyces*, *Lactobacillus* and *Bifidobacterium* bacteria and even fungal species like *Candida* (Fig 2), could account for a biofilm becoming cariogenic. Interestingly, fungal species such as *Candida albicans* have been shown to be capable of causing occlusal caries at a high rate in rats[51]. A recent study which used *in situ* imaging techniques to examine the architecture of the dental plaque biofilm on natural teeth demonstrated that *C. albicans* could form corn-cob structures with streptococcal species in the supragingival plaque, which may explain its important niche in the dental plaque[23]. Tanner et al suggested *Scardovia wiggsiae*, a new cariogenic bacteria, could be associated with dental caries[52]. Therefore, one must not be confined to the traditional thinking that a single species is the sole cause of diseases such as dental caries.

Recent advances in the understanding of the molecular microbiology of dental plaque biofilm have produced numerous clinical benefits. One such example is xylitol, which selectively inhibits the growth and metabolism of *S. mutans*. Clinical studies have shown the incorporation of xylitol into chewing gum to be effective in reducing mutans streptococci and lowering dental caries[53,54]. Conversely, some *in vitro* studies using multispecies cariogenic biofilm models have demonstrated that xylitol and sorbitol might not be as effective as claimed[55]. Use of probiotic bacteria such as *Lactobacillus rhamnosus* LB21 as a milk supplement is another idea that has been proposed for controlling dental caries[56]. However, some studies have shown this might not be effective in controlling colonisation of cariogenic bacteria in the caries-active adolescent[57]. Other strategies have been considered, such as the use of protease produced by early dental plaque biofilm colonisers to inhibit the colonisation of *S. mutans*[58]. However, the complete picture of the dental plaque biofilm is still not fully understood, which is the Achilles' heel in the task of finding a successful solution to the global epidemic of dental caries.

Dental plaque biofilm in periodontal disease

The main feature of periodontal disease is inflammation of the periodontal tissues in response to Gram-negative pathogenic bacteria such as *P. gingivalis* and spirochetes such *as T. denticola* in the dental plaque biofilm[59]. According to the ecological plaque hypothesis, it is assumed that secretion of gingival crevicular fluid is increased in response to inflammation of the periodontal tissues. This leads to a rise in the local pH above the normal neutral value. It has been suggested that even a minor rise in pH allows periodontopathic bacteria such as *P. gingivalis* to overgrow and override other microorganisms in the dental plaque[60]. *Porphyromonas gingivalis* is a hemin-dependent bacterium that acquires hemin from gingival crevicular fluid[61]. Secretory protease/hemagglutinins, such as gingipains, hemagglutinin B and hemagglutinin C, also aid *P. gingivalis* in acquiring hemin from erythrocytes. A rise in local hemin concentration, due to increased gingival crevicular fluid during periodontitis, provides a competitive advantage to the so-called red-complex bacteria, including *P. gingivalis*, over other commensal bacteria. Interestingly, recent studies have shown that *P. gingivalis* is able to shift its lipopolysaccharide (LPS) structure from penta-acylated

lipid A to tetra-acylated lipid A structures depending on the hemin concentration in the local environment[62]. This could be a strategy that pathogenic *P. gingivalis* uses to evade the human immune system as a tetra-acylated LPS structure 'paralyses' the local cytokine network, giving the bacterium an opportunity to invade the gingival tissue[63]. In addition to LPS, other virulent factors, such as type IV fimbriae of *P. gingivalis*, could also contribute to periodontal disease.

Intriguingly, this activity seems to be enhanced by heavy smoking[64]. Other studies have also shown that smoking could shift the microbial composition of dental plaque biofilm towards colonisation by periodontal pathogens such as *Tannerella forsythia*, *P. gingivalis*, *T. denticola*, *P. intermedia*, *Parvimonas micra*, *Prevotella nigrescens* and *Campylobacter rectus*[65,66].

Changes in microbiota in supra- and subgingival samples during *in vivo* development of dental plaque have been examined by several studies. Uzel et al studied the early developments that occurred in subgingival dental plaque biofilm in periodontally healthy and chronic periodontitis subjects who refrained from oral hygiene[67]. Within 2 days, microbial flora was re-established in the early dental plaque akin to that of the pre-cleaning state. Only subtle differences could be seen between the supragingival plaque of healthy and periodontally diseased subjects. Conversely, redevelopment of subgingival plaque was markedly different between the two groups; dental plaque was more rapidly formed in the latter compared with the healthy group.

Although the association of red-complex bacteria with periodontitis has been generally accepted by the scientific community, neither the exact role played by each bacterium nor the mechanism involved has been fully elucidated. Some studies have suggested other bacterial species are also involved in the pathogenesis of periodontal disease, complicating the already complex picture of pathogenesis. For instance, some studies have shown that *Selenomonas*, which was first described by van Leeuwenhoek, may have an association with periodontal disease[68].

Summary

In view of the foregoing information, it seems appropriate to conclude that the clinical picture of dental disease is a net result of an interaction between the pathogenic dental plaque biofilm and the host tissue response. In the healthy state, both plaque biofilm and adjacent tissues maintain a delicate balance and a harmonious cross-talk is established between the two counterparts. Recent advances in molecular microbiology have improved the understanding of dental plaque biofilm and produced numerous clinical benefits. Therefore, it is imperative that clinicians keep abreast with these new developments in the field of dentistry. Better insights into the molecular mechanism behind dental diseases will facilitate the development of novel therapeutic strategies to establish a 'healthy dental plaque biofilm' through modulating both host and microbial factors.

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