The Structure of Dental Plaque Microbial Communities in the Transition from Health to Dental Caries and Periodontal Disease

Alex M. Valm

Department of Biological Sciences, University at Albany, State University of New York, Albany, NY 12210, USA

Correspondence to Alex M. Valm: Department of Biological Sciences, University at Albany, State University of New York, Albany, NY 12210, USA. avalm@albany.edu
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Abstract

The human oral cavity harbors diverse communities of microbes that live as biofilms: highly ordered, surface-associated assemblages of microbes embedded in an extracellular matrix. Oral microbial communities contribute to human health by fine-tuning immune responses and reducing dietary nitrate. Dental caries and periodontal disease are together the most prevalent microbially mediated human diseases worldwide. Both of these oral diseases are known to be caused not by the introduction of exogenous pathogens to the oral environment, but rather by a homeostasis breakdown that leads to changes in the structure of the microbial communities present in states of health. Both dental caries and periodontal disease are mediated by synergistic interactions within communities, and both diseases are further driven by specific host inputs: diet and behavior in the case of dental caries and immune system interactions in the case of periodontal disease. Changes in community structure (taxonomic identity and abundance) are well documented during the transition from health to disease. In this review, changes in biofilm physical structure during the transition from oral health to disease and the concomitant relationship between structure and community function will be emphasized.

Introduction

Numerous molecular-based sequencing studies have resulted in a consensus among researchers that approximately 700 species or phylotypes comprise the bacterial component of the oral microbiome, while each individual human is estimated to carry a subset of between 50 and 200 species [1,2]. The human oral cavity includes different habitats for microbes including the epithelial mucosa; the papillary surface of the tongue dorsum; and the non-shedding, hard surfaces of the teeth, which themselves consist of two distinct compartments: the supragingival surface, that is, above the gum line, and the subgingival, that is, that below the gum line [2]. Site-specific, DNA sequencing studies of these different habitats have revealed that these different habitats support different microbial communities mediated by the characteristics of the surfaces available for attachment, oxygen availability, and exposure to host products delivered by saliva, to supragingival communities, and gingival crevicular fluid (GCF), to subgingival communities [3].

Dental caries are lesions of the tooth enamel and may involve the underlying dentin, which develop as a consequence of dietary sugar-driven microbial growth and carbohydrate metabolism that leads to localized acidification and disruption of tooth mineralization homeostasis [4]. Periodontitis is a chronic, progressive disease, characterized by expansion of the microbial biofilm at the gingival margin with the formation of an inflammatory infiltrate that contributes to destruction of connective tissue attachment to the tooth, alveolar bone resorption and may result in eventual tooth loss [5,6]. In addition, periodontal disease status is correlated with certain comorbid systemic diseases including cardiovascular disease, rheumatoid arthritis, adverse pregnancy outcome, and cancer through cellular and molecular mechanisms that are not well understood [6–12].

Dental caries and periodontal disease are both mediated by the oral microbiome and host interactions and inputs: diet in the case of caries and the immune system in the case of periodontal disease [4]. The transitions from health to caries pathology and to periodontal disease are both recognized to be caused not by introduction of exogenous pathogens, but by changes in microbial community structure, that is, taxonomic composition and relative abundance, that transform the communities into pathogenic states [13]. In fact, periodontal disease is correlated with an increase in microbial community diversity, in contrast to most diseases known to be mediated by the human microbiome [5,14,15]. The transition from oral health to disease is further recognized to be multi-factorial, interdependent between host and microbiota and dynamic [4].

Molecular sequencing-based approaches have revolutionized our understanding of the human microbiome. Next-generation DNA sequencing and other -omics technologies have permitted the assessment of the oral microbiome with enormous breadth and without the need for prior knowledge of the system, allowing the analysis of large sample sets and facilitating large-scale, longitudinal studies that together have greatly informed our understanding of the shift in microbial community structure that occurs in the transition from health to disease [5,16–18]. At the same time, the genetic and biochemical manipulation of individual organisms and small consortia under controlled laboratory conditions has permitted the identification of many of the molecular and cellular processes that underlie community function [19–24]. Importantly, the network of fine scale interactions that have been identified to date does not exist in an unstructured milieu. In fact, the highly non-random structure of supragingival dental plaque has been reported in a rich body of literature, with increasing taxonomic specificity [25]. Early electron and light microscopy-based studies allowed the development of two central hypotheses to the formation of dental plaque and its role in mediating disease, namely, that highly ordered communities result as a consequence of ecological succession and that no single pathogenic organism is responsible for periodontal disease [26,27]. This review presents a summary of the current state of knowledge regarding dental plaque structure and especially considers the importance of structure in the transition from dental health to caries pathology and periodontal disease.

Taxonomic Composition of Dental Plaque Communities in Health

The species composition and relative abundance of microbial communities is often referred to as community structure in the literature [3]. This type of structural information generated by sample homogenization and subsequent molecular identification through DNA sequencing is not to be confused with information on the physical architecture of microbial biofilms [28]. This type of structural information generated by direct observation with microscopy will be considered in detail subsequently. Particular emphasis will be given to the supra- and subgingival plaque communities as these communities are extraordinarily complex and species rich and have been observed to have highly non-random spatial structure, hypothesized to be due in part to the non-shedding nature of the tooth compared to the soft epithelium [4].

The oral microbiome of healthy subjects is dominated, like the human microbiome in general, by members of the phyla Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes as well as the additional orally abundant Fusobacteria and Spirochaetes [16]. A recent meta-analysis of Human Microbiome Project supra- and subgingival dental plaque data revealed that 13 genera are both highly abundant and have high prevalence within the sampled population: *Streptococcus*, *Corynebacterium*, *Capnocytophaga*, *Haemophilus/Aggregatibacter*, *Fusobacterium*, *Prevotella*, *Leptotrichia*, *Veillonella*, *Neisseria*, *Rothia*, *Actinomyces*, *Lautropia*, and *Porphyromonas* [29]. At the genus level, the taxonomic compositions of both the supra- and shallow-depth subgingival communities in states of health are similar, with marked differences in relative abundance; for example, the genus *Prevotella* is increased in subgingival communities, a reflection of the different environmental conditions experienced by the two microbial communities [16,29].

Together with bacteria, fungi also comprise the healthy human oral microbiome. Although the fungal load in healthy subjects is estimated to be orders of magnitude lower than the bacterial load, the size and morphology of fungal cells and their synergistic interactions with bacteria suggest an important role for these organisms in structuring dental plaque [30,31]. Assessment of fungal diversity has been hampered by both the incompleteness of fungal sequence databases, which itself is due partly to the inability to culture many species, necessitating *de novo* database construction, as well as a lack of standard protocols for fungal DNA extraction caused by the extreme heterogeneity in fungal cell wall composition [30,32]. Nonetheless, recent next-generation sequencing studies of oral fungal 18S rRNA internal transcribed spacer sequences identified numerous genera with both high abundance and prevalence in saliva and include *Candida*, *Cryptococcus*, *Fusarium*, *Aspergillus/Emericella/Eurotium*, and others [33,34].

Role of the Oral Microbiome in Health

Emerging evidence suggests that the human microbiome performs diverse functions that are

beneficial for the human host [35]. The beneficial effects of the human microbiome have primarily been observed in the gut; however, recent work has identified key beneficial functions of the oral microbiota [36–39]. It is hypothesized that a primary function of resident microbes is to act as a physical and biochemical barrier to prevent colonization or infection by exogenous organisms [40]. Indeed, the mouth is open to the external environment and receives countless airborne and droplet-associated microbes through breathing as well as food- and water-associated microbes through eating and drinking. By physically and chemically restricting access to the host epithelium through direct occlusion, by sequestering nutrients, and by secreting antimicrobials, the commensal oral microbiota may play an important role in directing foreign microbes to the flushing saliva and mucus, to be swallowed and delivered to the extremely low-pH, microbicidal environment of the stomach [40].

A second hypothesized function of the commensal microbiota is to promote maturation of both the innate and adaptive host immune systems, especially to achieve proper balance between pro- and anti-inflammatory processes in the absence of, and during, infection [41]. In the mouth, the study of patients with underlying primary immmuno-deficiency as well as mouse models of primary immmuno-deficiency has unraveled a complex interplay between subgingival microbiota and the host immune system including the importance of proper neutrophil recruitment to prevent potentially over compensatory inflammatory responses to oral microbiota. In addition, the crucial involvement of T_H17 cells in mediating microbiota-induced periodontal disease further supports the central involvement of neutrophils in periodontal disease [39,42]. In the context of beneficial microbes, it has been demonstrated that species of oral streptococci play an immuno-modulatory role and downregulate pro-inflammatory responses to beneficial oral microbes [43–47]. For example, *Streptococcus salivarius* was demonstrated to downregulate innate immune responses specifically by downregulating genes involved in the NF-kappaB pathway in oral epithelial cells [48].

In addition to roles that are common to all human associated microbiomes, the oral microbiome is hypothesized to contribute unique functions to human health and homeostasis. A key role for the oral microbiome in nitrate reduction and subsequent nitric oxide concentration in the systemic circulation has been proposed, implicating the oral microbiota in the maintenance of vascular health [43,49,50]. Dietary nitrate is concentrated in saliva and may be reduced by nitrate-reducing bacteria, which are abundant on the tongue dorsum [51]. The contribution that oral communities make to systemic nitric oxide homeostasis remains to be determined, but correlations between subgingival bacterial load and blood pressure have been described [52].

Dental Plaque Physical Structure in States of Oral Health

Early light and electron microscopy studies of intact dental plaque revealed these communities to be highly structured, with non-random distributions of morphologically and phenotypically different cells [26,27,53,54]. Dental plaque is now recognized as a polymicrobial biofilm, defined as a community of microbial cells embedded in an extracellular matrix, that grows on an interface between two phases of matter, for example, the solid tooth surface and liquid saliva or GCF [55]. The structure of dental plaque biofilms is hypothesized to be driven by specific inter-taxon physical and chemical interactions; environmental pressures including oxygen tension and host factors both growth promoting, for example, nutrients and growth-promoting factors in saliva and GCF, and growth inhibiting including antimicrobial peptides, lysozyme, secreted antibodies, and other immune mediators [56]. Coaggregation, the adhesion of taxonomically distinct cells to each other mediated by specific cell surface molecules, prevents the dislodging and loss of oral colonizers by mastication and shear forces generated by flowing saliva and GCF and also provides spatial proximity to facilitate microbial communication and chemical exchange [57–60]. The results of extensive *in vitro* binding assays using cultivable bacterial isolates were compiled to generate a hypothetical model for the spatial structure of dental plaque [61]. According to this model, a subset of microbes, namely species of the genera *Streptococcus* and *Actinomyces* are able to bind directly to the glycoprotein-rich salivary pellicle that coats the tooth, through bacterial surface receptor recognition [62]. A process of ecological succession then takes place by which these founding organisms and other early colonizers including species of the genus *Veillonella* serve as substrates for the subsequent attachment of later colonizing organisms culminating in a climax community, rich in diversity that includes organisms that are abundant in states of health as well as those enriched in states of periodontal disease, that is, cells of the genera, *Treponema* and *Tannerella* among others [63]. Importantly, according to this model, *Fusobacterium nucleatum* and to some extent *Porphyromonas gingivalis* serve as important bridging organisms that physically unite the early colonizers with late colonizing organisms, because these two species have demonstrated the ability to specifically coaggregate with both types of colonizers [4,60].

Lending support to this conceptual model, many of the inter-taxon associations hypothesized from *in vitro* coaggregation assays have been directly observed

using fluorescence microscopy with labeled antibodies or oligonucleotide probes in *in vitro*, co-culture experiments [57,64,65], on removable substrates worn by healthy volunteers [66], and in multiplex fluorescence *in situ* hybridization (FISH) on extracted dental plaque [67]. During development, the structure of dental plaque communities is further fine-tuned by the synergistic activities of the microbes themselves [68]. For example, members of the genus *Streptococcus* rapidly sequester oxygen at the apical, saliva-exposed surfaces of supragingival biofilms to create anaerobic niches within these health-associated structures [29].

Health-associated subgingival plaque biofilms have not yet been analyzed systematically with a high degree of taxonomic resolution due to the difficulty in obtaining intact, tooth-associated subgingival biofilms from healthy donors. However, multiplex FISH has recently been applied to supragingival dental plaque extracted from healthy volunteers in a manner that maintained the three-dimensional structure of the biofilms [29]. Within these undisturbed regions of supragingival biofilms, large, annular structures were repeatedly observed. Within these "hedgehog," structures, cells of the genus *Fusobacterium* were present; however, filamentous cells of the genus *Corynebacterium* were more abundant, and their distribution and colocalization with other taxa suggest a central role for this organism in structuring the community. Seven other bacterial taxa were consistently observed in hedgehog structures: *Streptococcus*, *Porphyromonas*, Pasturellaceae, Neisseriaceae, *Leptotrichia*, *Capnocytophaga*, and *Actinomyces*. Cells of the genus *Streptococcus* and sometimes *Porphyromonas* and family Pasturellaceae were observed to decorate the apical tips of some *Corynebacterium* filaments in "corncob" arrangements, a structure that had been described without taxonomic resolution in early electron microscopy studies of dental plaque. These observations were assimilated into an updated model for dental plaque structure, driven by synergistic inter-taxon interactions, ecological succession, and dynamic environmental and biochemical inputs that shape the physical structure of the health-associated community as it develops [29].

Community Structure in the Context of Dental Caries

Mutans streptococci, especially *Streptococcus mutans*, as well as lactobacilli are strongly correlated with caries [13]. *S. mutans* readily ferments sucrose and other sugars to produce ATP and lactic acid as a waste product [69]. The accumulation of lactate is thus responsible for the local acidification of the caries environment [70,71]. Species of the aciduric, that is, acid-tolerant genus *Veillonella*

utilize lactate as a carbon source and thus are involved in syntrophic metabolism of carbohydrate with *S. mutans* [19]. Molecular sequencing analyses have identified other acidogenic and aciduric organisms that are strongly correlated with different stages of caries progression *in vivo*, including *Bifidobacterium* spp. *Scardovia* spp., *Actinomyces*, and especially in the case of early childhood caries, *the fungus, Candida albicans* [13,31]. Other non-aciduric genera including *Corynebacterium*, *Granulicatella*, and *Propionibacterium* have also been found at increased abundance in caries-associated supragingival plaque [4]. Thus, the development of caries is marked by a shift in supragingival community composition from one that promotes health, to one that mediates disease (Fig. 1). This homeostasis breakdown in microbial community composition, or dysbiosis, is a common phenomenon of microbiome-mediated diseases [75].

Supragingival Plaque Biofilm Structure and Caries Development

Although the systems-level spatial analysis of caries-associated dental plaque biofilms with taxonomic resolution as described above for health-associated biofilms is yet to be achieved, mounting evidence assembled from different studies suggests an intrinsic contribution of spatial structure on the development of caries (Fig. 1). In the absence of abundant fermentable carbohydrates, Mitis group streptococci bind to the saliva-coated tooth with greater avidity than *S. mutans*, they grow more rapidly and they antagonize competitors with local secretion of hydrogen peroxide [4,76,77]. Thus, acidogenic and aciduric oral microbes are required but not sufficient for caries development; also needed is frequent consumption of dietary sugars by the host [4]. When ecological perturbation exceeds a threshold, interspecies interactions that shape the biofilm community during states of health are altered [21]. During frequent exposure to fermentable carbohydrates, localized regions of low pH generated by lactate producers further select for aciduric organisms resulting in a positive feedback loop, which ultimately leads to highly localized demineralization [78,79]. Dietary sucrose is especially cariogenic because its component 6-carbon sugars, glucose and fructose, are used to synthesize extracellular polymeric substances (EPS) in the form of glucans and fructans [80]. The extracellular biofilm matrix, which is comprised EPS, glycoproteins, and extracellular DNA, provides binding sites for embedded microbes, protects against biofilm removal during normal oral hygiene procedures, and contributes to the generation of highly localized regions of low pH by inhibiting exchange of saliva, which has a natural buffering capacity [79]. EPS also has the
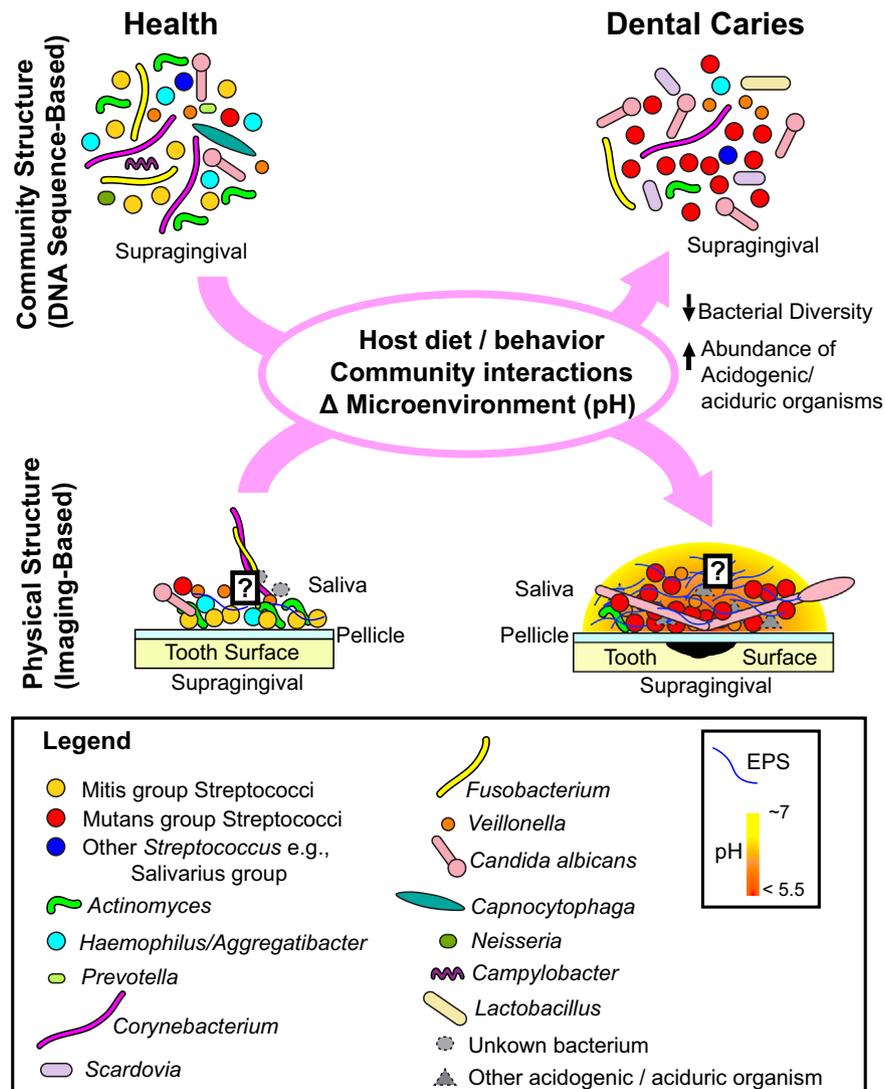
Fig. 1. Observed changes in dental plaque structure between states of health and sites of active caries. DNA sequencing provides a description of community structure in the form of taxonomic membership and abundance. Supragingival plaque communities from patients with dental caries experience shifts in community composition, marked by a general decrease in community diversity. The shift in microbial community structure is mediated by frequent dietary consumption of fermentable carbohydrates and synergistic interactions between increasingly abundant acidogenic and aciduric organisms (see Refs. [13,29,72]). The spatial structure of caries-associated communities also undergoes specific developmental changes during the transition to dysbiosis. The structure of caries associated biofilms is mediated by the increased production of EPS by Mutans Streptococci, including on the surface of *C. albicans* hyphae as seen in early childhood caries and by synergistic interactions among bacteria that create a highly localized low pH microenvironment mediated by secretion of lactic acid and its sequestration within dense EPS meshworks. Unknown bacterium and other acidogenic/aciduric organisms represent species that have been identified in molecular surveys but which have not been identified with taxonomic resolution in biofilm images. Small question marks in the diagram reflect recommended caution in drawing conclusions about inter-taxon associations because caries-associated supragingival biofilms have yet to be imaged after labeling with more than a few probes simultaneously (see Refs. [29,73,74]).

ability to alter diffusion and sequester antimicrobials, which has implications for therapeutic intervention [4,28,81,82]. Glucosyltransferase exoenzymes secreted by *S. mutans* have been observed to bind to the surface of *C. albicans* cells and function to synthesize glucans [31]. In this way, *C. albicans* hyphae act as important physical scaffolds for the development of EPS-embedded microcolonies of *S. mutans* and other organisms, which may contribute especially to early childhood caries which strongly correlate with *C. albicans* carriage [73]. Thus, diet and synergistic interactions shape the

microbial community in both its taxonomic makeup and physical structure by allowing the formation of highly localized acidic microenvironments [21]. These microenvironments in turn further shape the local structure of the biofilm to create a positive feedback loop, which can lead to highly localized acidification and tissue demineralization.

Community Structure in the Context of Periodontal Disease

As with caries, no single organism is implicated in the transition from health to periodontal disease, rather the subgingival microbial community present in states of periodontal health transitions to a state of dysbiosis in

Fig. 2 (*legend on next page*)

which the community structure, that is, species composition and abundance, shifts toward a pathogenic state [83]. Early culture-independent approaches first identified a three-member consortium of Gram negative organisms, called the "Red Complex" enriched in the subgingival microbiomes of patients with periodontal disease, consisting of *P. gingivalis*, *Treponema denticola*, and *Tannerella forsythia* [84]. These organisms have been termed pathobionts rather than pathogens because, although implicated in disease, they are normally present at lower abundances in the microbiota of subjects with no clinical markers of periodontitis [85]. The list of pathobionts has recently been expanded through modern culture-independent molecular surveys to include the Gram-positive *Filifacter alocis* and other anaerobes including cells of the genera *Parvimonas*, *Fusobacterium*, and *Prevotella* (Fig. 2) [5,16,88].

The cellular and molecular mechanisms that drive and reinforce the Jekyll to Hyde transition of subgingival communities toward a dysbiotic state are an active area of research. According to the Ecological Plaque Hypothesis, changes in environmental conditions, that is, nutrient availability, oxygen concentration, pH, and host inflammatory mediators, drive the community shift by selecting and enriching for pathobionts [89,90]. Consistent with this hypothesis, molecular sequencing-based approaches have confirmed that pathobionts are present in health-associated subgingival communities at low abundance; while, as ecological changes take place, these organisms expand within the communities above a threshold that initiates and reinforces periodontal disease pathology [5,16]. The newly described polymicrobial synergy and dysbiosis hypothesis builds on this ecological concept to include the dynamic and synergistic interactions between organisms and the host as a mechanism to shape and stabilize dysbiotic communities within their ecological context [91]. The gingival tissue destruction associated with inflammation is thought to contribute to dysbiosis by releasing nutrients including degraded collagen and other peptides as well as haem-containing compounds into the periodontal pocket in the form of GCF [4]. Supporting this hypothesis, periodontal pathobionts include organisms that require exogenous amino acids for growth in culture and a recent metatranscriptomic analysis of subgingival communities found enhanced expression of genes involved in iron uptake in periodontitis-associated communities [18,92].

That dysbiotic communities require inflammation for their nutritional support seems paradoxical because a localized inflammatory response by the host normally functions to inhibit microbial growth. This paradox is resolved by the demonstrated ability of *P. gingivalis* to manipulate the host immune response in such a fashion as to uncouple tissue destruction from microbicide during inflammation, through manipulation of the complement pathway mediated through gingipain exoenzymes that act on the inactive precursor complement protein, C5 to generate C5a [93]. *P. gingivalis* further promotes crosstalk between the complement C5a receptor 1 and Toll-like receptor 2 while bypassing the downstream effector Myd88 to achieve a pro-inflammatory, anti-phagocytic response in phagocytic cells [4]. Even further, *P. gingivalis* has demonstrated the ability to downregulate expression of interleukin-8 by epithelial cells, a chemokine that recruits phagocytic neutrophils and Th1 cells to the gingiva, as well as T-cell production of interferon-γ [39,94–96]. Thus, *P. gingivalis*, present in low abundance in both health- and disease-associated communities, acts as a keystone species in the developing dysbiotic community to create a pro-inflammatory, anti-phagocytic environment that favors the growth and expansion of pathobionts [97].

Subgingival Plaque Biofilm Structure and Periodontal Disease Development

As described above, an understanding of the synergistic activities of subgingival microbes and the host as these communities undergo dysbiosis is

Fig. 2. Observed changes in dental plaque structure between states of health and periodontal disease. Subgingival plaque communities from patients with periodontal disease have increased bacterial load and shifts in community composition that reflect a process of ecological succession. Importantly, the shift in community structure involves the expansion of a subset of organisms that are present in states of health without the displacement of other health-associated taxa (see Refs. [5,16]). The highly ordered physical structure of dental plaque communities has been probed with taxonomic resolution using FISH. In states of health, supragingival plaque biofilms have taxonomic distributions that reflect host and environmental inputs including oxygen concentration and salivary components as well as within-community interactions such as H_2O_2 production and oxygen sequestration to create anaerobic niches. Cells of the genus *Corynebacterium* were observed to play a central role in structuring the system. The spatial structure of subgingival plaque biofilms in states of health is not well studied due to the inaccessibility of these biofilms for imaging and is reflected in the graphic as a large question mark. FISH on extracted teeth from patients with periodontal disease revealed the spatial distribution of organisms with respect to the basal (tooth-associated) and apical (facing the gingival pocket) surfaces. Gingivitis is a reversible form of periodontal disease that is mediated by increased bacterial load at the gingival margin, characterized by a unique community structure not shown in this diagram. In susceptible hosts, gingivitis may progress to chronic periodontitis. Small question marks in the diagram reflect recommended caution in drawing conclusions about inter-taxon associations because subgingival biofilms have yet to be imaged after labeling with more than a few probes simultaneousl (see Refs. [29,86,87]).

emerging; however, what remains poorly understood is how these activities are distributed within the physical architecture of microbial communities [98]. The molecular cross-talk that takes place between the subgingival microbiota and the host immune system does not take place in an unstructured milieu, but rather within a highly ordered environment. The structure of biological systems, including both the involved host tissues and the subgingival microbial community, correlates with their function, and mounting evidence suggests the importance of biofilm structure in the transition through dysbiosis [86].

The close apposition of cells within polymicrobial biofilms allows biochemical interaction, signaling, and genetic exchange between cells. Facultative aerobes within dental plaque biofilms can sequester oxygen and create anaerobic niches [29]. This process may be important during the transition to dysbiosis because many pathobionts are strict anaerobes. During the progression of periodontal disease, the gingival pocket increases in volume, which may drive higher bacterial load within this niche [4,27]. That the total bacterial load is increased in patients with periodontal disease as newly dominant members of the community emerge and accumulate, rather than replace earlier colonizers suggests a dynamic physical interplay between biofilms and host tissue to create this new space [5]. How these newly dominant species are distributed within expanding biofilms is beginning to be explored. Extensive FISH labeling of subgingival biofilms on teeth extracted from patients with periodontal disease and systematic image analyses have provided a qualitative visual atlas of the spatial distributions of some dominant organisms including putative pathobionts (Fig. 2). Zijnge and colleagues [86] were able to describe four distinct layers in subgingival plaque from periodontitis-affected individuals. *Actinomyces* spp. were located primarily in the basal layer of the biofilm, that is, closest to the tooth surface. *Fusobacterium* was identified in the intermediate layers of the biofilms along with *Tannerella*, while *Prevotella* and *Porphyromonas* localized to both the apical and intermediate layers. Cells of the *Cytophaga–Flavobacterium–Bacteroides* cluster were observed in the apical layers, while *Treponema* were loosely arranged superior to the densely packed biofilm. Interestingly, some of these spatial arrangements superficially recapitulate those summarized in the conceptual model of Kolenbrander and colleagues [61] described earlier, reflecting the combined actions of ecological succession, coaggregation, and microbial growth during the transition from subgingival health to periodontal disease.

In addition to microbial physical interactions, including interkingdom interactions between fungi and bacteria, physical interactions between sub-

gingival biofilm microbes and host cells have been observed. Zijnge and colleagues [86] observed microbes of the genus *Synergistes* in close spatial proximity to polymorphonuclear leukocytes in multiplex FISH images of extracted teeth with associated gingival tissue, suggesting direct physical interaction between these biofilm resident microbes and host immune cells. In fact, close apposition of host leukocytes, identified by their characteristic lobed nuclei, to subgingival biofilm microbes had previously been observed in transmission electron micrographs of human and canine subgingival tissue [99].

Early Events During the Transition from Health to Periodontal Disease

In a longitudinal metatranscriptomic study of long-term healthy subgingival sites and sites that progressed to periodontal disease in human subjects, *P. gingivalis* displayed upregulation of a large number of virulence genes in healthy sites that later progressed to disease, while *T. denticola* and *T. forsythia* did not upregulate any but one or a few virulence genes until later timepoints during disease progression, suggesting that when present, *P. gingivalis* serves as a microbial driver in the transition from periodontal health to disease [4,100]. Furthermore, this transition may involve an intermediate state. Gingivitis is a reversible form of periodontal disease, marked by microbiota-induced inflammation at the apical gingival border, that is, the zone that distinguishes the supra- and subgingival compartments [101]. Gingivitis may be induced experimentally in volunteers who have abstained from normal oral hygiene for a short period of time and usually resolves upon resumption of normal oral hygiene practices [102]. However, in susceptible individuals in non-experimental situations, gingivitis may progress to chronic periodontal disease (Fig. 2) [103]. Molecular analyses of dental plaque communities in volunteers with experimentally induced gingivitis reveal a unique cohort of microbes that are associated with gingivitis but not health or chronic periodontitis, which comprised *F. nucleatum* subsp. polymorphum, *Lachnospiraceae* spp., *Lautropia* sp., and *Prevotella oulorum* [102]. Importantly, the mature and highly ordered hedgehog structures previously described in supragingival plaque biofilms were extracted from volunteers who abstained from normal oral hygiene for 12–48 h [29], and these structures or others like them that project tens of microns above the enamel surface may be involved in the induction of gingivitis, especially if they locate at the gingival border, again suggesting a role for plaque biofilm structure in mediating the transition from periodontal health to disease [104].

Conclusions and Future Directions

Both dental caries and periodontal disease are highly prevalent within the human population. Both diseases are polymicrobial in their etiology and result when the supra- and subgingival microbial communities associated with states of health experience a homeostasis breakdown and undergo dysbiosis. The etiologies of these diseases are multifactorial and depend on synergistic activities, both chemical and physical, within the microbial communities and between the host diet and behavior in the case of caries and the immune system in the case of periodontal disease.

As described here, descriptive studies of dental plaque structure in states of health and disease are beginning to be achieved with systems level taxonomic resolution. What remains to be achieved is a mechanistic understanding of how fine-scale intercellular interactions lead to large-scale physical structures. To that end, *in vitro* biofilm culture systems and animal models may be exploited for controlled laboratory testing of structure-related hypotheses [105,106]. The analysis of complex and highly heterogeneous image data is not a straightforward task, and the development of image analysis tools that can be shared and standardized by researchers is tremendously valuable [107]. Furthermore, the development of mature dental plaque biofilms is a dynamic process that results as a consequence of ecological succession. Analysis of static images of fully developed communities provides limited information on the developmental processes that take place to achieve the climax community structure. To this end, live cell imaging will provide crucial information to "connect the dots" in plaque structure development over time [108].

To achieve a deep understanding of the forces that drive dysbiosis within dental plaque communities, collaboration will be required among scientists with diverse expertise including microbiology, biochemistry, immunology, ecology, imaging, and genomics as well as concerted effort among researchers to synthesize emerging knowledge into unified theories.

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