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Dental biofilm: ecological interactions in health and disease

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Abstract

Background: The oral microbiome is diverse and exists as multispecies microbial communities on oral surfaces in structurally and functionally organized biofilms.

Aim: To describe the network of microbial interactions (both synergistic and antagonistic) occurring within these biofilms and assess their role in oral health and dental disease.

Methods: PubMed database was searched for studies on microbial ecological interactions in dental biofilms. The search results did not lend themselves to systematic review and have been summarized in a narrative review instead.

Results: Five hundred and forty-seven original research articles and 212 reviews were identified. The majority (86%) of research articles addressed bacterial–bacterial interactions, while inter-kingdom microbial interactions were the least studied. The interactions included physical and nutritional synergistic associations, antagonism, cell-to-cell communication and gene transfer.

Conclusions: Oral microbial communities display emergent properties that cannot be inferred from studies of single species. Individual organisms grow in environments they would not tolerate in pure culture. The networks of multiple synergistic and antagonistic interactions generate microbial inter-dependencies and give biofilms a resilience to minor environmental perturbations, and this contributes to oral health. If key environmental pressures exceed thresholds associated with health, then the competitiveness among oral microorganisms is altered and dysbiosis can occur, increasing the risk of dental disease.

Key words: ecology; gene transfer; interactions; metabolism; oral microbiome; signalling

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The mouth supports the growth of diverse communities of microorganisms – viruses, mycoplasmas, bacteria, *Archaea*, fungi and protozoa (Wade 2013). These communities persist on all surfaces as multispecies biofilms and form the resident oral

microbiome, which generally exists in harmony with the host, and delivers important benefits that contribute to overall health and well-being. The microorganisms found within these oral biofilms live in close proximity with one another, which results in a wide range of potential interactions, which can be synergistic or antagonistic. The composition of the microbiome is influenced by the oral environment, and changes in local conditions can affect the microbial interactions within these oral communities and determine, in part, whether the relationship between the oral

microbiome and the host is symbiotic or potentially damaging (dysbiotic), thereby increasing the risk of diseases such as caries or periodontal diseases (Marsh 2003, Roberts & Darveau 2015). Our aim was to review systematically the literature on microbial interactions in dental biofilms in health and disease. However, the search strategy and outcomes, presented below, led to a conclusion that the topic is too broad for a systematic report and so the results are presented as a narrative review, highlighting the main microbial interactions in dental biofilms in health and introducing the

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environmental drivers for ecological dysbiosis towards disease.

Literature search

A PubMed search procedure was performed on 19-07-2016. The query combined four separate search items: (i) “microbiota,” including either bacteria, viruses, *Archaea*, fungi, protozoa or mycoplasma; (ii) “oral,” including distinct oral niches; (iii) interactions, including either “ecology,” “interaction,” “synergy,” “inhibition,” “co-occurrence,” “communication,” “metabolism,” “nutrients,” “gene transfer” or “quorum sensing”; and (iv) “plaque,” “biofilm,” “community” or “consortium” (Table S1). This resulted in 3758 hits. Of these, 3593 passed the English language filter. After the screening of the titles and abstracts, the entries that did not relate to the topic were excluded, leaving 759 articles. Among these were 212 reviews.

The vast majority (86%) of the original research articles ($N = 547$) addressed bacterial interactions (Table 1). These included physical (e.g. co-aggregation, co-adhesion) and nutritional synergistic interactions, antagonistic interactions such

as production of bacteriocins and other inhibitory substances, cell-to-cell communication and gene transfer. The bacterial species involved ranged from primary colonizers to taxa associated with caries and periodontal disease. Only 45 (8.2%) of the studies involved fungi, while interactions involving viruses (18 studies), *Archaea* (four studies) and protozoa (three studies) were the least studied. Inter-kingdom interactions were addressed in 71 studies, with the majority of these focusing on *Candida albicans* and oral streptococci (Table 1).

Due to the high number of articles included and the broad range in the methods and the outcomes among the studies found, it was not possible to report on the results in the form of a systematic review or meta-analysis. Instead, the articles that were identified by the described search procedure were used as the basis of the narrative review below.

Microbial interactions in health

The close physical proximity of microorganisms within oral biofilms inevitably increases the probability of interactions occurring. The most common types of interaction are

listed in Table 2 and can be synergistic or antagonistic to the participating species (Hojo et al. 2009, Huang et al. 2011, Kolenbrander 2011, Diaz 2012, Guo et al. 2014, Jakubovics 2015a, Nobbs & Jenkinson 2015, Ng et al. 2016).

Synergistic interactions

Physical interactions and biofilm architecture

Oral microorganisms must attach to surfaces if they are to persist in the mouth and avoid being lost by swallowing. Evidence primarily derived from laboratory studies suggests that early colonizers adhere via specific adhesin-receptor mechanisms to molecules in the conditioning films that coat oral surfaces (Hojo et al. 2009), although, ultimately, microbial growth is the major contributor to the increase in biofilm biomass (Dige et al. 2007). Oral microorganisms have a natural tendency to adhere to other microbes and this process (co-adhesion – the adherence of planktonic cells to already attached organisms on a surface) facilitates the formation of multispecies biofilms (Kolenbrander 2011). In addition to anchoring a cell to a surface, co-adhesion also promotes microbial interactions by co-locating organisms next to physiologically relevant partner species, thereby facilitating nutritional co-operation and food chains, gene transfer and cell–cell signalling. Substantial changes in gene expression occur when cells are in close proximity or physical contact with one another (Wright et al. 2013), while functional consequences can result, such as the protection of obligately anaerobic bacteria in aerobic environments by neighbouring species that either consume oxygen (Bradshaw et al. 1994) or are oxygen-tolerating (Diaz et al. 2002). *Candida albicans* can also co-aggregate with oral streptococci and can form synergistic partnerships in which the yeast promotes streptococcal biofilm formation, while streptococci enhance the invasive property of *Candida* (Diaz et al. 2012, Xu et al. 2014). These physical and functional associations can manifest themselves in some of the complex multispecies arrangements observed in oral biofilms formed in vivo, such as “corn cob,” “test-tube brush” and

Table 1. Details on original research articles ($N = 547$) obtained in PubMed search described in Table S1

Members of the interaction(s)	Details
Bacteria–Bacteria ($N = 473$)	Oral health: $N = 205$; caries pathogen (CP): $N = 107$; periodontal pathogen (PP): $N = 149$; CP and PP: $N = 6$; Oral versus non-oral species: $N = 6$ Antagonism (A): $N = 116$; synergy (S): $N = 214$; A and S: $N = 3$; metabolism: $N = 98$; communication: $N = 32$; gene transfer: $N = 10$
Bacteria–Fungi ($N = 45$)	<i>Candida albicans</i> : $N = 40$; <i>C. albicans</i> and other <i>Candida</i> species: $N = 3$; undefined <i>Candida</i> spp.: $N = 2$ Bacteria involved: <i>Streptococcus mutans</i> : $N = 11$; <i>Streptococcus gordonii</i> : $N = 10$; other oral streptococci: $N = 9$; <i>Actinomyces</i> : $N = 5$; <i>Staphylococcus aureus</i> $N = 2$; <i>Aggregatibacter actinomycetemcomitans</i> , <i>Enterococcus faecalis</i> , <i>Fusobacterium nucleatum</i> $N = 1$ each; probiotic lactobacilli: $N = 1$; microbial consortia or microcosm: $N = 8$ Antagonism: $N = 11$; synergy: $N = 33$; communication: $N = 5$
Bacteria–Viruses ($N = 18$)	Bacteriophages: $N = 6$; Herpes viruses: $N = 7$; virome: $N = 3$; CRISPR: $N = 3$
Bacteria– <i>Archaea</i> ($N = 4$)	Methanogenic <i>Archaea</i> and periodontal pathogens
Fungi–Fungi ($N = 7$)	Different <i>Candida</i> species: $N = 6$; <i>Pichia</i> versus opportunistic fungi
Fungi–Viruses ($N = 1$)	HSV enhances <i>C. albicans</i> adherence
Bacteria–Protozoa ($N = 3$)	<i>Bdellovibrio bacteriovorus</i> – bacterial predator

Table 2. Types of synergistic and antagonistic microbial interactions that occur among oral microorganisms growing in dental plaque biofilms

Interactions	
Synergistic	Antagonistic
Enzyme complementation/enzyme sharing	Bacteriocin production
Food chains (food webs)	Hydrogen peroxide production
Co-adhesion	Organic acid production/generation of inhibitory pH conditions
Cell-cell signalling	Bacteriophage release
Gene transfer	Competition for essential nutrients
Environmental modification	Predation

“hedgehog” structures (Zijnge et al. 2010, Dige et al. 2014, Mark Welch et al. 2016).

Nutritional interactions

The primary nutrients for oral microorganisms are host proteins and glycoproteins, and these are obtained mainly from saliva for organisms in supragingival plaque (for a review, see Jakubovics 2015b) and from gingival crevicular fluid (GCF) for those located in subgingival biofilms (Wei et al. 1999). Pure cultures of oral microorganisms grow poorly or not at all on these structurally complex substrates, and consortia of interacting species are needed for their catabolism. Proteins are broken down by the action of mixtures of proteases and peptidases, but the catabolism of glycoproteins (consisting of a protein backbone decorated with linear or branched oligosaccharide side chains) involves the sequential removal of terminal sugars from side chains before the protein backbone becomes accessible to proteolytic attack (Takahashi 2015). Oral bacteria express glycosidases with different specificities so that the concerted action of several species is necessary for the complete degradation of host glycoproteins (Bradshaw et al. 1994). Similarly, combinations of mutans streptococci, *Streptococcus oralis* and *Fusobacterium nucleatum*, degraded albumin more effectively than any of the three species alone (Homer & Beighton 1992). The biofilm matrix is another potential source for carbon and energy for interacting consortia of oral bacteria. Fructans and soluble glucans in dental plaque can be metabolized by combinations of bacteria that produce exo- and/or endohydrolytic enzymes (Bergeron &

Burne 2001, Koo et al. 2013). Individual bacteria are dependent therefore on the metabolic capability of other species for access to essential nutrients.

Further complex nutritional inter-relationships develop in microbial communities when the products of metabolism of one organism (primary feeder) become the main source of nutrients for another (secondary feeder), resulting in the development of food chains or food webs (Hojo et al. 2009) (some examples are illustrated in Fig. 1). These food webs can result in the complete and energetically efficient catabolism of complex host molecules to the simplest end products of metabolism (e.g. CO₂, CH₄, H₂S). Numerous synergistic metabolic interactions occur among bacteria in subgingival

biofilms in order to enable them to degrade host proteins and glycoproteins as nutrient sources (ter Steeg et al. 1987, ter Steeg & van der Hoeven 1989). These interactions are discussed in more detail later in the section on “Ecological drivers towards dysbiosis and disease”.

Nutritional inter-dependencies such as those described above contribute to the temporal stability and resilience of oral microbial communities, while a consequence of the reliance of resident oral bacteria on the metabolism of these complex substrates is that species avoid direct competition for individual nutrients, and hence are able to co-exist and maintain a stable equilibrium, also termed microbial homeostasis (Alexander 1971, Marsh 1989). This has been elegantly demonstrated in a computational study on KEGG pathway-based metabolic distances between 11 oral bacteria that are known to interact (Mazumdar et al. 2013). Metabolism was a major factor driving the order of colonization, with specific metabolic pathways associated with different layers in the biofilm, resulting in a functionally structured community. However, in such a structured community, there was an optimal trade-off between their resource sharing and functional synergy (Mazumdar et al. 2013).

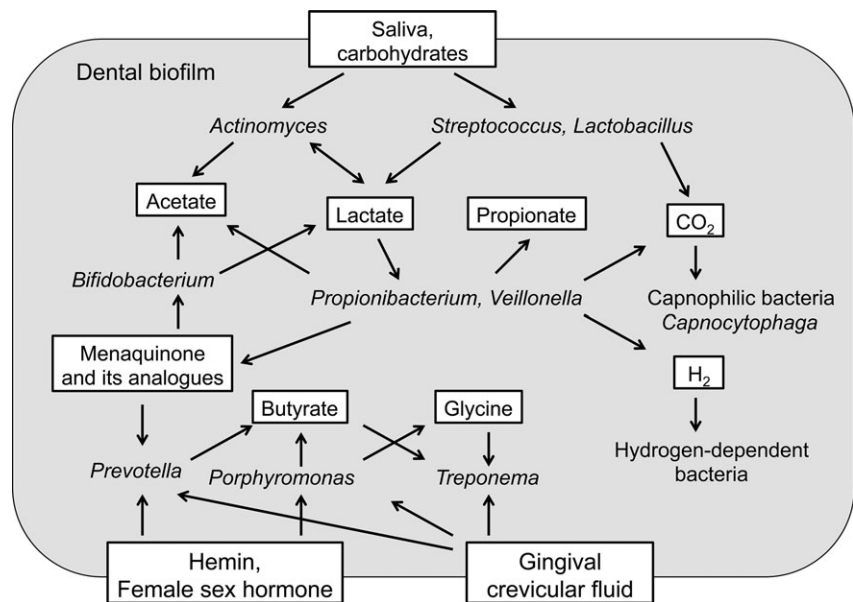


Fig. 1. Examples of nutritional interactions among oral microorganisms (figure modified from Hojo et al. 2009).

Cell-cell signalling

Laboratory studies have shown that microbial cells are able to communicate with, and respond to, neighbouring cells in biofilms by means of small, diffusible, effector molecules. Gram-positive cells produce peptides that generally have a narrow spectrum of activity. In *Streptococcus mutans*, two peptides [competence-stimulating peptide (CSP) and sigma X-inducing peptide] promote genetic competence in other cells of *S. mutans*; production of these peptides is influenced by the local pH (Guo et al. 2014) and carbohydrate source (Moye et al. 2014). CSP-mediated quorum sensing has also been identified in *Streptococcus gordonii* and *Streptococcus intermedius*. The function of CSPs is to alter gene transcription and protein synthesis involved in biofilm formation, competence development, bacteriocin synthesis, stress resistance, and autolysis (Senadheera & Cvitkovitch 2008, Guo et al. 2014). Some streptococci can inactivate CSPs and thereby inhibit biofilm formation by *S. mutans* (Wang et al. 2011). CSP produced by *S. gordonii* can also inhibit biofilm formation by *C. albicans* (Jack et al. 2015), so it is possible that a complex network of signalling interactions will exist in a multispecies biofilm such as dental plaque.

Autoinducer-2 (AI-2) is produced by several genera of oral Gram-positive and Gram-negative bacteria and may be a “universal language” for inter-species and inter-kingdom communication in dental biofilms, and the efficiency of signalling might be enhanced by co-adhesion. Biofilm formation with two co-adhering species – *S. oralis* and *Actinomyces naeslundii* – was inhibited when an AI-2 knockout of *S. oralis* was used instead of the wild type (Rickard et al. 2006), while AI-2 produced by *Aggregatibacter actinomycetemcomitans* inhibited hyphae formation and biofilm formation by *C. albicans* (Bachtiar et al. 2014). AI-2 produced by *F. nucleatum* had a differential effect on biofilm formation when cultured with two different species of oral streptococci; biofilm formation was enhanced with *S. gordonii* but reduced with *S. oralis* (Jang et al. 2013). Some of these responses are

dependent on the concentration of the signalling molecules. These cell-cell signalling strategies could enable cells to sense and adapt to various environmental stresses and, thereby, regulate (and coordinate) the expression of genes that influence the ability of pathogens to cause disease.

Gene transfer

The close proximity of cells in biofilms provides ideal conditions for horizontal gene transfer (HGT). HGT involves either acquisition of DNA from co-resident species or from exogenous sources (Petersen et al. 2005, Roberts & Kreth 2014). DNA can be transferred through: transduction by bacterial viruses (bacteriophages), conjugation by bacterial pili and transformation by DNA uptake involving naturally competent bacteria; in addition to the mechanisms above, DNA can also be transferred via membrane vesicles in Gram-negative bacteria (Olsen et al. 2013). HGT allows oral bacteria to sample from an immense metagenome and in this way increase their adaptive potential to changes in the oral environment (Roberts & Kreth 2014). For instance, metabolic adaptability to carbohydrate-rich environments such as the oral cavity and gut has been found in a *Lactobacillus salivarius* strain carrying a plasmid with genes involved in glycolysis (Roberts & Kreth 2014). HGT is thought to be the main mechanism in acquiring antibiotic resistance genes (ARGs), which are richly present in the oral cavity (Sukumar et al. 2016).

As described earlier, signalling molecules such as CSP markedly increase the ability of recipient cells to take up DNA (Senadheera & Cvitkovitch 2008). Extracellular DNA (eDNA) is a component of the biofilm matrix and plays a critical role in adhesion and in possible nutrient storage and as a potential source of phosphate and other ions (Jakubovics & Burgess 2015). eDNA release has been demonstrated in dual species experiments with *S. mutans* and *S. gordonii* through *S. mutans* competence-induced bacteriocin production (Kreth et al. 2005); Gram-negative bacteria also release eDNA, including *Veillonella* spp. (Hannan et al. 2010),

Porphyromonas gingivalis and *F. nucleatum* (Ali Mohammed et al. 2013).

Evidence for HGT in dental biofilms has come from the discovery that both resident (*S. mitis*, *S. oralis*) and pathogenic (*S. pneumoniae*) bacteria isolated from the naso-pharyngeal area possess genes conferring penicillin resistance that display a common mosaic structure (Chi et al. 2007). Similar evidence suggests sharing of genes encoding for penicillin-binding proteins among resident oral and pathogenic *Neisseria* species (Bowler et al. 1994), and IgA protease encoding genes among a range of oral streptococcal species (Poulsen et al. 1998).

Antagonistic interactions

A considerable number of studies addressed antagonistic interactions involving inter-species and inter-kingdom competition or “warfare”. The production of antagonistic compounds such as bacteriocins, hydrogen peroxide, organic acids, different enzymes and release of lytic phages are just a few examples of “weapons” that can give an organism a competitive advantage during colonization and when competing with other microbes (Table 2).

Bacteriocins and bacteriocin-like substances are produced by both Gram-positive and Gram-negative bacteria, with the most studied oral species being streptococci, and examples include mutacin produced by *S. mutans* (Merritt & Qi 2012), sanguicin by *S. sanguinis* and salivarin by *S. salivarius* (Jakubovics et al. 2014). Two types of mutacin have been detected; lantibiotics, which have a broad spectrum of activity, and the more common non-lantibiotics, which have a narrower antimicrobial range (Merritt & Qi 2012). Lactobacilli also produce bacteriocins and are being evaluated as potential oral probiotics largely due to their antimicrobial properties; for example, reuterin from *Lactobacillus reuteri* was active against selected periodontal and cariogenic bacteria (Kang et al. 2011).

Bacterial “warfare” implies that one of the interacting partners benefits at the expense of the other. This has been shown with two taxa occupying the same niche – *S. gordonii*

and *S. mutans*, where *S. gordonii* had a competitive advantage over *S. mutans* when using amino sugars from salivary glycoproteins as an energy source: *S. gordonii* released hydrogen peroxide that inhibited transcription of *S. mutans* genes responsible for the metabolism of these compounds (Zeng et al. 2016). Indeed, hydrogen peroxide is one of the most studied agents produced in dental biofilms, but its impact on the oral microbiota is complex and difficult to predict. Under aerobic conditions (as could occur during early stages of biofilm formation), *Streptococcus sanguinis* produces high concentrations of hydrogen peroxide that are capable of inhibiting a range of Gram-positive species (Holmberg & Hallander 1972, 1973, Kretz et al. 2016); much lower concentrations are generated during anaerobic growth. *Streptococcus mutans* is susceptible to hydrogen peroxide, but strains that produce mutacin are able to inhibit other streptococci (Ryan & Kleinberg 1995, Ashby et al. 2009). Hydrogen peroxide production has been proposed as a major mechanism for controlling the levels of putative periodontopathic bacteria in dental plaque (Hillman et al. 1985, Hillman & Shivers 1988). However, other bacteria in the supragingival biofilms (e.g. *Neisseria*, *Haemophilus* and *Actinomyces* species) are also able to degrade hydrogen peroxide, and little free peroxide can be detected in plaque (Ryan & Kleinberg 1995). Thus, there may be varying concentrations of hydrogen peroxide in different regions of the biofilm, and the balance between symbiosis and dysbiosis may depend on the complex interplay between multiple antagonistic microbial interactions.

Counter-intuitively, antagonistic interactions might also be beneficial to both partners involved and might even stimulate the fitness of the microbial community (Stacy et al. 2014). In the presence of oxygen, *A. actinomycetemcomitans* that cross-feeds with lactate produced by *S. gordonii* has to survive high concentrations of hydrogen peroxide released by *S. gordonii* (Fig. 2). To ameliorate oxidative stress, *A. actinomycetemcomitans* not only expresses catalase (H_2O_2 -detoxifying enzyme), but also responds to

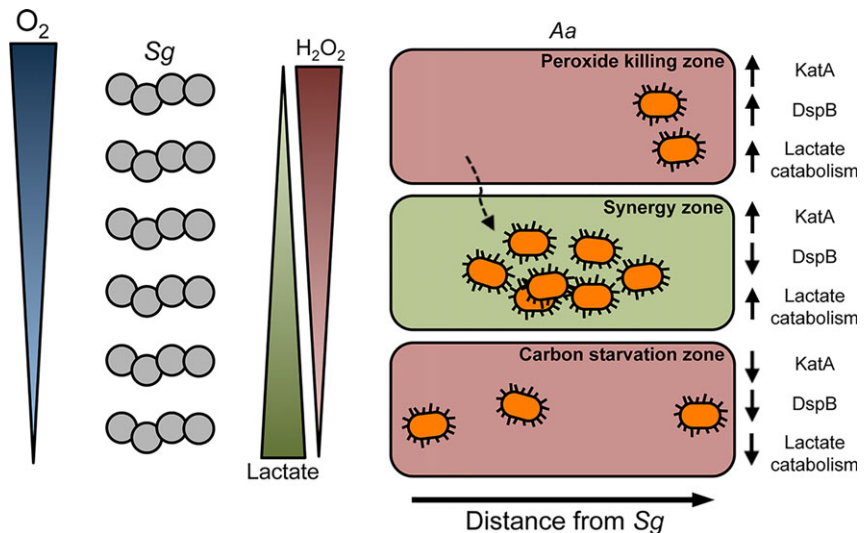


Fig. 2. Model for *Streptococcus gordonii* and *Aggregatibacter actinomycetemcomitans* interactions: hydrogen peroxide production by *S. gordonii* (Sg) supports lactate consumption by *A. actinomycetemcomitans* (Aa) (figure S8 from Stacy et al. 2014). *A. actinomycetemcomitans* expresses H_2O_2 -detoxifying enzyme catalase (KatA), which also protects *S. gordonii* from self-inflicted oxidative stress. Dispersin B (DspB) is an enzyme that promotes dispersal of *A. actinomycetemcomitans* biofilms and results in increased distance between the *A. actinomycetemcomitans* and the H_2O_2 -producing *S. gordonii*. The three zones (Peroxide killing zone, Synergy zone and Carbon starvation zone) correspond to different concentrations in oxygen, hydrogen peroxide and lactate in the biofilm, as indicated with the respective triangles.

elevated H_2O_2 by induction of Dispersin B – an enzyme that promotes dispersal of *A. actinomycetemcomitans* biofilms, resulting in increased physical distance between the *A. actinomycetemcomitans* and the H_2O_2 -producing *S. gordonii*. On the other hand, *S. gordonii*, which does not make its own catalase, is cross-protected by *A. actinomycetemcomitans* from self-inflicted oxidative stress.

A highly diverse oral bacteriophage gene pool has been discovered through a metagenomics approach (Pride et al. 2012, Naidu et al. 2014, Dalmaso et al. 2015, Edlund et al. 2015a). Phages are bacterial viruses that may lyse competing cells. The production of antagonistic factors will not necessarily lead to the complete exclusion of sensitive species as the presence of distinct microhabitats within a biofilm such as plaque enable bacteria to survive under conditions that would be incompatible to them in a homogeneous environment. Noteworthy, although parasitic by their nature, phages might have a beneficial role in the oral ecosystem: a recent comparison of the bacteria-phage network revealed that phages supported a complex

microbial community structure in health that was absent during periodontal disease (Wang et al. 2016).

Antagonism will also be a mechanism whereby exogenous species are prevented from colonizing the oral cavity (bacterial interference or colonization resistance). Oral streptococci have been shown to interfere with colonization by *Pseudomonas aeruginosa* through nitrite-mediated interference (Scofield & Wu 2015, 2016), while a sophisticated colonization resistance structure has been described in an in vitro murine oral microbial community with the “Sensor” (*Streptococcus saprophyticus*) sensing the intruding non-oral *Escherichia coli* strain and producing diffusible signals to the “Mediator” (*Streptococcus infantis*) that depresses the capacity of the “Killer” (*Streptococcus sanguinis*) to produce hydrogen peroxide, resulting in inhibition of the invading *E. coli* (He et al. 2014).

Ecological drivers towards dysbiosis and disease

When the oral environment changes, the ecology of the ecosystem is affected. This has an impact on the

outcome of the interactions among the microorganisms in the biofilms, which will affect the proportions of the members of the community, and can increase the risk of disease (dysbiosis). Two scenarios will be dissected below: one leading towards a cariogenic and the other towards a periodontopathogenic ecosystem.

Dental caries is associated with an increased frequency of dietary sugar intake. These sugars are metabolized rapidly to acid (mainly lactic acid), and a low pH is generated within the biofilm. Lactate can be utilized by *Veillonella* spp., and other species, for example *Neisseria* (Hoshino & Araya 1980), *Haemophilus* (Traudt & Kleinberg 1996), *Aggregatibacter* (Brown & Whiteley 2007), *Porphyromonas* (Lewis et al. 2009) and *Actinomyces* (Takahashi & Yamada 1996), and converted to weaker acids. Fewer carious lesions and less lactate in plaque were measured in rats inoculated with *S. mutans* and *Veillonella alcalescens* than in animals infected with *S. mutans* alone (van der Hoeven et al. 1978). Higher proportions of *Veillonella* spp. have been detected in samples from caries lesions when compared to plaque from healthy enamel (Gross et al. 2012), perhaps because of the increased glycolytic activity and higher levels of lactate at these sites. Symbiosis between *Veillonella* and *S. mutans* has been demonstrated in mixed cultures: when *Veillonella parvula* was added to the pair of antagonists (*S. mutans* and *S. gordonii*), it mitigated the inhibitory effects of *S. gordonii* on sugar metabolism and growth of *S. mutans* (Liu et al. 2011).

The frequent conditions of low pH in biofilms associated with caries are inhibitory to the growth of many of the bacteria associated with enamel health, resulting in decreased microbial diversity (Gross et al. 2012, Jiang et al. 2011, Li et al. 2007, Peterson et al. 2013). Repeated conditions of low pH alter the competitiveness of members of the biofilm community and select for increased proportions of acidogenic and acid-tolerating bacteria including mutans streptococci, lactobacilli (Bradshaw et al. 1989), low-pH non-*S. mutans* streptococci and bifidobacteria (Marsh 1994, Takahashi & Nyvad 2008). Sucrose-induced

dysbiosis results not only in reduced taxonomic diversity, but also in a changed metaproteome, as recently shown in microcosms where proteins involved in acid tolerance and acid production dominated the dysbiotic biofilms (Rudney et al. 2015).

A counter mechanism against acidification of the ecosystem is alkali production by the members of the community, mainly through ammonia production from arginine and urea (Burne & Marquis 2000, Shu et al. 2003, Liu et al. 2012, Huang et al. 2015, Takahashi 2015). Recently, by applying a metatranscriptomics and metabolomics approach, a much higher diversity in alkali-generating pathways within complex oral biofilms has been discovered, including glutamate dehydrogenase, threonine and serine deaminase, and upregulation in membrane proteins involved in ammonia gas conduction besides the urease activity and arginine deiminase system (Edlund et al. 2015b). Additionally, this study revealed that *Veillonella* species are well adapted towards acid stress by upregulating various pathways that contributed to pH recovery.

Thus, unlike health, dental caries is associated with a shift in the composition of the biofilm to a community that is dominated by a strongly saccharolytic and acid-tolerant microbiota leading to a loss of diversity, and a reduction in levels and activity of beneficial bacteria (Gross et al. 2012, Jiang et al. 2011, Li et al. 2007, Peterson et al. 2013), although the diversity may increase when the lesion penetrates dentine, perhaps reflecting important environmental changes (Simón-Soro et al. 2014).

In contrast, the accumulation of microbial biomass around the gingival margin induces an inflammatory response. This results in an increased flow of GCF, which delivers not only components of the host defences (e.g. immunoglobulins, complement, neutrophils, cytokines) (Ebersole 2003), but, inadvertently, host molecules that can act as substrates for proteolytic bacteria. Some of these host molecules also contain haemin (e.g. haptoglobin, haemopexin, haemoglobin), which is an essential cofactor for the growth of potential periodontopathogens such

as *P. gingivalis* (Olczak et al. 2005). The change in local environmental conditions associated with inflammation will alter the competitiveness and outcome of multiple interactions among the microbes that make up the subgingival microbiota, leading to substantial changes in the microbial composition of the biofilm. Although there is agreement that there are major changes in the proportions of individual species in biofilms from inflamed sites (e.g. see reviews by Pérez-Chaparro et al. 2014, Diaz et al. 2016), there are conflicting reports on whether the diversity of the resultant microbial communities is altered. The diversity may increase in gingivitis (Kistler et al. 2013, Schincaglia et al. 2017), but the evidence for chronic periodontitis is more contentious (Abusleme et al. 2013, Hong et al. 2015, Kirst et al. 2015, Park et al. 2015).

The inflammatory response can influence the subgingival microbiota in two ways: (i) via the impact of the host defences and (ii) by the resultant changes to the environment. The innate defences will inhibit susceptible species, but a number of periodontal pathogens, such as *P. gingivalis*, can subvert the host response, for example, by degrading complement, interfering with neutrophil function and blocking phagocytosis (for reviews, see Slaney & Curtis 2008, Hajishengallis & Lamont 2014, Mysak et al. 2014). Thus, sensitive species will be eliminated (although some may survive due to cross-protection from neighbouring organisms), but those that can tolerate the inflammatory response will flourish. It has been argued that the microbial consortia that are associated with periodontitis are “inflammophilic” in that they have adapted to not only endure inflammation but also to exploit the altered environmental conditions (Hajishengallis 2014), such as small rises in pH and temperature (Eggert et al. 1991, Fedi & Killoy 1992, Hafajee et al. 1992, Nyako et al. 2005). Such small changes to the local environment can alter gene expression and increase the competitiveness of species such as *P. gingivalis* within microbial communities (Marsh et al. 1993). However, a more substantial change to the inflamed pocket is the altered nutrient status as a result of

the increased flow of GCF. To study the impact of this, laboratory studies have been performed using serum as a surrogate for GCF, and complex nutritional inter-relationships among subgingivally derived microbial consortia have been observed (ter Steeg et al. 1987, ter Steeg & van der Hoeven 1989). When biofilms from patients with chronic periodontitis were inoculated into pre-reduced (i.e. anaerobic) heat-inactivated human serum, the microbial composition of the consortia changed over time and these changes correlated with distinct stages in glycoprotein breakdown involving bacteria with different metabolic capabilities. Initially, carbohydrate side chains were removed by organisms with complementary glycosidase activities; this was followed by the hydrolysis of the protein core by obligately anaerobic bacteria leading to extensive amino acid fermentation. Significantly, individual species grew only poorly in pure culture on serum (ter Steeg & van der Hoeven 1989).

Numerous nutritional inter-dependencies and physical interactions will develop among the species coping with the array of novel host factors produced during the inflammatory response. For example, a complex but symbiotic metabolic relationship has been demonstrated in laboratory studies of *P. gingivalis* and *Treponema denticola* (Grenier 1992, Tan et al. 2014). Early studies demonstrated that isobutyric acid produced by *P. gingivalis* stimulated the growth of *T. denticola*, while succinic acid generated by *T. denticola* enhanced the growth of *P. gingivalis* (Grenier 1992). More recent studies have shown that the biomass is higher when both species are grown in co-culture, and glycine produced by *P. gingivalis* is utilized by the spirochaete (Tan et al. 2014). Both species respond to the presence of the other as seen by changes in global gene expression in both species. Similarly, the growth of certain species that have been previously described as being “unculturable” (e.g. *Fretibacterium fastidiosum*, *Prevotella* HOT-376, *Tannerella* HOT-286) has been shown recently to be due to their dependence on siderophores and to the close physical proximity of “helper” strains (Vartoukian et al. 2016a,b). Other studies

have demonstrated the importance of close physical associations to biofilm formation by interacting species of Gram-negative anaerobic bacteria (Sharma et al. 2005, Okuda et al. 2012).

Periodontal diseases may be an example of “pathogenic synergism” (van Steenberg et al. 1984), in which disease is a consequence of the combined activity of an interacting consortium in which each member is only weakly virulent. Different species would undertake a distinct role or function in order for the consortium to persist and cause disease. This is consistent with the recent concept of low abundance species (“keystone pathogens”) having a disproportionate effect of the virulence of the whole community (Hajishengallis et al. 2011, Hajishengallis & Lamont 2012). Gene transfer can occur within these communities; this can include not only mobile elements that code for drug resistance but also larger stretches of DNA that effect the virulence of recipient cells, for example, *P. gingivalis* possesses a “pathogenicity island” (Curtis et al. 1999).

Evidence for the role of the entire community and not just a few pathogens in dysbiosis has recently been delivered by metatranscriptome analysis of dental biofilms from sites with active periodontal disease (Yost et al. 2015): various streptococci, *Veillonella parvula* and *Pseudomonas fluorescens*, were highly active in transcribing putative virulence factors besides periodontal pathogens such as *Tannerella forsythia* and *P. gingivalis*. The genes that were over-represented at these sites were related to cell motility, lipid A and peptidoglycan biosynthesis, and transport of iron, potassium and amino acids.

Microbial interactions in such complex consortia could influence treatment outcomes. Although not advocated for routine use in periodontal disease, antibiotics are frequently used as adjunctive treatment to mechanical debridement in cases with severe or recurrent disease (Jepsen & Jepsen 2016). However, care needs to be taken as, apart from the existence and inter-species transfer of resistance genes within microbial communities, β -lactamase producing bacteria are commonly present in subgingival biofilms and they could protect neighbouring organisms that

should be susceptible to the action of the drug (Walker et al. 1987, van Winkelhoff et al. 1997, Rams et al. 2013).

Attempts have also been made to exploit antagonistic interactions to resolve both periodontal disease and caries. For periodontal therapy, either bacterial interference has been applied by deliberately implanting beneficial oral bacteria into a treated pocket (van Essche et al. 2013, Teughels et al. 2013) or using predatory protozoa, such as *Bdellovibrio* species (Dashiff & Kadouri 2011, van Essche et al. 2011, Loozen et al. 2015) or bacteriophage (reviewed by Allaker & Douglas 2009), while for caries prevention, different approaches (e.g. lozenges, milk, yoghurt) with probiotic bacteria that are antagonistic against *S. mutans* have been tried (Cagetti et al. 2013). A recent systematic review on the use of probiotics in managing oral diseases concluded that there is sufficient evidence for supporting the use of probiotics in the case of gingivitis and periodontitis but not for caries (Gruner et al. 2016), although this is an area in which more research is required.

Conclusions

Microbial communities, such as those found in dental biofilms, display “emergent properties”; that is, their properties are more than the sum of the component species, and their characteristics cannot be inferred from studies of individual organisms (Diaz et al. 2014). The microbiota is structurally and functionally organized, and it has been argued that such microbial communities could be considered as primitive multicellular organisms (Caldwell et al. 1997, Ereshefsky & Pedroso 2015). In health, numerous interactions contribute to stability and resilience of the ecosystem against environmental perturbations (Alexander 1971, Marsh 1989).

If certain key environmental pressures exceed thresholds that vary from patient to patient, then the competitiveness of certain bacteria is altered and dysbiosis can occur, leading to caries or periodontal diseases. In caries and periodontal diseases, changes in the nutrient status at the site due to increases in

fermentable carbohydrates (and the resultant acidic conditions) and host proteins (including haemin-containing molecules), respectively, disrupt the microbial interactions that control the balance of the microbial communities in health. Effective prevention of dental disease will require interference with these factors that drive dysbiosis (Marsh 2003), and a greater understanding of microbial interactions could lead to strategies to actively promote oral health.

The current literature search led us to the following conclusions: (i) oral microbial interactions belong to a highly studied and diverse topic, which was too broad for a systematic review; (ii) most oral microbial interactions have been investigated in laboratory systems, and occasionally animal models, and therefore some caution should be exercised when extrapolating these findings to events in humans; (iii) the majority of the interactions studied involve bacteria only, while other segments of the oral microbiota (fungi, *Archaea*, viruses, protozoa) are understudied; (iv) current technological advances (e.g. metagenomics, metatranscriptomics, metaproteomics, metabolomics, spectral imaging fluorescence in situ hybridization) enable the study of more complex community level interactions, including those among members of the microbiota from different kingdoms (Diaz et al. 2014) rather than just the conventional dual species studies; (v) both synergistic and antagonistic interactions contribute to the ecological stability of the microbial community that characterizes oral health; and (vi) more attention needs to be focussed on what microorganisms are doing within these microbial communities (Takahashi 2015), rather than just cataloguing which ones are present. The oral microbiome in health and disease might be better described by a series of functions and interactions, rather than as a list of individual organisms, as these functions might not be provided by the same microbes in different people (Lloyd-Price et al. 2016).

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Table S1. PubMed query search terms and results.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Clinical Relevance

Scientific rationale for the study: Microorganisms persist in the mouth as multispecies biofilms that deliver important benefits to the host. Microbes will interact because of their physical proximity, and the outcome will

influence oral biofilm composition and activity.

Principal findings: A literature review confirmed that numerous synergistic and antagonistic interactions occur among the resident microbes, resulting in tightly integrated communities that are resilient against minor

environmental perturbations, which contributes to oral health.

Practical implications: Treatment strategies should also include reducing environmental pressures that drive dysbiosis so that a favourable ecological balance is maintained.