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Role of microbial communities in the pathogenesis of periodontal diseases and caries

Alex Mira[1], A. Simon-Soro[1] and M. A. Curtis[2]

[1]Department of Health and Genomics, Center for Advanced Research in Public Health, FISABIO Foundation, Valencia, Spain; [2]Institute of Dentistry, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK

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Abstract

: The microbiological characteristics of both caries and periodontal disease show significant change from those in health. In both instances, there is evidence of co-association of different organisms into consortia.

Aim: We review and summarize a number of issues pertinent to the community organization and functional activity of the bacterial populations resident on supra- and subgingival tooth surface and the influence of these populations on disease.

Methods: A literature review was undertaken with a particular emphasis on recent publications involving high-throughput, deep sequencing approaches to the analysis of microbial populations and their functional activity.

Results: There is increasing evidence to suggest that both caries and periodontal disease represent dysbiotic states of the oral microbiome. The mode of acquisition of the oral microbial communities may be less passive than previously recognized but once established remains relatively stable within an individual although there are very significant site variations. A repertoire of stable dysbiotic states may occur in both caries and periodontitis involving different microbial community structures with potentially similar functional properties.

Conclusions: The processes which underlie the development and stability of microbial populations in the healthy mouth are fundamental to understanding how these populations are transformed into a dysbiotic state in disease.

Key words: caries; microbial communities; oral microbiome; periodontal disease

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The biofilms on the supra- and subgingival surfaces of the teeth are composed of complex microbial communities which have evolved to inhabit these specialized oral environments. The complexity of these communities was evident from the first time they were subject to scientific evaluation some 350 years ago by Antonie van Leeuwenhoek using the very first microscopes and is increasingly appreciated as the current methods of high-throughput and culture independent molecular methods are applied. Sequence analysis of 16S ribosomal RNA is one of the most recent and most powerful of these investigative techniques because of the universal presence of the 16S rRNA gene in all bacteria, flexibility of use to describe either all the species present in a given sample or to target specific genera and finally the speed and cost efficiency of the approach. Its application has led to the description of 11 phyla in the domain Bacteria in the oral microbiome in addition to methanogenic species of the Methanobrevibacter genus from the domain Archaea. The phyla of the domain Bacteria that are reliably present include Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes, Tenericutes, Fusobacteria, Proteobacteria,

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Spirochaetes, Synergistetes and two currently unnamed phyla, SR1 and TM7. Several hundred, distinct species are contained within these divisions representing the highly diverse microbial communities of the mouth (Human Microbiome Project Consortium 2012). The periodontal microbiota is particularly heterogeneous, and over 400 species have been described in this habitat alone using a 16S rRNA amplification, cloning and Sanger sequencing approach (Dewhirst et al. 2010). When clonal variation within a bacterial species is also taken into account, the composition of these bacterial communities attains an even higher degree of complexity. In addition, the fungal oral communities have recently been shown to be very diverse and go well beyond the classically reported presence of *Candida* species (Ghannoum et al. 2010). Fungi have a close interplay with bacteria, and some authors consider this interplay beneficial in the maintenance of oral homoeostasis (Krom et al. 2014). However, this manuscript focuses on the role of bacterial communities, for which information is more complete.

The evolutionary adaptations developed by individual microbes within these highly diverse communities on the supra- and subgingival tooth surfaces will include: the ability to adhere; to gain nutrients from the environment for catabolic and anabolic needs; to evade elimination by microbe and host mediated offensive strategies; and to disperse to new environments within and between individuals. However, given that the overriding characteristic of life in these oral habitats is in the context of a community organizational structure, it is likely that many of these essential parameters of survival will be met, either in whole or in part through a web of community interactions. The cooperative degradation (and subsequent consumption) of complex macromolecules by the concerted and sequential activity of enzymes produced by different microorganisms and the potential protection from host derived antimicrobials such as host defence peptides and complement by the extracellular proteolytic enzyme activity of a neighbouring member of the consortium (Bradshaw et al.1994, Lamont & Hajishengallis 2015) are two well-described examples. More detailed descriptions of these cooperative and mutually beneficial microbial interactions are described in the paper by Zaura and Marsh (2017, this issue).

Against this background of a highly evolved, highly complex and highly cooperative microbial society, it is pertinent to ask what is the role played by microbial communities in the pathogenesis of the two most prevalent diseases at these sites in the mouth: dental caries and periodontal disease? It is worth noting at this point that the overwhelming majority of studies of the pathogenesis of these conditions using in vitro and animal models and the interpretation of human studies of the microbiology in health versus disease have taken a reductionist approach: the pathological entity is a single organism or group of organisms acting independently rather than a coordinated, inter-dependent consortium.

In this study, we review and summarize a number of issues pertinent to the community organization and functional activity of the bacterial populations resident on the supra- and subgingival tooth surface and the influence of these populations on disease. In the first instance, a description of the microbiology of both diseases is presented with particular focus on more recent non-cultivation high-throughput approaches. The process of acquisition of the oral microbiome is examined as well as the temporal appearance and potential source(s) of some of the community components that are frequently associated with the two diseased states. Recent data which challenge the notion that acquisition is an entirely passive event are also presented. Next, the stability of these established bacterial communities in health is described with particular reference to the comparative resilience of microbial populations at other sites of the human body. Given that a characteristic of both diseases is their site specificity, the site variation of the bacterial communities both supra- and subgingivally within a single individual is also reviewed. To understand the maintenance of health and the pathogenesis of disease at the population level, the inter-individual variation in microbial community structures is also discussed.

These preceding descriptions aim to provide a contextual background against which to rationalize the well-known shifts in microbial population structure which occur co-incident with the alteration from health to disease in both caries and periodontal disease as well as the potential importance of these shifts for diagnostic and potentially therapeutic purposes. Finally, this work questions whether the changes in microbial population structure that are so characteristic of both diseases are actually responsible for disease initiation and progression as opposed to inevitable outcomes of the altered environments that clearly pertain to a carious lesion or an inflamed periodontal pocket. Is dysbiosis of the microbial communities of the mouth simply a consequence rather than a cause of these diseases?

Characteristics of the Microbiology of Caries and Periodontal Disease

The microbiology of caries

Perhaps influenced by the specific aetiology of most infectious diseases, the characterization of dental caries causing agents was reduced for decades to mutans streptococci, especially *Streptococcus mutans* and *S. sobrinus* (the latter first described by Louis Pasteur). The epidemiological evidence for their association with the disease, its acidogenicity and aciduricity is extensive (Loesche 1986), and in fact, most preventive strategies against the disease, including immunization approaches, were aimed at these bacteria (Zhang 2014). However, this mutans-centric paradigm was challenged when other acidogenic bacterial species were isolated from carious lesions and found to be strongly associated with the disease. These included *Bifidobacterium* (Mantzourani et al. 2009), *Lactobacillus* (Badet & Thebaud 2008) and *Scardovia wiggsiae*, the latter associated with early childhood caries (Tanner et al. 2011). In addition, a sensitive test like PCR amplification failed to detect *S. mutans* in a significant proportion of cavities (Aas et al. 2008). However, PCR amplification of DNA from

carious lesions confirmed the presence of many other bacterial species, like *Atopobium*, *Prevotella* or *Corynebacterium*, among others (Aas et al. 2008, Gross et al. 2012, Torlakovic et al. 2012, Simón-Soro et al. 2013). Metagenomic studies in which DNA from carious lesions was directly sequenced without any cloning or PCR step also revealed a complex community, including different species of non-mutans streptococci as well as bacteria like *Veillonella* or *Capnocytophaga*, which appeared to be at high proportions in some cases (Belda-Ferre et al. 2012, Simón-Soro et al. 2013). Thus, a new picture emerged in which complex bacterial communities appeared to be associated with the disease. The estimated number of bacterial species in supragingival dental plaque on teeth sound surfaces reached values of 500–600, whereas in dentin cavities decreased to 200 and this diversity dropped on average to 125 in non-cavitated enamel "white-spot" lesions (Simón-Soro et al. 2013). Thus, although bacterial diversity was considerably reduced under disease conditions, probably due to the highly acidic environment, the number of bacteria found in caries lesions was consistently large, suggesting a polymicrobial aetiology.

Given that DNA-based molecular methods like PCR can potentially detect inactive or even dead microorganisms, the application of RNA-based techniques was crucial to confirm the presence of complex microbial communities in caries lesions. RNA is highly unstable and has a short half life and therefore can only be detected if bacteria are alive and actively growing (Amann et al. 1995). RNA-based PCR studies identified again dozens of bacterial species on individual enamel or dentin caries lesions (Simón-Soro et al. 2014, Simón-Soro & Mira 2015), confirming the activity of many bacteria that could have a joined or even synergistic effect. When total RNA was sequenced by a metatranscriptomics approach, the gene expression profiles of individual bacteria could be identified, revealing a complex pattern of bacterial activity (Peterson et al. 2014, Do et al. 2015).

The concept of within-individual variability extends to the various diseases and disease processes which have a different clinical terminology, but for which specific microbiological features must be described. Different carious sites, for instance, probably imply different microbial niches, and the same can be true for cases of chronic and aggressive periodontitis, or those exacerbated by systemic causes. For example, in root caries, where enamel is absent, a different bacterial composition has been described, with *Actinomyces viscosus* or *Propionibacterium acidifaciens* being some of the microorganisms proposed to be associated with its aetiology (Nyvad & Kilian 1990, Hashimoto et al. 2011, Chen et al.2015). Furthermore, when different caries lesions are sampled, dramatic differences in bacterial composition are found, both by DNA- and RNA-based methods (Gross et al. 2012, Jiang et al. 2013, Simón-Soro et al. 2014), indicating that caries aetiology is not only polymicrobial but also extremely variable among individuals. In addition, when different caries lesions have been sampled from the same individual, bacterial composition has also been shown to vary significantly (Simón-Soro et al. 2014). This highly variable polymicrobial feature of oral diseases makes the development of vaccines against oral diseases extremely difficult, as a single immunization target cannot be identified (Simón-Soro & Mira 2015).

Nevertheless, some patterns are starting to emerge, and several bacteria appear to be associated with specific lesions or stages. Lactobacilli, for instance, were initially associated with advanced stages of the disease (Shah et al. 2011), a feature that has been confirmed by molecular methods, which failed to detect lactobacilli in non-cavitated enamel lesions and found this genus to be associated with dentin cavities (Simón-Soron et al. 2013, Jiang et al. 2014). When present, lactobacilli appear to be extremely dominant in the lesion, perhaps be displacing other species (Obata et al. 2014). In some cases, a few species of *Lactobacillus* accounted for 99% of the lesion activity (Simón-Soro & Mira 2015). Except for these cases, different caries lesions appear to harbour different bacterial consortia, making it extremely hard to identify a concrete "core" of caries causing agents. This disagrees with the specific-plaque hypothesis of dental caries (Emilson & Krasse 1985) and supports a view of a non-specific community associated with the disease (Theilade 1986) where an ecological change triggered by external factors like sugar promotes the dysbiosis (Marsh 2003, Takahashi & Nyvad 2011). Nevertheless, when bacterial composition profiles in different dentin cavities were compared, Obata et al. (2014) detected different "clusters" of bacteria. One was dominated by *Lactobacillus* spp., whereas when lactobacilli were absent, a consortia of *Propionibacterium*, *Atopobium* and *Prevotella* could be identified in several lesions. Thus, the co-occurrence of different bacterial species that may form part of stable consortia cannot be discarded and more work should be directed in the future to identify and characterize those potential cariogenic consortia.

Given the strong evidence linking mutans streptococci to caries, it was surprising to find that *S. mutans* accounted for <1% of the total caries lesion community in both DNA- (Gross et al. 2012) and RNA-based studies (Simón-Soro et al. 2014). However, microbial ecology studies highlight that minorities should not be underestimated, as bacteria found at small proportions could have a vital role in the community. In periodontitis, for instance, the presence of *P. gingivalis* at <1% of the total has been shown to be sufficient to develop the disease, by eliciting an immune response in the host that allows the establishment of dysbiotic community of the normally benign commensal community which ultimately are responsible for the intense inflammation (Darveau et al. 2012). Thus, although gum disease is the outcome of a microbial dysbiosis, *P. gingivalis* has been proposed to be a "keystone" pathogen whose activity is necessary for the development of the disease (Hajishengallis et al. 2012). The role of *S. mutans* as a keystone caries pathogen is more difficult to imagine given the inert nature of the tooth surface, the only region of the oral cavity without a constitutive mucosal immune response. Nevertheless, its potential role in the initiation of ecological

change by promoting acidification or adherence and triggering the dysbiotic state cannot be discarded. From an applied viewpoint, the dramatic variation in caries-associated consortia suggests that traditional antimicrobial strategies may not be effective and new approaches directed towards restoration of the ecological balance of dental plaque have been proposed (Fejerskov 2004, Marsh et al. 2015).

The microbiology of periodontal disease

Our understanding of the defining features of the microbiology of periodontal disease has similarly evolved. Nonetheless, there are some important principles which appear irrefutable. These principles can be summarized as the 3Cs of the microbiology of periodontal disease – **C**ommunity change, microbial **C**omplexes and **C**ommensal involvement.

First, the qualitative and quantitative composition of the microbial populations in periodontal disease represents community-wide changes to the composition in health. These changes represent global shifts in the overall population structure and include the emergence of organisms not normally encountered in health and a disappearance or reduction in others. The cultivation studies of Moore and colleagues some thirty years ago (Moore et al. 1982) provide a convincing demonstration of this principle. In this investigation of the bacteriology of severe generalized periodontitis in 21 individuals, they described the isolation and characterization by biochemical techniques of 2723 individual isolates representing 190 bacterial species, subspecies or serotypes. Of these, 11 species exceeded 1% of the subgingival flora and were most closely associated with disease and 11 others were also sufficiently frequently isolated to be deemed suspected agents of tissue destruction. This study highlights the marked difference between the overall microbiota in periodontally diseased, subgingival sites compared to the adjacent supragingival microbiota. Whilst the supragingival microbiota was dominated by the *Actinomyces* spp., *Streptococcus* spp and *Veillonella* spp., which comprised some 40% of the total cultivatable bacteria, the same genera represented only approximately 10% of the organisms at subgingival diseased sites. Conversely, members of the *Bacteroides* and *Fusobacteria* were present to approximately 20% of the subgingival microbiota but to only approximately 5% supragingivally.

This early work has been confirmed and re-emphasized by more recent high-throughput non-cultivation approaches (see e.g. Colombo et al. 2009). A recent systematic review of these studies (Pérez-Chaparro et al. 2014) concluded those organisms previously shown to be periodontitis-associated – *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Fusobacterium nucleatum, F. periodonticum, Prevotella intermedia, P. nigrescens, Parvimonas micra, Campylobacter gracilis, C. rectus, C. showae, Eubacterium nodatum, Streptococcus constellatus* and *Aggregatibacter actinomycetemcomitans* (Proceedings of the World Workshop 1996, Socransky et al. 1998, Teles et al. 2013) represented a far too restricted list. On review of 41 different studies, they provided evidence to support the potential involvement of 17 new additional candidate organisms based on their reported association with periodontitis in 3–5 independent investigations. These organisms include several members of bacterial phyla previously implicated (*Bacteroidetes, Firmicutes, Proteobacteria, Spirochaetes* and *Synergistetes*), a new phylum (*Candidatus Saccharibacteria)* and also members of the Archaea domain of life, which has not previously been associated with any infectious disease of humans. This seemingly endlessly increasing list of disease-associated organisms adds further weight to the notion that the changes in the microbiology of periodontal diseases represent a community-wide perturbation of the entire microbial population structure and function of subgingival plaque (Curtis 2014).

The second principle which has emerged from the study of microbial populations in periodontal disease is the clear co-association of different organisms in the microbiota in health and disease. This co-association, the tendency of some groups of organisms to be consistently detected together in the same sample, has been interpreted as the existence of consortia of bacterial species acting in concert with one another in mutualistically beneficial manner. The highly influential study of Socransky et al. (1998) analysed approximately 13,000 plaque samples from 185 subjects using whole genomic DNA probes to 40 bacterial species. Associations were sought among species using cluster analysis and community ordination techniques. One of the key and fundamentally important findings of this study, which has shaped our understanding of periodontal infections ever since, was the description of bacterial complexes, as opposed to individual bacterial species, that were associated with either periodontal health or periodontal disease. The complex most strongly associated with periodontal disease, the "red complex", was composed of three bacterial species which subsequently became the focus of intense investigation: *Porphyromonas gingivalis, Treponema denticola* and *Tannerella forsythia*. Other complexes, for example the yellow complex which comprised predominantly different streptococcal species, and the green complex which contained a preponderance of capnocytophaga species, represented early colonizers of dental plaque which were more closely associated with health. The orange complex contained those organisms generally considered to colonize dental plaque later: fusobacteria species, members of the prevotella and the campylobacter. More recent deep sequencing approaches have expanded these original observations (e.g. Griffen et al. 2012). In a recent study, Kirst et al. (2015) used 16S rRNA sequencing to survey the subgingival microbiota in 25 individuals with periodontal disease and 25 healthy controls. Through cluster analysis techniques, they demonstrated the presence of two predominant clusters. One cluster characterized by high levels of *Fusobacterium* and *Porphyromonas* bacterial species was strongly associated with the diseased population, whereas a second cluster, this time dominated by *Rothia* and streptococci was representative of periodontal health. Analogous to different gut enterotypes, based on the abundance of key bacteria in the

gut microbiota, the authors suggested that these discrete clusters in periodontal health and disease may represent different periodonto-types which differentiate between periodontal health and disease. Hence, as might be predicted from the metabolic and cooperative interactions described in the paper by Zaura and Marsh (2017), discrete consortia of bacterial species within the overall complex microbial population structure appear to be the norm in dental plaque in health and disease.

A third emerging principle concerns the contributory role of organisms normally considered to be commensal or health associated. The absolute requirement of the commensal microbiome in order for *P. gingivalis* to induce periodontal disease in the murine model (Hajishengallis et al. 2011) underscores the functional importance of the microbial community associated with health to the progress of disease. Furthermore, the results of transcriptional profiling of microbial samples from periodontally healthy versus diseased sites (Duran-Pinedo et al. 2014b) have demonstrated that the majority of potential virulence factors upregulated in human disease are derived not from the traditional group of periodontal bacteria but instead from those bacteria normally associated with health. This may be explained by the effect of altered environmental conditions or, potentially, by a direct effect of a periodontal bacterium such as *P. gingivalis* on the pattern of gene expression of neighbouring organisms as demonstrated in mixed biofilm in vitro studies (Frias-Lopez & Duran-Pinedo 2012, Duran-Pinedo et al. 2014a). Hence, our classification of an organism as a commensal, and therefore invariably in harmonious balance with the host, is almost certainly too rigid a definition. The key determinant to describe the association of any bacterium with health (or disease) is dictated by the functional activities produced by that cell at any given time – and these may change dependent upon the environmental context including the presence of other bacteria with the ability to manipulate the transcriptional profile of community neighbours (Darveau et al. 2012).

Acquisition of Oral Microbial Communities

Some understanding of the temporal acquisition of microbial populations and the development of the complex oral biofilms in the mouth (Gibbons 1989 Könönen et al. 1999) may be required in order to determine the susceptibility of these populations to dysbiosis. These steps in the development of the oral microbiome to a mature a stable health state are illustrated in Fig. 1.

The conventional view of bacterial transmission to the child involves initial "passive" transfer from the mother through the birth canal. This view has been confirmed by high-throughput sequencing data in children born by vaginal and caesarean delivery (Dominguez-Bello et al. 2010). The microbial composition of the gut of babies born via C-section resembled the mother's skin bacteria whereas babies born via conventional delivery had different communities with some similarity to the vaginal microbiota. In longitudinal studies of faecal samples, the initial bacterial composition at birth has been shown to influence the subsequent development of a healthy gut microbiota, where colonization by some key components of the microbiota, such as the *Bacteroides*, is significantly delayed in babies born by C-section (Rutayisire et al. 2016), and a similar process may occur in the oral cavity. For instance, using a DNA chip, three-month-old babies born by C-section were found to harbour a reduced bacterial diversity in the mouth (54 species) compared to vaginally delivered children (79 species), with some bacteria like *Slackia exigua* being exclusively found in the latter (Holgerson et al. 2011). Very recently, it has been shown that experimental oral exposure of babies born by C-section to maternal vaginal fluids increases the colonization of vaginal bacteria in the oral cavity, the gut and the skin of the babies, and partially restores their oral microbiota, which shifted towards that found in babies born by vaginal delivery (Dominguez-Bello et al. 2016). Given the higher epidemiological risk of immune-related diseases in children born by C-section (Huurre et al.

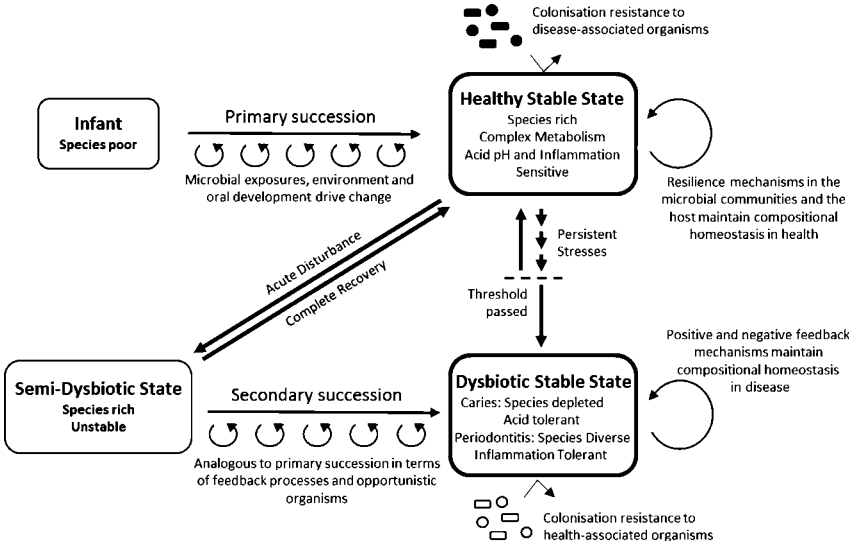

Fig. 1. Compositional transitions in the oral microbiome. The microbial communities of the mouth undergo compositional changes from birth and throughout development. Health is associated with a relatively stable community structure which is largely resilient to change by external stresses. Dysbiosis occurs when a threshold in the resilience mechanisms is overcome due to dietary influences in caries and predominantly, although not exclusively, host factors in periodontal disease. The dysbiotic state displays functional differences which contribute to disease and may also be resistant to compositional change. Acute changes may also occur in health leading to an unstable semi-dysbiotic state which can be reversed to the original healthy community structure or alternatively undergo further compositional change to a stable dysbiosis. Adapted from Lozupone CA, Stombaugh J, Gordon J, Jansson JK, Knight R. Nature. 2012 489(7415):220–230.

2008), together with the important role played by the immune system in oral diseases (Silva et al. 2015), it is not unreasonable to think that acquisition of an aberrant microbial composition at birth may have longer term consequences on the development of dysbiotic oral microbial communities.

After this initial colonization process, sequential development has traditionally been shown to be governed by saliva exchange and other potential environmental sources. This is supported by work showing relatedness of infant bacterial strains to family members or children caregivers from whom microorganisms may have been transmitted (Li & Caufield 1995), although this association has been shown to be weaker than initially expected (Cephas et al. 2011). In addition, the dramatic changes in the anatomy of oral tissues throughout childhood, the immune response and dietary changes have long been known to influence microbial composition. For instance, the eruption of teeth has been proposed to influence the colonization by microorganisms, especially those living on hard tissues, and an increase in bacterial diversity has been observed at this point (Rotimi & Duerden 1981, Brailsford et al. 2005). To these environmental parameters, we have to add the potential impact of prosthodontic/orthodontic appliances, which have a profound impact in bacterial colonization (Koopman et al. 2015). In the case of well-studied oral pathogens like mutans streptococci, a "window of infectivity" was proposed around the time of first teeth eruption for initial colonization (Caufield et al. 1993), and also for subsequent periods of teeth eruption, like those of primary molars or permanent first molars (Brailsford et al. 2005). However, several manuscripts have challenged this concept, as mutans streptococci have been found in infants before teeth eruption (Milgrom et al. 2000, Wan et al. 2001). In addition, other authors have found a constant increase in mutans streptococci with time without a discrete window of infectivity (see Law et al. 2007 for a review). This could be relevant for disease, because some authors have found a correlation of *S. mutans* initial colonization with delivery mode and perinatal factors (Li et al. 2005), and have proposed a link with the onset and severity of caries disease (Köhler et al. 1988, Li et al. 1994, Lai et al. 1997). In other studies, the oral microbiota at 3 months of age could not be related to caries development at 3 years (Holgerson et al. 2015). Finally, not only disease-associated species but also health-associated ones have been detected in longitudinal studies, like *Neisseria flavescens* and *Porphyromonas catoniae*, which have been proposed as biomarkers of caries-free children (Crielaard et al. 2011). The acquisition of oral anaerobes has been addressed by Könönen et al. 1999, and suggests again the colonization of given species in a sequential, timely manner.

In recent years, driven by the application of high-throughput sequence analyses, two important new concepts have emerged in addition to these established views of passive acquisition of oral microbial communities. First, it is proposed that human breastmilk harbours several hundred bacterial species that change from colostrum through mature milk. This microbiota includes many oral microorganisms such as streptococci, *Veillonella* or *Leptotrichia* (Cabrera-Rubio et al. 2012, Jeurink et al. 2013). The breastmilk-associated microbiota has been shown to be transmitted to the infant gut (Collado et al. 2016), and therefore, the same would be expected to happen in the oral cavity. In fact, although oral bacteria could be transmitted from the infant's oral cavity to the mammary glands during breastfeeding, the appearance of oral bacteria in precolostrum suggests that breastmilk could in fact be an important source for directed microbial colonization of the oral cavity (Mira & Rodriguez 2016). Given that different oral microbial profiles have been found between breastfed and formula-fed infants and that some bacteria associated with breastfeeding have been found to inhibit the growth of oral pathogens (Holgerson et al. 2013), the influence of breastfeeding on the risk of caries and periodontitis is an area for further investigation.

Secondly, although the topic is not without controversy, several authors have proposed that the developing foetus may be exposed to microorganisms before birth. This new concept is based on the identification of a microbiota in the placenta which appears to resemble that of the oral cavity (Aagaard et al. 2014) and to be influenced by preterm delivery and by infections during the first term of pregnancy (Prince et al. 2016). In a study of infants born by C-section, the bacterial composition of the placenta appeared to resemble that of the amniotic fluid and the meconium of the infant (Collado et al. 2016) opening up the possibility that the infants were exposed to a given set or microorganisms in utero. Although no oral samples were taken in this experiment, faecal material from the infant at 3 days of age had a similar bacterial composition to that of breastmilk, suggesting that the oral cavity could also undergo a similar process of microbial succession to that reported in the gut. It has to be kept in mind, however, that the microbial load detected in these samples is extremely low and therefore the possibility for contamination at the moment of sampling, sample processing and DNA extraction cannot be excluded, as even the traces of DNA in commercial kits have been shown to be sufficient to provide positive bacterial PCR amplification (Biesbroek et al. 2012, Lauder et al. 2016). Thus, the potential presence of microbes in utero would appear to be at really low densities that could be confounded with contamination, and appropriate quality controls, as well as careful sample handling should be performed to establish whether there really is a pre-birth microbial exposure.

If the presence of microorganisms in utero is confirmed, the consequences for the development of the infant immune system and for oral diseases will also be important. A change of paradigm has been proposed in which the placental trophoblasts (main constituents of the placenta), which have been shown to respond to bacterial components (Koga et al. 2009), would interact with commensal microorganisms, which could induce tolerance rather than rejection (Mor & Kwon 2015), especially if their antigens are present in small amounts (Sabatos-

Peyton et al. 2010). Thus, the potential presence of antigens from oral bacteria in the placenta has been proposed to develop foetal tolerance towards the maternal oral microbiome during pregnancy (Zaura et al. 2014). Co-incidentally, many physiological changes during human pregnancy facilitate bacterial translocation into the maternal circulation, including increased mucosal permeability and gum inflammation (Mira & Rodriguez 2016). Furthermore, injection of oral samples (saliva and plaque) into pregnant mice resulted in colonization of placenta by oral microorganisms (Fardini et al. 2010). In this way, the immune system of the new born infant would be conditioned to accept the normal microbiome of the mother in preference to bacteria from other sources. This interesting hypothesis would have important consequences, as the maternal oral health status during pregnancy could directly influence the acquisition, post-partum colonization and immune recognition of the infants' oral microbiome. This could be especially relevant for pregnant women with periodontitis, as the presence of periodontal pathogens in breastmilk and placenta could be facilitated, increasing infant immune tolerance to a disease-prone microbiota. Supporting this view, a recent qPCR-based study has detected several periodontal pathogens in placenta, which were found at higher levels in samples from mothers with periodontitis (Blanc et al. 2015). From an applied point of view, the confirmation of the placental microbiota-mediated tolerance would have consequences for public health policies directed towards improving oral health in pregnant and breastfeeding mothers, as this could reduce the prevalence of oral diseases of children later in life. Nevertheless, more longitudinal studies are required to provide solid epidemiological evidence showing that early use of antibiotics, mode of delivery, breastfeeding habits and other factors impacting early life microbiota acquisition are linked to oral diseases later in life.

Within-individual Variability

The oral cavity harbours different microbial communities which are specific to each of the multiple niches, for example, the tongue, cheek mucosa, dental tissues, gingival crevice or hard palate (Fejerskov et al. 1994, Babaahmady et al. 1997, Aas et al. 2005, Zaura et al. 2009). The initial work, performed by culturing, DNA hybridization and molecular cloning of the 16S gene has been confirmed by the enormous effort carried out by the Human Microbiome Consortium, which sampled several hundred individuals at different body parts, including six oral locations and analysed them by massive sequencing and metagenomic techniques (Human Microbiome Project Consortium 2012). However, within specific tissues, several authors have also unravelled significant heterogeneity in microbial composition. This was not unexpected because the earlier classical studies had already shown striking changes in environmental features like pH between different teeth (Kleinberg & Jenkins 1964), and changes in oxygen availability, redox potential, chewing forces and even hygiene differences, among others, have been proposed to influence and fragment a given oral site into multiple micro-niches (Zaura et al. 2009). When different teeth were sampled at the same mesio-buccal location, astonishing differences were observed in bacterial composition, with twenty species being associated with specific tooth locations (Haffajee et al. 2009). In another study, Simón-Soron et al. (2013) sampled plaque from over 100 locations per individual, observing significant differences in bacterial composition between teeth of the same individual and above all between vestibular and lingual surfaces of the same tooth. A repeated feature included a clear increase of streptococci at the vestibular surfaces of tooth and gingival sulci, where they accounted on average for 48% and 43% of all bacteria, whereas on the lingual surfaces, they reached only 24% and 12% in supra- and subgingival plaque samples, respectively. In addition, *Streptococcus* and *Fusobacterium* appeared to have opposite frequency patterns, suggesting adaptation of individual bacteria to specific microniches (Simón-Soron et al. 2013) (Fig. 2). Unfortunately, there is very limited information regarding intra-individual variability in fungal composition.

It is interesting to note that when saliva has been collected from the same individuals for which supra- and subgingival dental plaque samples were available, very different bacterial profiles were obtained (see, for instance Segata et al. 2012, Simón-Soron et al. 2013). This has important applied value, because saliva has traditionally been a preferred sample for epidemiological and even aetiological studies, due to its non-invasive nature and its convenience. This could be the reason why some studies have found an association between microbial composition and disease in plaque samples but not saliva, in both dental caries and gingivitis (Ling et al., 2010; Huang et al., 2011), as saliva contains microbes from all oral locations, including those not related to the disease. This probably introduces a large microbial diversity which undermines the diagnostic value of salivary microorganisms and highlights the importance of appropriate sampling for microbial-based diagnostic tests of caries and periodontitis (Belda-Ferre et al. 2015).

The dramatic level of variability in microbial composition detected between healthy sites, and also between disease sites, within the same individual underscores the importance of proper lesion classification for microbiological studies and that an important effort must be put into appropriate sampling at specific sites (discussed by Nyvad et al. 2013), as opposed to the frequent pooling of material for subsequent analysis. This includes also the distinction between active and inactive caries lesions, clinically diagnosed by texture, brightness and colour, but not fully characterized at the microbiological level (Nyvad & Fejerskov 1997).

The microbiology of dental caries and periodontitis at different stages of the disease has shown that they are both better understood as a dynamic process, and not as a classical infectious disease with a well-defined aetiology. In dental caries, different microbial communities have been found at the initial (enamel-degrading) and advanced (dentin expansion) stages (Gross et al. 2012, Simón-Soro et al. 2012, Simón-Soro

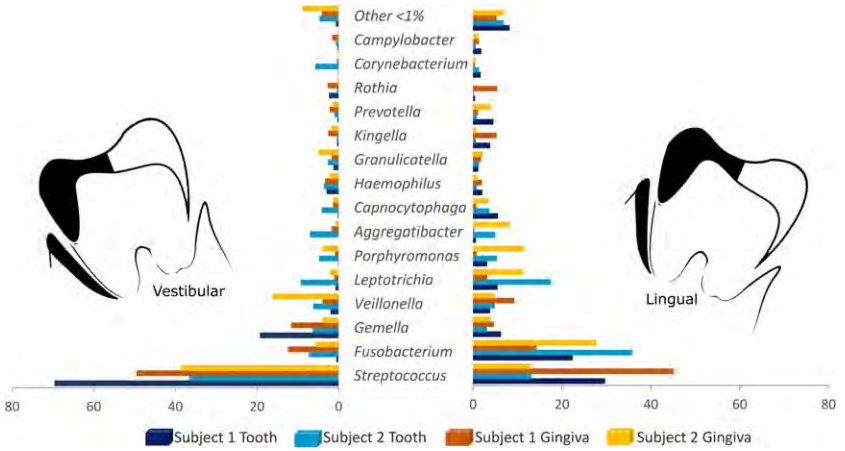

Fig. 2. Intra- and inter-individual variability in oral microbiota composition. Bar graphs show the proportion of different bacterial genera in supragingival and subgingival dental plaque from two healthy individuals in the same molar (tooth 4.6), collected either at the vestibular (buccal) or lingual surfaces. DNA was extracted from the samples, which were subject to pyrosequencing of the 16S rRNA gene to determine bacterial composition. Important differences are found at the same site and location between the two individuals, as well as between supra- and subgingival plaque samples. In addition, large differences in the proportion of some bacteria were found between the vestibular and lingual surfaces of the same tooth. For instance, *Streptococcus* dominated the vestibular surface of tooth and gingival sulcus in individual 2, but its proportion was significantly lower on the lingual surface, where *Fusobacterium* was the most frequent organism in this individual. The data illustrate the enormous variability in microbial populations not only between individuals but also within well-defined intra-oral niches. Sequences were downloaded from Simón-Soro et al. 2012.

& Mira 2015, Obata et al. 2014). In some cases, both carious lesions at different stages and samples from sound surfaces of the same individual were available and confirm a different bacterial composition, indicating that the shift in microbiota is not due to inter-individual differences (Simón-Soro et al. 2014). The same was demonstrated in an induced gingivitis cohort where all 20 patients in the study developed the disease after 14 days of absence in toothbrushing (Kistler et al. 2013). In these individuals, subgingival bacterial composition at baseline (healthy gingival sulcus) and gingivitis within the same patients was studied by massive sequencing of 16S rRNA genes, detecting differences in composition and activity through time. Bacterial taxa associated with gingivitis included *Fusobacterium nucleatum* subsp. *polymorphum*, Lachnospiraceae sp., *Lautropia* sp. and *Prevotella oulorum*.

Variability of caries and periodontitis microbial aetiology at different ages must also be better studied, as specific organisms like *S. wiggsiae* have been associated with early childhood caries lesions (Tanner et al. 2011) but had passed previously unnoticed in adult cavities. In adolescent aggressive periodontitis, *Aggregatibacter actinomycetemcomitans* has been identified as a causative agent (Åberg et al. 2015) and other bacteria potentially involved in this disease are being examined (Clerehugh & Tugnait 2001, Oh et al. 2002, Shaddox et al. 2012, Könönen & Müller 2014) and compared to the classical disease complexes in adult forms of the disease.

Stability of the Microbial Communities in Health

Stahringer et al. (2012) analysed the DNA sequences of saliva samples (representing bacteria from different mouth locations) from over 200 adolescents over a 10-year period and detected a "core" salivary microbiota that can be defined by eight bacterial genera which were detected in >95% of all samples. These included *Streptococcus*, *Veillonella*, *Gemella*, *Granulicatella*, *Neisseria*, *Prevotella*, *Rothia* and *Fusobacterium*. In this study, there appeared to be a bacterial species which was present in all samples, namely *Streptococcus mitis*, in agreement with other DNA-based studies performed with a lower number of samples (Aas et al. 2005). In addition, another 13 genera were found in more than 50% of the samples. Utter et al. (2016) also detected that a few bacterial genera accounted for most of the dental plaque community in American and Chinese populations, and the different genera varied in their stability, which fluctuated around a given mean. Although bacterial composition was found to be fairly stable at the genus and species level through time, a nucleotide resolution level analysis revealed that the proportion of different strains varied within each bacterial taxa, and that some of these strains could be described as bacterial "fingerprints" in dental plaque, that is individual-specific strains fluctuating around a stable mean over time. For instance, over a dozen *Corynebacterium* strains were detectable in each individual, of which a different subset reached high abundance in any given person. In another study performed in adults, significant differences were found in the estimated salivary bacterial density over a period of 1 year, suggesting possible seasonal changes (Cameron et al. 2015). However, salivary microbiota composition in those individuals was also found to be fairly stable. These features contrast to other human body habitats like the gut, where a higher degree of temporal variability was found and a where clear core, conserved composition could not be found (Turnbaugh et al. 2009). This is surprising given the high exposure of the oral cavity to external contamination and shows that the oral microbiome has an important degree of stability under health conditions.

Certain stability in the oral microbiota has also been observed in shorter time periods when compared to other human niches. For instance, tongue samples from two individuals were collected on a daily basis for a one-year period (Caporaso et al. 2011). Bacterial diversity was determined by pyrosequencing, and its analysis showed that the tongue's microbiota was clearly distinguishable from that of other body parts

from the same individual, such as the gut or the skin. The fact that oral samples normally appear together in principal components analyses (PCAs) when other human samples are included suggests that oral microbial communities are more stable than other body habitats. However, this does not imply that there is low intra-oral variability. In fact, a large variation was observed within these tongue samples through time, as only 10% of the species were estimated to be part of the stable or "core microbiome". Exposure to different foods, medication, travel and changes in immune response, among others, were hypothesized to be linked to changes in microbial composition, including blooms of individual taxa that were frequently observed (Costello et al. 2009). Thus, from a practical viewpoint, defining an individual's microbiota based on the results obtained from a single sample may not be representative and will be influenced by the relative presence of transient community members.

Although more longitudinal studies are needed to address the question of oral microbial composition stability throughout life, current data suggest a progressive increase in complexity and diversity from birth to the first years of life, with important alterations during teeth eruption, end of breastfeeding, introduction of solid food, permanent teeth eruption and maturation of the immune system. Oral microbiota during adolescence and adulthood appears to be more stable than in other well-studied niches like the gut, but still with an important degree of within-individual variability. That variability occurs in the first place due to dental plaque development from the moment of toothbrushing, as the dental biofilm increases in complexity within hours to a mature state in a few days (Kolenbrander et al. 2006). But there are also important intra-individual changes in microbiota composition at a longer scale, which suggests that environmental effects are the drivers of that variability. Whether the environment or human genetic background is behind the large inter-individual variability was elegantly addressed by studying saliva samples in a set of 45 twin pairs through time, which showed similar degrees of intra-individual variation in microbial composition in monozygotic and dizygotic twin pairs (Stahringer et al. 2012). In fact, twins' salivary bacterial composition was more similar to each other than to the whole population at all timepoints, but become more different when they aged and no longer cohabited. Lifestyle was also found to be the driver of variability in other studies (David et al. 2014). Thus, the data suggest that it is the environment, and not only the genetic makeup, which accounts for the large inter-individual variability and for perturbations in within-individual stability.

The Concept of Symbiosis versus Dysbiosis

The overall conclusions of the studies listed above are that the composition and structure of oral microbial communities are flexible throughout development but attain a relatively stable state – more stable than gut, the nares and the skin (Costello et al. 2009). Equally, they are responsive to the lifestyle of the individual and to environmental effects which can elicit changes in composition and abundance of different species over both extremely short and prolonged timescales. Shifts or differences in the population structure of human-associated microbiota do not necessarily equate to a dysbiosis, rather they are representative of the flexibility of these populations which are entirely compatible with health. On a more general note, given the apparent flexibility of the microbiota associated with humans, there must be equal levels of flexibility in the recognition and response systems which interface with these microbiota, the full details of which have yet to be elucidated.

If flexibility represents the norm, how is dysbiosis defined? The critical differentiating factor is the response of the host. Dysbiosis is now recognized as a definitive change in the microbiota at a given site in the body, crucially, accompanied by a breakdown of host–microbial mutualism. Dysbiosis of human-associated microbiota is now thought to be the defining event of multiple inflammatory and systemically driven pathologies and this can be extended to caries and periodontal disease (Fig. 1). Whilst no single organism or collection of organisms have been identified as a consistent marker in any human-associated microbiota, several defining features of dysbiosis have emerged through investigation of these pathology-associated microbial populations in the last decade.

First, in several examples, dysbiosis is reflected in a reduction in the overall microbial diversity of the corresponding symbiotic community. For example, a reduction in taxonomic diversity and species membership of the microbiota has been observed in multiple studies of the human gut microbiota in disease in addition to animal infection models (Pham & Lawley 2014). Dysbiosis in the gut generally leads to a depletion of obligate anaerobic bacteria such as *Bacteroides* and *Ruminococcus* spp., and conversely, an increase in facultative anaerobes including the family Enterobacteriaceae (e.g. *Eschericia coli, Klebsiella* spp., *Proteus* spp.). Manichanh et al.(2006) reported a decrease in microbial diversity in the faecal microbiota in Crohn's disease. Lepage et al.(2011) reported a similar reduction in ulcerative colitis, and Chang et al.(2008) described decreased diversity of the faecal microbiota in recurrent *Clostridium difficile*-associated diarrhoea. A functional consequence of a less diverse gut microbiota appears to be a reduced metabolic capacity, exemplified by a decline in short chain fatty acid production. These physiological by-products of carbohydrate fermentation by the microbiota are important energy sources for the host and also enhance the mucosal barrier, inhibit intestinal inflammation and oxidative stress. Hence, a reduction in the metabolic capacity of the microbiota may contribute to the impairment of host defences and thereby promote the stability of a dysbiotic community (Pham & Lawley 2014).

The microbial changes that are characteristic of the development of dental caries adhere to this feature of dysbiosis: the overall richness of the supragingival community is diminished as those bacteria with a more acidophilic nature predominate whilst those less able to tolerate low

pH are reduced. However, the obverse appears the case in periodontal disease. Here, the overall diversity of the subgingival microbial population frequently is increased relative to that in health (Griffen et al. 2012, Camelo-Castillo et al. 2014), although this view has been challenged in other investigations (Kirst et al.2015). The apparent divergence from the norm in the case of periodontal disease may have underlying significance for the aetiopathogenesis of the disease.

What might provide a mechanistic explanation for the distinction between decreased diversity in dysbiosis in caries versus increased diversity in periodontal diseases? One possibility is that the environment of a diseased periodontal pocket may represent a site of immune dysfunction which permits the proliferation of species normally controlled by the host defence. Lowered efficiency of the innate and adaptive response in periodontal disease due to host or bacterially mediated effector mechanisms is not inconsistent with many in in vitro and animal investigations. Inhibition of IL-8 activity by *P. gingivalis* – so-called chemokine paralysis (Darveau et al.1998) provides one such example. Support for this mechanistic explanation comes from a surprising source: the mucosal microbiota associated with colorectal cancer tumours and polyps – sites of immune suppression in the colon – also demonstrate elevations in population diversity compared to healthy mucosal surfaces (Mira-Pascual et al. 2015). An alternative explanation may be simply that the increased nutrient supply due to the ingress of gingival crevicular fluid in periodontal disease provides such a rich environment for enhanced bacterial growth rate that this is sufficient to outweigh even the very significant challenge presented by the elevated inflammatory response. However, given that an elevated nutrient supply is also likely to be a feature at sites of disease in inflammatory disorders of gut, where microbial diversity decreases compared to health, this explanation appears to have less credibility. Finally, one could argue that a diseased site in the periodontium actually represents multiple geographically dispersed niches descending from the cemento-enamel junction to the migrating front of the pocket epithelium. Perhaps this diversity of niches is then reflected in the increased richness of the microbial populations.

The second emerging feature of dysbiotic communities is a preferential loss of organisms considered beneficial to human health and a corresponding increase in pathobionts, members of the normal commensal microbiota with the potential to cause pathology. In the case of dysbiosis of the gut, these changes are exemplified by well documented reductions in the levels of obligate anaerobic members of the Firmicutes phylum and increased representation of members of the Proteobacteria, in particular the family *Enterobacteriaceae* (recently reviewed in Walker & Lawley (2013) and Hold et al. 2014). Most human pathogens belong to the Proteobacterium phylum including members of the Enterobacteriaceae which contains a number of frank and opportunistic pathogens including *Salmonella* spp., *Shigella* spp., *Klebsiella* spp., *Proteus* spp. and *E. coli*. Similarly in caries and periodontal disease, marked shifts in the microbial population structure are observed as described in a preceding section. What is less clear in the case of these two conditions, however, is which organisms or consortia of organisms we should consider to be beneficial organisms and which should be considered the pathobionts. Identification of the former may have significant therapeutic potential for both conditions. For example, new bacterial species with antimicrobial and pH buffering capacity have been found to be associated with caries-free conditions and have been proposed to be used as oral health promoting probiotics (Camelo-Castillo et al. 2014, Huang et al. 2016) and a similar strategy has been followed for periodontal disease (Teughels et al. 2011).

The overriding consensus of investigations of dysbiotic microbiota in human disease is that rather than seeking to describe a single bacterium or even group of organism responsible for the disease in a hugely complex microbial system, the microbiota as a whole should be viewed as the pathological determinant. Whilst this may be the case, it is not to diminish the potentially pivotal role played by individual organisms in driving dysbiosis in a host-associated microbiota. Recent observations using the mouse model of periodontal disease have demonstrated that one of the presumed important pathobionts of the disease, *Porphyromonas gingivalis* may contribute to disease by altering the normal oral microbiota to a dysbiotic state (Hajishengallis et al.2011). These studies in mice demonstrated that the oral commensal microbiota is responsible for the tissue and bone destruction associated with periodontitis when just low numbers of *P. gingivalis* are present. This organism was accordingly described as a community activist able to orchestrate the normal benign periodontal microbiota into a dysbiotic community structure (Darveau et al. 2012).

Functional Properties of the Microbial Communities in Caries and Periodontal Disease

The polymicrobial nature of periodontitis and dental caries not only implies that several organisms can be responsible for the onset of disease, but also that they could have synergistic effects. Synergy in relation to virulence has been demonstrated in several complex microbial communities, ranging from abscesses to otitis media or chronic wounds, as well as oral diseases (Murray et al. 2014). In dental caries, an example is given by consortia between *Veillonella alcalescens* and *S. mutans*. Veillonellae and streptococci appear together in microscopy images of dental plaque and coaggregate in vitro (Hughes et al. 1992, Dige et al. 2014), which has been interpreted as a metabolic dependency, because *S. mutans* produces lactate as a result of sugar fermentation and *Veillonella* utilizes lactate as carbon source (Mikx & van der Hoeven 1975). The interesting feature is that when these species were grown together in an artificial mouth model, they were shown to produce more acid than any one of them separately (Noorda et al. 1988), suggesting a synergistic cariogenic effect.

Similarly, there are a growing number of examples of synergistic interactions between different organisms in the periodontal microbiota

which may contribute to disease. Animal studies over the last 20 years have consistently demonstrated that poly-microbial infections with, for example, combinations of *P. gingivalis, T.denticola*, *T. forsythia* and *F. nucleatum* cause significantly higher levels of pathology than mono-infections with these bacteria (Feuille et al. 1996, Ebersole et al. 1997, Kesavalu et al. 2007, Verma et al. 2010a, b). Some mechanistic explanations for these synergies are now emerging: for example *S. gordonii* may enhance the virulence of *Aggregatibacter actinomycetemcomitans* by a cross-feeding mechanism (Stacy et al. 2014); *T. denticola* and *P. gingivalis* have the potential to cooperate metabolically (Tan et al.2014); *Prevotella intermedia* and *P. gingivalis* can act synergistically in the acquisition of haemin from haemoglobin (Byrne et al. 2013).

These in vitro and animal model studies are now being complemented by data from human studies on the functional activities of oral microbial consortia in health and disease. Until recently, most studies on the oral microbiology of caries and periodontitis have focused on the bacterial composition of samples under health and disease conditions, showing that both diseases involve a clear dysbiosis, or change in the balanced proportion of different microorganisms. However, the composition of microbial consortia associated with health and disease varies considerably between individuals and between sites. This shows the limitation of taxonomy-based studies, because the large redundancy and plasticity of microbes allows different bacterial consortia to perform the same functions (see e.g. Vaishampayan et al. 2010). Thus, some authors have emphasized the importance of identifying what are oral microbes doing rather than what taxonomic groups are present at a given time (Belda-Ferre et al. 2012, Duran-Pinedo & Frias-Lopez 2015, Takahashi 2015). Also, presence of a given organism does not imply that it is metabolically active. An example is given by some bacteria which were present at low levels in 24-h supragingival dental plaque, like *Corynebacterium*, but which was found to be among the most active in the community (Benítez-Páez et al. 2014). In addition, although the presence of some bacteria, like *Fusobacterium*, was found to be similar in healthy gingival sulci and periodontal pockets, its pattern of gene expression was found to be radically different between the two conditions (Yost et al. 2015). This indicates that the microbial community as a whole behaves in a different way under both conditions, and emphasizes the importance of functional analysis, which are now possible due to the advances in metagenomic (whole DNA analysis, providing the genetic repertoire of the microorganisms) and metatranscriptomic (whole RNA analysis, providing the expression pattern of the community) approaches (Nyvad et al. 2013).

In enamel caries lesions, for example, DNA-based functional studies have shown an over-representation of genes encoding pH stress proteins, as well as enzymes for the degradation of dietary sugars, which is consistent with the established role of cariogenic microbes to develop a cavity. However, when dentin caries lesions were studied, over-represented genes included those for osmotic stress, allantoin degradation, proteolytic activity, and utilization of glycans, among others (Belda-Ferre et al. 2012, Simón-Soron et al. 2013). This suggests that the microbial communities in initial, enamel lesions perform different functions than those of more advanced, dentin cavities, where bacteria appear to be specialized in utilizing sugars associated with the dentinary tissue and to degrade proteins, given rise to the hypothesis that dental caries is not a single disease but a tissue-dependent process of different aetiologies (Simón-Soron et al. 2013). Among the detected proteases, it was particularly relevant the identification of bacterial collagenases (as well as other proteases like glycoproteases, serine-proteases, carboxy-terminal proteases and metalloproteases) suggesting that, in addition to human metalloproteinases (Tjäderhane et al. 1998), this large microbial-encoded proteolytic potential may significantly contribute to dentinal protein degradation (Takahashi & Nyvad, 2016, although experimental evidence with dentine isolates should confirm that these microbial collagenases can degrade the important protein component of dentin.

In the case of periodontitis, whole RNA and DNA sequencing has been performed on healthy and disease sites, which has allowed identifying those functions overexpressed under each condition (Duran-Pinedo et al. 2014b, Yost et al. 2015). These studies show a large diversity of active functions in the healthy gingival sulcus, whereas periodontal pockets showed a congruence of several gene functions that appeared to be overexpressed in all patients. One of the activities which could be influencing virulence was that of gingipain, a protein synthetized by *P. gingivalis* which cleaves the complement component 5 (C5) generating high levels of C5a locally, which has a potent inflammatory effect (Hajishengallis et al. 2012). Other overexpressed functions in chronic periodontitis included biological processes related to flagellar motility, peptide transport, iron acquisition, beta-lactam degradation and biosynthesis of the lipid A component of endotoxins from Gram-negative bacteria. In one study, a metatranscriptomic analysis of active and inactive sites from patients with chronic periodontitis was performed (Yost et al. 2015). The over-represented functional signatures at active, progressing sites included cell motility, transport (citrate, iron, potassium, chloride and amino acids), lipid A and peptidoglycan biosynthesis, and synthesis of aromatic compounds, among others.

If we focus on the red-complex species of periodontal disease, the above-mentioned functional studies showed that all three species undergo an activation of metalloproteases, peptidases and proteins involved in iron metabolism, suggesting that these may represent important virulence factors in these well-established oral pathogens. However, those studies showed, surprisingly, that most upregulated virulence factors in periodontitis came from organisms which are generally not considered major periodontal pathogens (Duran-Pinedo et al. 2014b) in agreement with the idea that the whole microbial community could be providing the virulence output (Berezow & Darveau 2011).

Microbial Community Structure as a Cause versus a Consequence of Caries and Periodontal Disease

To summarize, dysbiosis in caries and periodontal disease may be defined as deleterious perturbations to the health-associated microbial population structures of the oral microbiota and accompanying breakdown in the normal homoeostatic balance between the host and the resident microbes. Multiple factors are likely to be able to effect the perturbations. In the case of caries, dietary sugar and its metabolism by acid tolerant bacteria appears to have a profound influence on this dysbiosis and a direct linkage to the disease process.

In the case of periodontal diseases, apart from the specific role of the JP2 clone of *A. actinomycetemcomitans* in cases of aggressive periodontitis (Haubek et al. 2008), the situation is less clear that dysbiosis is causative or consequential of the disease. It is feasible to argue that alterations to the inflammatory status of a site will lead to the selection of those members of the microbial community most well adapted to survive the increased inflammatory pressure and/or take maximal advantage of the altered nutritional environment presented by such an alteration. Bacteria less well adapted to the injurious properties of the innate response or ill-equipped to compete metabolically in this new environment will diminish in their overall proportions in the population. In this scenario, the dysbiosis is consequential not causative. Further evidence that the environmental changes induced by the diseased status of the host drive and sustain the alterations to the microbiota rather than the reverse comes from the efficacy, in certain instances, of immuno-modulatory drugs which target the inflammatory systems of the host as opposed to direct intervention at the level of the microbiota (Delima et al. 2002).

In other fields, there is now accumulating evidence to support the counter argument that dysbiosis in itself is sufficient to both initiate and drive disease. Perhaps the most compelling data has come from studies in animals over the last decade which have demonstrated that transfer of a dysbiotic microbiome from a diseased animal into a healthy recipient can be sufficient to recapitulate both the dysbiosis and the disease phenotype. For example, transplantation experiments where the disease-associated gut microbiota is transferred into healthy germ free animals have demonstrated that the disease phenotype can be reproduced in the recipients in a variety of conditions including adiposity, metabolic syndrome and colitis (Spor et al. 2011).

Equally, the restoration of a normal healthy microbiota following successful faecal transplantation for the treatment of *Clostridium difficile* infection suggests that restoration of a normal balanced microbiota in the gut is sufficient to restore intestinal health (van Nood et al. 2013). Faecal transplantation treatment of the other major inflammatory bowel diseases, ulcerative colitis and Crohn's disease has so far yielded less successful outcomes. However, the principle of treating inflammatory diseases associated with a perturbed microbiome by modalities aimed entirely at conversion of the dysbiotic microbiota to the previously harmonious and balanced system is now firmly established. Such an approach may have some benefit in the treatment of periodontal disease (Pozhitkov et al. 2015).

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Address:
Alex Mira
Department of Health and Genomics
Center for Advanced Research in Public Health
FISABIO Foundation
Valencia
Spain
E-mail: mira_ale@gva.es

Clinical Relevance

Scientific rationale: Dental plaque is composed of a complex mixture of different bacterial species which appear to be organized into an organized community structure with the potential to have an impact on the maintenance of health and the development of disease.

Principal findings: Review of the literature indicated that the directed acquisition of the oral microbial communities may vary between individuals. There are highly significant differences in the microbiology, both between individuals and between sites in the same individual but an overall temporal stability at a given environmental site. Oral pathogens appear to be present at low frequencies in healthy individuals. However, stressors on these normally stable systems can lead to dramatic compositional and functional changes in the microbiome and harmful effects to the oral tissues.

Practical implications: An improved understanding of the mechanisms behind the compositional changes in the microbial communities of the mouth could enable the development of preventative approaches and/or methods to reverse disease-associated microbial community structures to their status in health, although specific antimicrobial strategies (i.e. passive or active immunization) may not be effective.