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.

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

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 ($N = 45$)	*Candida albicans*: $N = 40$; *C. albicans* and *other Candida* species: $N = 3$; undefined *Candida* spp.: $N = 2$
	Bacteria involved: *Streptococcus mutans*: $N = 11$; *Streptococcus gordonii*: $N = 10$; other oral streptococci: $N = 9$; *Actinomyces*: $N = 5$; *Staphylococcus aureus* $N = 2$; *Aggregatibacter actinomycetemcomitans*, *Enterococcus faecalis*, *Fusobacterium nucleatum* $N = 1$ 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–*Archaea* ($N = 4$)	Methanogenic *Archaea* and periodontal pathogens
Fungi–Fungi ($N = 7$)	Different *Candida* species: $N = 6$; *Pichia versus* opportunistic fungi
Fungi–Viruses ($N = 1$)	HSV enhances *C. albicans* adherence
Bacteria–Protozoa ($N = 3$)	*Bdellovibrio bacteriovorus* – 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. CO_2, CH_4, H_2S). 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).
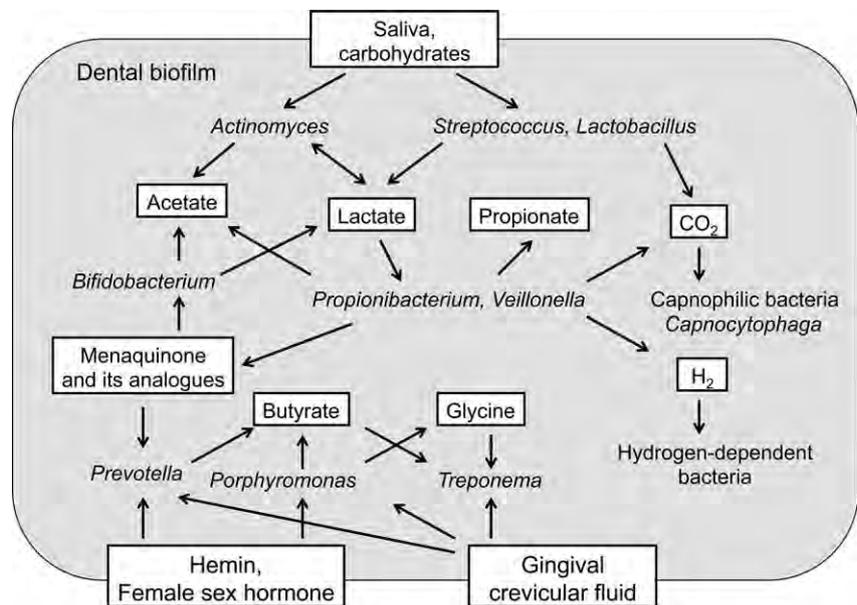

Fig. 1. Examples of nutritional interactions among oral microorganisms (figure modified from Hojo et al. 2009).

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 salivaricin 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, Kreth 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
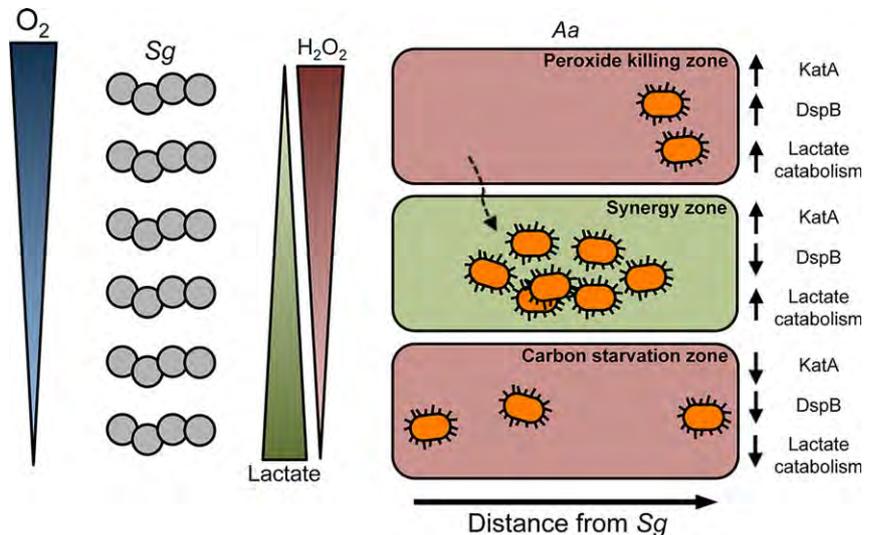

Fig. 2. Model for *Streptococcus gordonii* and *Aggregatibacter actinomycetemcomitans* interactions: hydrogen peroxide production by *S. gordonii* (Sg) supports lactate consumption by *A. actinomycetemcomitans* (Aa) (figure S8 from Stacy et al. 2014). *A. actinomycetemcomitans* expresses H_2O_2-detoxifying 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, Dalmasso 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 (Scoffield & 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 derepresses 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 "inflammo-philic" 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, Haffajee 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 interdependencies 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 Steenbergen 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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References

Abusleme, L., Dupuy, A. K., Dutzan, N., Silva, N., Burleson, J. A., Strausbaugh, L. D., Gamonal, J. & Diaz, P. I. (2013) The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. *ISME Journal* 7, 1016–1025.

Alexander, M. (1971). *Microbial Ecology*, pp. 207–223. London: Wiley.

Ali Mohammed, M. M., Nerland, A. H., Al-Haroni, M. & Bakken, V. (2013) Characterization of extracellular polymeric matrix, and treatment of *Fusobacterium nucleatum* and *Porphyromonas gingivalis* biofilms with DNase I and proteinase K. *Journal of Oral Microbiology* 5: 20015. doi:10.3402/jom.v5i0.20015.

Allaker, R. P. & Douglas, C. W. I. (2009) Novel anti-microbial therapies for dental plaque-related diseases. *International Journal of Antimicrobial Agents* 33, 8–13.

Ashby, M. T., Kreth, J., Soundarajan, M. & Sivuilu, L. S. (2009) Influence of a model human defensive peroxidase system on oral streptococcal antagonism. *Microbiology* 155, 3691–3700.

Bachtiar, E. W., Bachtiar, B. M., Jarosz, L. M., Amir, L. R., Sunarto, H., Ganin, H., Meijler, M. M. & Krom, B. P. (2014) Ai-2 of *Aggregatibacter actinomycetemcomitans* inhibits *Candida albicans* biofilm formation. *Frontiers in Cellular and Infection Microbiology* 4, 94.

Bergeron, L. J. & Burne, R. A. (2001) Roles of fructosyltransferase and levanase-sucrase of *Actinomyces naeslundii* in fructan and sucrose metabolism. *Infection and Immunity* 69, 5395–5402.

Bowler, L. D., Zhang, Q. Y., Riou, J. Y. & Spratt, B. G. (1994) Interspecies recombination between the pena genes of *Neisseria meningitidis* and commensal *Neisseria* species during the emergence of penicillin resistance in *N. meningitidis*: natural events and laboratory simulation. *Journal of Bacteriology* 176, 333–337.

Bradshaw, D. J., Homer, K. A., Marsh, P. D. & Beighton, D. (1994) Metabolic cooperation in oral microbial communities during growth on mucin. *Microbiology* 140, 3407–3412.

Bradshaw, D. J., McKee, A. S. & Marsh, P. D. (1989) Effects of carbohydrate pulses and pH on population shifts within oral microbial communities *in vitro*. *Journal of Dental Research* 68, 1298–1302.

Brown, S. A. & Whiteley, M. (2007) A novel exclusion mechanism for carbon resource partitioning in *Aggregatibacter actinomycetemcomitans*. *Journal of Bacteriology* 189, 6407–6414.

Burne, R. A. & Marquis, R. E. (2000) Alkali production by oral bacteria and protection against dental caries. *FEMS Microbiology Letters* 193, 1–6.

Cagetti, M. G., Mastroberardino, S., Milia, E., Cocco, F., Lingstrom, P. & Campus, G. (2013) The use of probiotic strains in caries prevention: a systematic review. *Nutrients* 5, 2530–2550.

Caldwell, D. E., Wolfaardt, G. M., Korber, D. R. & Lawrence, J. R. (1997). Do bacterial communities transcend darwinism? In: Jones, J. G. (ed.). *Advances in Microbial Ecology*, pp. 105–191, Boston, MA: Springer US.

Chi, F., Nolte, O., Bergmann, C., Ip, M. & Hakenbeck, R. (2007) Crossing the barrier: evolution and spread of a major class of mosaic pbp2x in *Streptococcus pneumoniae*, *S. mitis* and *S. oralis*. *International Journal of Medical Microbiology* 297, 503–512.

Curtis, M. A., Hanley, S. A. & Aduse-Opoku, J. (1999) The rag locus of *Porphyromonas gingivalis*: a novel pathogenicity island. *Journal of Periodontal Research* 34, 400–405.

Dalmasso, M., de Haas, E., Neve, H., Strain, R., Cousin, F. J., Stockdale, S. R., Ross, R. P. & Hill, C. (2015) Isolation of a novel phage with activity against *Streptococcus mutans* biofilms. *PLoS One* 10, e0138651.

Dashiff, A. & Kadouri, D. E. (2011) Predation of oral pathogens by *Bdellovibrio bacteriovorus* 109J. *Molecular Oral Microbiology* 26, 19–34.

Diaz, P. I. (2012) Microbial diversity and interactions in subgingival biofilm communities. *Frontiers of Oral Biology* 15, 17–40.

Diaz, P. I., Hoare, A. & Hong, B. Y. (2016) Subgingival microbiome shifts and community dynamics in periodontal diseases. *Journal of the California Dental Association* 44, 421–435.

Diaz, P. I., Strausbaugh, L. D. & Dongari-Bagtzoglou, A. (2014) Fungal-bacterial interactions and their relevance to oral health: linking the clinic and the bench. *Frontiers in Cellular and Infection Microbiology* 4: 101. doi:10.3389/fcimb.2014.00101.

Diaz, P. I., Xie, Z., Sobue, T., Thompson, A., Biyikoglu, B., Ricker, A., Ikonomou, L. & Dongari-Bagtzoglou, A. (2012) Synergistic interaction between *Candida albicans* and commensal oral streptococci in a novel *in vitro* mucosal model. *Infection and Immunity* 80, 620–632.

Diaz, P. I., Zilm, P. S. & Rogers, A. H. (2002) *Fusobacterium nucleatum* supports the growth of *Porphyromonas gingivalis* in oxygenated and carbon-dioxide-depleted environments. *Microbiology* 148, 467–472.

Dige, I., Gronkjar, L. & Nyvad, B. (2014) Molecular studies of the structural ecology of natural occlusal caries. *Caries Research* 48, 451–460.

Dige, I., Nilsson, H., Kilian, M. & Nyvad, B. (2007) In situ identification of streptococci and other bacteria in initial dental biofilm by confocal laser scanning microscopy and fluorescence in situ hybridization. *European Journal of Oral Sciences* 115, 459–467.

Ebersole, J. L. (2003) Humoral immune responses in gingival crevice fluid: local and systemic implications. *Periodontology 2000* 31, 135–166.

Edlund, A., Santiago-Rodriguez, T. M., Boehm, T. K. & Pride, D. T. (2015a) Bacteriophage and their potential roles in the human oral cavity. *Journal of Oral Microbiology* 7, 27423.

Edlund, A., Yang, Y., Yooseph, S., Hall, A. P., Nguyen, D. D., Dorrestein, P. C., Nelson, K. E., He, X., Lux, R., Shi, W. & McLean, J. S. (2015b) Meta-omics uncover temporal regulation of pathways across oral microbiome genera during *in vitro* sugar metabolism. *ISME Journal* 9, 2605–2619.

Eggert, F. M., Drewell, L., Bigelow, J. A., Speck, J. E. & Goldner, M. (1991) The pH of gingival crevices and periodontal pockets in children, teenagers and adults. *Archives of Oral Biology* 36, 233–238.

Ereshefsky, M. & Pedroso, M. (2015) Rethinking evolutionary individuality. *Proceedings of the National Academy of Sciences of the United States of America* 112, 10126–10132.

van Essche, M., Loozen, G., Godts, C., Boon, N., Pauwels, M., Quirynen, M. & Teughels, W. (2013) Bacterial antagonism against periodontopathogens. *Journal of Periodontology* 84, 801–811.

van Essche, M., Quirynen, M., Sliepen, I., Loozen, G., Boon, N., van Eldere, J. & Teughels, W. (2011) Killing of anaerobic pathogens by predatory bacteria. *Molecular Oral Microbiology* 26, 52–61.

Fedi, P. F. & Killoy, W. J. (1992) Temperature differences at periodontal sites in health and disease. *Journal of Periodontology* 63, 24–27.

Grenier, D. (1992) Nutritional interactions between two suspected periodontopathogens, *Treponema denticola* and *Porphyromonas gingivalis*. *Infection and Immunity* 60, 5298–5301.

Gross, E. L., Beall, C. J., Kutsch, S. R., Firestone, N. D., Leys, E. J. & Griffen, A. L. (2012) Beyond *Streptococcus mutans*: dental caries onset linked to multiple species by 16S rRNA community analysis. *PLoS One* 7, e47722.

Gruner, D., Paris, S. & Schwendicke, F. (2016) Probiotics for managing caries and periodontitis: systematic review and meta-analysis. *Journal of Dentistry* 48, 16–25.

Guo, L., He, X. & Shi, W. (2014) Intercellular communications in multispecies oral microbial communities. *Frontiers in Microbiology* 5, 328.

Haffajee, A. D., Socransky, S. S. & Goodson, J. M. (1992) Subgingival temperature (i). Relation to baseline clinical parameters. *Journal of Clinical Periodontology* 19, 401–408.

Hajishengallis, G. (2014) Immunomicrobial pathogenesis of periodontitis: keystones, pathobionts, and host response. *Trends in Immunology* 35, 3–11.

Hajishengallis, G. & Lamont, R. J. (2012) Beyond the red complex and into more complexity: the polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. *Molecular Oral Microbiology* 27, 409–419.

Hajishengallis, G. & Lamont, R. J. (2014) Breaking bad: manipulation of the host response by *Porphyromonas gingivalis*. *European Journal of Immunology* 44, 328–338.

Hajishengallis, G., Liang, S., Payne, M. A., Hashim, A., Jotwani, R., Eskan, M. A., McIntosh, M. L., Alsam, A., Kirkwood, K. L., Lambris, J. D., Darveau, R. P. & Curtis, M. A. (2011) Low-abundance biofilm species orchestrates inflammatory periodontal disease through the commensal microbiota and complement. *Cell Host & Microbe* 10, 497–506.

Hannan, S., Ready, D., Jasni, A. S., Rogers, M., Pratten, J. & Roberts, A. P. (2010) Transfer of antibiotic resistance by transformation with eDNA within oral biofilms. *FEMS Immunology and Medical Microbiology* 59, 345–349.

He, X., McLean, J. S., Guo, L., Lux, R. & Shi, W. (2014) The social structure of microbial community involved in colonization resistance. *ISME Journal* 8, 564–574.

Hillman, J. D. & Shivers, M. (1988) Interaction between wild-type, mutant and revertant forms of the bacterium *Streptococcus sanguis* and the bacterium *Actinobacillus actinomycetemcomitans* in vitro and in the gnotobiotic rat. *Archives of Oral Biology* 33, 395–401.

Hillman, J. D., Socransky, S. S. & Shivers, M. (1985) The relationships between streptococcal species and periodontopathic bacteria in human dental plaque. *Archives of Oral Biology* 30, 791–795.

van der Hoeven, J. S., Toorop, A. I. & Mikx, R. H. (1978) Symbiotic relationship of *Veillonella alcalescens* and *Streptococcus mutans* in dental plaque in gnotobiotic rats. *Caries Research* 12, 142–147.

Hojo, K., Nagaoka, S., Ohshima, T. & Maeda, N. (2009) Bacterial interactions in dental biofilm development. *Journal of Dental Research* 88, 982–990.

Holmberg, K. & Hallander, H. O. (1972) Interference between gram-positive microorganisms in dental plaque. *Journal of Dental Research* 51, 588–595.

Holmberg, K. & Hallander, H. O. (1973) Production of bactericidal concentrations of hydrogen peroxide by *Streptococcus sanguis*. *Archives of Oral Biology* 18, 423–434.

Homer, K. A. & Beighton, D. (1992) Synergistic degradation of bovine serum albumin by mutans streptococci and other dental plaque bacteria. *FEMS Microbiology Letters* 69, 259–262.

Hong, B.-Y., Furtado Araujo, M. V., Strausbaugh, L. D., Terzi, E., Ioannidou, E. & Diaz, P. I. (2015) Microbiome profiles in periodontitis in relation to host and disease characteristics. *PLoS One* 10, e0127077.

Hoshino, E. & Araya, A. (1980) Lactate degradation by polysaccharide-producing *Neisseria* isolated from human dental plaque. *Archives of Oral Biology* 25, 211–212.

Huang, R., Li, M. & Gregory, R. L. (2011) Bacterial interactions in dental biofilm. *Virulence* 2, 435–444.

Huang, X., Schulte, R. M., Burne, R. A. & Nascimento, M. M. (2015) Characterization of the arginolytic microflora provides insights into ph homeostasis in human oral biofilms. *Caries Research* 49, 165–176.

Jack, A. A., Daniels, D. E., Jepson, M. A., Vickerman, M. M., Lamont, R. J., Jenkinson, H. F. & Nobbs, A. H. (2015) *Streptococcus gordonii* comCDE (competence) operon modulates biofilm formation with *Candida albicans*. *Microbiology* 161, 411–421.

Jakubovics, N. S. (2015a) Intermicrobial interactions as a driver for community composition and stratification of oral biofilms. *Journal of Molecular Biology* 427, 3662–3675.

Jakubovics, N. S. (2015b) Saliva as the sole nutritional source in the development of multispecies communities in dental plaque. *Microbiology Spectrum* 3, doi: 10.1128/microbiolspec.MBP-0013-2014.

Jakubovics, N. S. & Burgess, J. G. (2015) Extracellular DNA in oral microbial biofilms. *Microbes and Infection* 17, 531–537.

Jakubovics, N. S., Yassin, S. A. & Rickard, A. H. (2014) Community interactions of oral streptococci. *Advances in Applied Microbiology* 87, 43–110.

Jang, Y.-J., Sim, J., Jun, H.-K. & Choi, B.-K. (2013) Differential effect of autoinducer 2 of *Fusobacterium nucleatum* on oral streptococci. *Archives of Oral Biology* 58, 1594–1602.

Jepsen, K. & Jepsen, S. (2016) Antibiotics/antimicrobials: systemic and local administration in the therapy of mild to moderately advanced periodontitis. *Periodontology 2000* 71, 82–112.

Jiang, W., Jiang, Y., Li, C. & Liang, J. (2011) Investigation of supragingival plaque microbiota in different caries status of chinese preschool children by denaturing gradient gel electrophoresis. *Microbial Ecology* 61, 342–352.

Kang, M.-S., Oh, J.-S., Lee, H.-C., Lim, H.-S., Lee, S.-W., Yang, K.-H., Choi, N.-K. & Kim, S.-M. (2011) Inhibitory effect of *Lactobacillus reuteri* on periodontopathic and cariogenic bacteria. *Journal of Microbiology* 49, 193–199.

Kirst, M. E., Li, E. C., Alfant, B., Chi, Y.-Y., Walker, C., Magnusson, I. & Wang, G. P. (2015) Dysbiosis and alterations in predicted functions of the subgingival microbiome in chronic periodontitis. *Applied and Environment Microbiology* 81, 783–793.

Kistler, J. O., Booth, V., Bradshaw, D. J. & Wade, W. G. (2013) Bacterial community development in experimental gingivitis. *PLoS One* 8, e71227.

Kolenbrander, P. E. (2011) Multispecies communities: interspecies interactions influence growth on saliva as sole nutritional source. *International Journal of Oral Science* 3, 49–54.

Koo, H., Falsetta, M. L. & Klein, M. I. (2013) The exopolysaccharide matrix: a virulence determinant of cariogenic biofilm. *Journal of Dental Research* 92, 1065–1073.

Kreth, J., Giacaman, R. A., Raghavan, R. & Merritt, J. (2016) The road less traveled – defining molecular commensalism with *Streptococcus sanguinis*. *Molecular Oral Microbiology* doi: 10.1111/omi.12170.

Kreth, J., Merritt, J., Shi, W. & Qi, F. (2005) Coordinated bacteriocin production and competence development: a possible mechanism for taking up DNA from neighbouring species. *Molecular Microbiology* 57, 392–404.

Lewis, J. P., Iyer, D. & Anaya-Bergman, C. (2009) Adaptation of *Porphyromonas gingivalis* to microaerophilic conditions involves increased consumption of formate and reduced utilization of lactate. *Microbiology* 155, 3758–3774.

Li, Y., Ge, Y., Saxena, D. & Caufield, P. W. (2007) Genetic profiling of the oral microbiota associated with severe early-childhood caries. *Journal of Clinical Microbiology* 45, 81–87.

Liu, J., Wu, C., Huang, I.-H., Merritt, J. & Qi, F. (2011) Differential response of *Streptococcus mutans* towards friend and foe in mixed-species cultures. *Microbiology* 157, 2433–2444.

Liu, Y.-L., Nascimento, M. & Burne, R. A. (2012) Progress toward understanding the contribution of alkali generation in dental biofilms to inhibition of dental caries. *International Journal of Oral Science* 4, 135–140.

Lloyd-Price, J., Abu-Ali, G. & Huttenhower, C. (2016) The healthy human microbiome. *Genome Medicine* 8, 51.

Loozen, G., Boon, N., Pauwels, M., Slomka, V., Rodrigues Herrero, E., Quirynen, M. & Teughels, W. (2015) Effect of *Bdellovibrio bacteriovorus* HD100 on multispecies oral communities. *Anaerobe* 35, 45–53.

Mark Welch, J. L., Rossetti, B. J., Rieken, C. W., Dewhirst, F. E. & Borisy, G. G. (2016) Biogeography of a human oral microbiome at the micron scale. *Proceedings of the National Academy of Sciences of the United States of America* 113, E791–E800.

Marsh, P. (1989) Host defenses and microbial homeostasis: role of microbial interactions. *Journal of Dental Research* 68, 1567–1575.

Marsh, P. D. (1994) Microbial ecology of dental plaque and its significance in health and disease. *Advances in Dental Research* 8, 263–271.

Marsh, P. D. (2003) Are dental diseases examples of ecological catastrophes? *Microbiology* 149, 279–294.

Marsh, P. D., McKee, A. S. & Mothershaw, A. S. (1993) Continuous culture studies of *Porphyromonas gingivalis*. In: Shah, H. N., Mayrand, D. & Genco, R. J. (eds). *Biology of the Species Porphyromonas gingivalis*, pp. 105–123, Boca Raton, FL: CRC Press.

Mazumdar, V., Amar, S. & Segrè, D. (2013) Metabolic proximity in the order of colonization of a microbial community. *PLoS One* 8, e77617.

Merritt, J. & Qi, F. (2012) The mutacins of *Streptococcus mutans*: regulation and ecology. *Molecular Oral Microbiology* 27, 57–69.

Moye, Z. D., Zeng, L. & Burne, R. A. (2014) Fueling the caries process: carbohydrate metabolism and gene regulation by *Streptococcus mutans*. *Journal of Oral Microbiology* **6**, doi: 10.3402/jom.v6.24878.

Mysak, J., Podzimek, S., Sommerova, P., Lyuya-Mi, Y., Bartova, J., Janatova, T., Prochazkova, J. & Duskova, J. (2014) *Porphyromonas gingivalis*: major periodontopathic pathogen overview. *Journal of Immunology Research* **2014**, 476068.

Naidu, M., Robles-Sikisaka, R., Abeles, S. R., Boehm, T. K. & Pride, D. T. (2014) Characterization of bacteriophage communities and CRISPR profiles from dental plaque. *BMC Microbiology* **14**, 175.

Ng, H. M., Kin, L. X., Dashper, S. G., Slakeski, N., Butler, C. A. & Reynolds, E. C. (2016) Bacterial interactions in pathogenic subgingival plaque. *Microbial Pathogenesis* **94**, 60–69.

Nobbs, A. H. & Jenkinson, H. F. (2015) Interkingdom networking within the oral microbiome. *Microbes and Infection* **17**, 484–492.

Nyako, E. A., Watson, C. J. & Preston, A. J. (2005) Determination of the ph of peri-implant crevicular fluid in successful and failing dental implant sites: a pilot study. *Archives of Oral Biology* **50**, 1055–1059.

Okuda, T., Kokubu, E., Kawana, T., Saito, A., Okuda, K. & Ishihara, K. (2012) Synergy in biofilm formation between *Fusobacterium nucleatum* and *Prevotella* species. *Anaerobe* **18**, 110–116.

Olczak, T., Simpson, W., Liu, X. & Genco, C. A. (2005) Iron and heme utilization in *Porphyromonas gingivalis*. *FEMS Microbiology Reviews* **29**, 119–144.

Olsen, I., Tribble, G. D., Fiehn, N.-E. & Wang, B.-Y. (2013) Bacterial sex in dental plaque. *Journal of Oral Microbiology* **5**, doi: 10.3402/jom.v5i0.20736.

Park, O.-J., Yi, H., Jeon, J. H., Kang, S.-S., Koo, K.-T., Kum, K.-Y., Chun, J., Yun, C.-H. & Han, S. H. (2015) Pyrosequencing analysis of subgingival microbiota in distinct periodontal conditions. *Journal of Dental Research* **94**, 921–927.

Pérez-Chaparro, P. J., Gonçalves, C., Figueiredo, L. C., Faveri, M., Lobão, E., Tamashiro, N., Duarte, P. & Feres, M. (2014) Newly identified pathogens associated with periodontitis: a systematic review. *Journal of Dental Research* **93**, 846–858.

Peterson, S. N., Snesrud, E., Liu, J., Ong, A. C., Kilian, M., Schork, N. J. & Bretz, W. (2013) The dental plaque microbiome in health and disease. *PLoS ONE* **8**, e58487.

Petersen, F. C., Tao, L. & Scheie, A. A. (2005) DNA binding-uptake system: a link between cell-to-cell communication and biofilm formation. *Journal of Bacteriology* **187**, 4392–4400.

Poulsen, K., Reinholdt, J., Jespersgaard, C., Boye, K., Brown, T. A., Hauge, M. & Kilian, M. (1998) A comprehensive genetic study of streptococcal immunoglobulin A1 proteases: evidence for recombination within and between species. *Infection and Immunity* **66**, 181–190.

Pride, D. T., Salzman, J., Haynes, M., Rohwer, F., Davis-Long, C., White, R. A. III, Loomer, P., Armitage, G. C. & Relman, D. A. (2012) Evidence of a robust resident bacteriophage population revealed through analysis of the human salivary virome. *ISME Journal* **6**, 915–926.

Rams, T. E., Degener, J. E. & van Winkelhoff, A. J. (2013) Prevalence of *β*-lactamase-producing bacteria in human periodontitis. *Journal of Periodontal Research* **48**, 493–499.

Rickard, A. H., Palmer, R. J., Blehert, D. S., Campagna, S. R., Semmelhack, M. F., Egland, P. G., Bassler, B. L. & Kolenbrander, P. E. (2006) Autoinducer 2: a concentration-dependent signal for mutualistic bacterial biofilm growth. *Molecular Microbiology* **60**, 1446–1456.

Roberts, A. P. & Kreth, J. (2014) The impact of horizontal gene transfer on the adaptive ability of the human oral microbiome. *Frontiers in Cellular and Infection Microbiology* **4**, 124.

Roberts, F. A. & Darveau, R. P. (2015) Microbial protection and virulence in periodontal tissue as a function of polymicrobial communities: symbiosis and dysbiosis. *Periodontology 2000* **69**, 18–27.

Rudney, J. D., Jagtap, P. D., Reilly, C. S., Chen, R., Markowski, T. W., Higgins, L., Johnson, J. E. & Griffin, T. J. (2015) Protein relative abundance patterns associated with sucrose-induced dysbiosis are conserved across taxonomically diverse oral microcosm biofilm models of dental caries. *Microbiome* **3**, 69.

Ryan, C. S. & Kleinberg, I. (1995) Bacteria in human mouths involved in the production and utilization of hydrogen peroxide. *Archives of Oral Biology* **40**, 753–763.

Schincaglia, G. P., Hong, B. Y., Rosania, A., Barasz, J., Thompson, A., Sobue, T., Panagakos, F., Burleson, J. A., Dongari-Bagtzoglou, A. & Diaz, P. I. (2017) Clinical, immune, and microbiome traits of gingivitis and peri-implant mucositis. *Journal of Dental Research* **96**, 47–55.

Scofield, J. A. & Wu, H. (2015) Oral streptococci and nitrite-mediated interference of *Pseudomonas aeruginosa*. *Infection and Immunity* **83**, 101–107.

Scofield, J. A. & Wu, H. (2016) Nitrite reductase is critical for *Pseudomonas aeruginosa* survival during co-infection with the oral commensal *Streptococcus parasanguinis*. *Microbiology* **162**, 376–383.

Senadheera, D. & Cvitkovitch, D. G. (2008) Quorum sensing and biofilm formation by *Streptococcus mutans*. *Advances in Experimental Medicine and Biology* **631**, 178–188.

Sharma, A., Inagaki, S., Sigurdson, W. & Kuramitsu, H. K. (2005) Synergy between *Tannerella forsythia* and *Fusobacterium nucleatum* in biofilm formation. *Oral Microbiology and Immunology* **20**, 39–42.

Shu, M., Browngardt, C. M., Chen, Y.-Y. M. & Burne, R. A. (2003) Role of urease enzymes in stability of a 10-species oral biofilm consortium cultivated in a constant-depth film fermenter. *Infection and Immunity* **71**, 7188–7192.

Simón-Soro, A., Guillen-Navarro, M. & Mira, A. (2014) Metatranscriptomics reveals overall active bacterial composition in caries lesions. *Journal of Oral Microbiology* **6**, doi: 10.3402/jom.v6.25443.

Slaney, J. M. & Curtis, M. A. (2008) Mechanisms of evasion of complement by *Porphyromonas gingivalis*. *Frontiers in Bioscience* **13**, 188–196.

Stacy, A., Everett, J., Jorth, P., Trivedi, U., Rumbaugh, K. P. & Whiteley, M. (2014) Bacterial fight-and-flight responses enhance virulence in a polymicrobial infection. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 7819–7824.

ter Steeg, P. F. & van der Hoeven, J. S. (1989) Development of periodontal microflora on human serum. *Microbial Ecology in Health and Disease* **2**, 1–10.

ter Steeg, P. F., van der Hoeven, J. S., de Jong, M. H., van Munster, P. J. & Jansen, M. J. (1987) Enrichment of subgingival microflora on human serum leading to accumulation of Bacteroides species, peptostreptococci and Fusobacteria. *Antonie van Leeuwenhoek* **53**, 261–272.

van Steenbergen, T. J., van Winkelhoff, A. J. & de Graaff, J. (1984) Pathogenic synergy: mixed infections in the oral cavity. *Antonie van Leeuwenhoek* **50**, 789–798.

Sukumar, S., Roberts, A. P., Martin, F. E. & Adler, C. J. (2016) Metagenomic insights into transferable antibiotic resistance in oral bacteria. *Journal of Dental Research* **95**, 969–976.

Takahashi, N. (2015) Oral microbiome metabolism: from "who are they?" to "what are they doing?". *Journal of Dental Research* **94**, 1628–1637.

Takahashi, N. & Nyvad, B. (2008) Caries ecology revisited: microbial dynamics and the caries process. *Caries Research* **42**, 409–418.

Takahashi, N. & Yamada, T. (1996) Catabolic pathway for aerobic degradation of lactate by *Actinomyces naeslundii*. *Oral Microbiology and Immunology* **11**, 193–198.

Tan, K. H., Seers, C. A., Dashper, S. G., Mitchell, H. L., Pyke, J. S., Meuric, V., Slakeski, N., Cleal, S. M., Chambers, J. L., McConville, M. J. & Reynolds, E. C. (2014) *Porphyromonas gingivalis* and *Treponema denticola* exhibit metabolic symbioses. *PLoS Pathogens* **10**, e1003955.

Teughels, W., Durukan, A., Ozcelik, O., Pauwels, M., Quirynen, M. & Haytac, M. C. (2013) Clinical and microbiological effects of *Lactobacillus reuteri* probiotics in the treatment of chronic periodontitis: a randomized placebo-controlled study. *Journal of Clinical Periodontology* **40**, 1025–1035.

Traudt, M. & Kleinberg, I. (1996) Stoichiometry of oxygen consumption and sugar, organic acid and amino acid utilization in salivary sediment and pure cultures of oral bacteria. *Archives of Oral Biology* **41**, 965–978.

Vartoukian, S. R., Adamowska, A., Lawlor, M., Moazzez, R., Dewhirst, F. E. & Wade, W. G. (2016a) Cultivation of 'unculturable' oral bacteria, facilitated by community culture and media supplementation with siderophores. *PLoS One* **11**, e0146926.

Vartoukian, S. R., Moazzez, R. V., Paster, B. J., Dewhirst, F. E. & Wade, W. G. (2016b) First cultivation of health-associated *Tannerella* sp. Hot-286 (BU063). *Journal of Dental Research* **95**, 1308–1313.

Wade, W. G. (2013) The oral microbiome in health and disease. *Pharmacological Research* **69**, 137–143.

Walker, C. B., Tyler, K. Z., Low, S. B. & King, C. J. (1987) Penicillin-degrading enzymes in sites associated with adult periodontitis. *Oral Microbiology and Immunology* **2**, 129–131.

Wang, B.-Y., Deutch, A., Hong, J. & Kuramitsu, H. K. (2011) Proteases of an early colonizer can hinder *Streptococcus mutans* colonization *in vitro*. *Journal of Dental Research* **90**, 501–505.

Wang, J., Gao, Y. & Zhao, F. (2016) Phage-bacteria interaction network in human oral microbiome. *Environmental Microbiology* **18**, 2143–2158.

Wei, G. X., van der Hoeven, J. S., Smalley, J. W., Mikx, F. H. & Fan, M. W. (1999) Proteolysis and utilization of albumin by enrichment cultures of subgingival microbiota. *Oral Microbiology and Immunology* **14**, 348–351.

van Winkelhoff, A. J., Winkel, E. G., Barendregt, D., Dellemijn-Kippuw, N., Stijne, A. & van der Velden, U. (1997) B-lactamase producing bacteria in adult periodontitis. *Journal of Clinical Periodontology* **24**, 538–543.

Wright, C. J., Burns, L. H., Jack, A. A., Back, C. R., Dutton, L. C., Nobbs, A. H., Lamont, R. J. & Jenkinson, H. F. (2013) Microbial interactions in building of communities. *Molecular Oral Microbiology* **28**, 83–101.

Xu, H., Jenkinson, H. F. & Dongari-Bagtzoglou, A. (2014) Innocent until proven guilty: mechanisms and roles of *Streptococcus-Candida* interactions in oral health and disease. *Molecular Oral Microbiology* **29**, 99–116.

Yost, S., Duran-Pinedo, A. E., Teles, R., Krishnan, K. & Frias-Lopez, J. (2015) Functional signatures of oral dysbiosis during periodontitis progression revealed by microbial metatranscriptome analysis. *Genome Medicine* **7**, 27.

Zeng, L., Farivar, T. & Burne, R. A. (2016) Amino sugars enhance the competitiveness of beneficial commensals with *Streptococcus mutans* through multiple mechanisms. *Applied and Environment Microbiology* **82**, 3671–3682.

Zijnge, V., van Leeuwen, M. B. M., Degener, J. E., Abbas, F., Thurnheer, T., Gmür, R. & Harmsen, H. J. M. (2010) Oral biofilm architecture on natural teeth. *PLoS One* **5**, e9321.

Supporting Information

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

Table S1. PubMed query search terms and results.

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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.