The Role of Bacterial Biofilms in Dental Caries and Periodontal and Peri-implant Diseases: A Historical Perspective

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Abstract

Over the last hundred years, groundbreaking research in oral microbiology has provided a broad and deep understanding about the oral microbiome, its interactions with our body, and how the community can affect our health, be protective, or lead to the development of dental diseases. During this exciting journey, hypotheses were proposed, and concepts were established, discarded, and later revisited from updated perspectives. Dental plaque, previously considered a polymicrobial community of unspecific pathogenicity, is recognized as microbial biofilms with healthy, cariogenic, or periodontopathogenic profiles, resulting from specific ecologic determinants and host factors. The "one pathogen, one disease" paradigm of oral infections has been replaced by a holistic concept of a microbial community as the entity of pathogenicity. Cutting-edge technology can now explore large microbial communities related to different clinical conditions, which has led to finding several novel disease-associated species and potential pathobionts and pathobiomes. This vast amount of data generated over time has widened our view of the etiology of caries and periodontal and peri-implant diseases and has promoted updated strategies to treat and prevent the oral diseases.

Keywords: microbiota, peri-implantitis, periodontitis, plaque biofilms, gingivitis, tooth decay

Introduction

Science and technology frequently appear to recycle concepts, inventions, and discoveries, as described by Steven Poole (2016) in *Rethink: The Surprising History of New Ideas*: old is the new new. For example, the 1960s description of the amphibiontic relationship between the indigenous oral flora and the human host (Rosebury 1962) has resurfaced under the concept of keystone pathogens, pathobionts, and dysbiosis as triggers of oral diseases (Marsh 1994; Hajishengallis et al. 2012). Advances in biotechnology have facilitated revisiting topics in oral microbiology to unravel key features and mechanisms that were not previously appreciated.

The diversity of the oral microbiota and the ability of oral microorganisms to form dental plaque on tooth, implants, and oral mucosal surfaces in a sophisticated manner have been characterized (Moore and Moore 1994; Paster et al. 2001; Zaura et al. 2009; Dewhirst et al. 2010; Charalampakis and Belibasakis 2015). Complex interactions among microorganisms, the host, and the oral environment were demonstrated, which can translate into the clinical symptoms of white spot lesions or inflamed gingiva/peri-implant mucosa (Marsh 2005; Charalampakis and Belibasakis 2015). The oral microbiome is understood to have a dynamic "biofilm lifestyle" (Marsh 2005). In celebration of the *Journal of Dental Research* centennial, this review highlights findings that, over a century of technological progress, contributed to the evolutionary improvement of our knowledge of dental biofilms and their roles in oral health and diseases.

Oral Microbiology 100 y Ago

The 19th century was an active period in medical sciences, and major discoveries included cultivation of microorganisms and identification of etiologic agents of infectious diseases. Koch's postulates described criteria whereby microorganisms could be related to diseases. During this era, reputed dentists, including Riggs, Harlan, and Miller reinforced the microbial etiology of caries and periodontal diseases and promoted the concept of proper oral hygiene for managing disease. Periodontal disease (pyorrhea alveolaris) was renamed "Riggs's disease." Riggs introduced the mechanical debridement of teeth with irrigation of periodontal pockets with antiseptics. Harlan recognized that subgingival microorganisms were mainly anaerobic and proposed injection of hydrogen peroxide into periodontal pockets to provide "nascent oxygen to destroy the unclassified microorganisms there present." The first oral microbiologist,

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Willoughby Dayton Miller, reported that caries was caused by acids produced by oral bacteria following fermentation of sugars, stating that "a clean tooth never decays." Miller (1890) described the chemoparasitic theory of caries, the nonspecificity of dental plaque associated with periodontal diseases, methods for cultivation and identification of oral microorganisms, and the concept of focal infection. Considering the limited knowledge of human biology and biotechnology of that era, the extraordinary theories and findings of these dedicated scientists provide the basis for modern oral microbiology.

Association of Bacteria with Oral Diseases

The 20th-century explosion in understanding of oral microorganisms parallels the increased knowledge of the human microbiome (Table 1). Early microscopy techniques revealed the fusospirochetal component of necrotizing ulcerative gingivitis—an association that was confirmed >50 y later through electron microscopy (Listgarten 1965). Based on studies of rampant caries in children, the disease-associated oral microbiota included species now recognized in *Lactobacillus* and *Bifidobacterium* (Howe and Hatch 1917). Within a few years, the British microbiologist Clarke (1924) isolated *S. mutans* from carious lesions. Nevertheless, for several decades, lactobacilli were considered the primary caries pathogens.

After a period in the 1930s when the relationship of microbes to disease was questioned, bacteria regained importance in the etiology of oral diseases in the 1950s, due mainly to studies in experimental animals. Extraoral anaerobic mixed infections revealed an essential role of *Bacteroides melaninogenicus* (Socransky and Gibbons 1965), most likely *Porphyromonas gingivalis* (personal communication, S. S. Socransky). Experiments in caries demonstrated that lesions did not occur in the absence of microorganisms, even under high-sucrose caries-inducing diets (Orland et al. 1954). Moreover, caries associated with acidogenic streptococci (Fitzgerald and Keyes 1960) could be transferred to caries-free animals by microbial plaques (Keyes 1960). The caries-inducing *Streptococcus* was subsequently recognized to be *S. mutans* (Edwardsson 1968) and was able to induce caries, including rampant caries with a very high-sucrose diet, in a monkey model (Bowen 1969). In the same decade, alveolar bone loss was induced in hamsters by inoculating plaque from animals with periodontitis (Jordan and Keyes 1964).

Studies of humans that used an experimental gingivitis model established the etiologic role of dental biofilm in the onset of gingival inflammation (Löe et al. 1965). Conversely, resolution of inflammation and caries prevention were observed after vigorous dental plaque control and antimicrobial treatment, further reinforcing a bacterial etiology of caries and periodontal diseases (Loesche 1979; Axelsson and Lindhe 1978).

Nonspecific to Specific Plaque Hypothesis

The "nonspecific plaque hypothesis" (Rosebury 1947) was replaced by a "specific plaque hypothesis" based on the association of *S. mutans* with caries and linking individual species or sets of species with different clinical conditions (Loesche 1979). In the 1970s and early 1980s, advancements in sampling, culture, and bacterial taxonomy equipped researchers with better tools to identify bacteria and their association with clinical conditions. Differences in microbial composition in health and disease were now evident with identification of an increasing number of species associated with different clinical conditions (Listgarten 1976; Tanner et al. 1979; Mombelli et al. 1987; Moore and Moore 1994). Studies focused on detecting and characterizing potential pathogens and their virulence factors. This reductionist view of single species as etiologic agents of caries and periodontal diseases, however, frequently failed to fulfill the criteria of classical infectious diseases, and by the end of the 20th century, oral bacteria were seen as a part of a complex and interactive microbial community that could take different roles in the pathogenesis of oral diseases.

Current Ecologic View of Dental Plaque Biofilm

The concept of biofilm (Costerton et al. 1987) described the natural environment for microorganisms as an organized complex of multispecies communities in an extracellular matrix attached to surfaces (Hall-Stoodley et al. 2004). Bacterial growth and properties in biofilms differed from the more artificial environment of growth in liquid media, particularly in resilience to environmental change, including reduced sensitivity to antimicrobial agents. Studies in the 1960s and 1970s by Gibbons, Listgarten, and collaborators and in the 1990s by Kolenbrander and coworkers demonstrated that bacteria adhere selectively to oral surfaces, the polysaccharide matrix, and other bacteria in specific spatial arrangements during biofilm development (Gibbons and Van Houte 1971; Listgarten 1976; Kolenbrander et al. 1993). Complex attachment mechanisms included specific hidden (cryptitopes) salivary proline-rich proteins that are exposed only during bacterial-surface interactions or as a result of enzymatic action in the gingival fluid (Gibbons et al. 1990). Microbial complexes included description of "corn cob" formations from coaggregation among streptococci, rods, and filamentous bacteria (Listgarten 1976). Subsequent research on dental biofilm structures that used high-resolution laser scanning confocal microscopy and in situ hybridization with species-specific probes illustrated the architecture of dental biofilm with bacteria arranged into specific clusters (Zijnge et al. 2010; Mark Welch et al. 2016).

Dental biofilm development and linking with clinical conditions have been examined via culture and genetic approaches.

Table 1. Investigations of Dental Biofilm and Oral Bacteria Associated with Caries and Periodontal/Peri-implant Diseases in the Last 100 y.

Reference (Institution)	Key Findings
Howe and Hatch 1917 (The Forsyth Dental Infirmary for Children, Boston, MA, USA)	*Bacillus acidophilus* (*Lactobacillus acidophilus*) and bifidobacteria isolated in high numbers from advanced carious lesions.
Clarke 1924 (Institute of Pathology and Research, London, UK)	Description of *Streptococcus mutans* and its association with early caries lesion.
Orland et al. 1954 (University of Chicago, Chicago, IL, USA); Fitzgerald and Keyes 1960 and Keyes 1960 (NIDR, Bethesda, USA)	Studies using germ-free and gnotobiotic rodents found that caries did not develop in the absence of microorganisms, even under a cariogenic diet; caries is infectious and transmissible and is associated with acidogenic oral streptococci.
Rosebury 1962 (Columbia University, New York, NY, USA)	A textbook on the human indigenous microbiota: the concept of amphibiotic oral microbiota is presented.
Jordan and Keyes 1964 (NIDR, Bethesda, MD, USA)	Destructive periodontal disease is an infectious and transmissible disease in hamsters.
Macdonald et al. 1963; Socransky and Gibbons 1965 (The Forsyth Dental Infirmary for Children, Boston, MA USA)	"*Bacteroides melaninogenicus*" was found to be a key pathogen in anaerobic mixed groin infections in rodents. The pathogenic strain was later recognized as *Porphyromonas gingivalis*.
Listgarten 1965 (University of Toronto, Toronto, Canada; Harvard School of Dental Medicine, Boston, MA, USA)	Description of spirochetes and fusiforms by electron microscopy of NUG biopsies.
Löe et al. 1965 (Royal Dental College, Aarhus, Denmark)	Experimental gingivitis in humans: deliberate withdrawal of oral hygiene leads to biofilm accumulation and gingival inflammation that is reversible with reintroduction of oral hygiene methods.
Krasse et al. 1968 (University of Gothenburg, Gothenburg, Sweden)	High levels of *S. mutans* in dental plaque is related to high caries risk.
Bowen 1969 (Royal College of Surgeons of England, London, UK)	Infection with *S. mutans* in the presence of high sucrose led to development of rampant caries in monkeys.
Gibbons and Van Houte 1971 (Forsyth Dental Center, Boston, MA, USA)	Selective adherence of oral bacteria on epithelial surfaces.
Listgarten 1976 (School of Dental Medicine, University of Pennsylvania, Philadelphia, PA, USA)	Microscopic study of dental plaque associated with periodontal health and disease shows differences in structure and composition between supra- and subgingival biofilms and in varying degrees of disease.
Loesche 1979 (University of Michigan, Ann Arbor, MI, USA)	Nonspecific and specific hypothesis of dental plaque.
Hardie et al. 1977 (The London Hospital Medical College, London, UK)	A 2-y longitudinal study in children failed to show a strong association between *S. mutans* and development of caries.
Tanner et al. 1979 (Forsyth Dental Center, Boston, MA, USA)	A study of the bacteria associated with advanced progressing periodontitis in humans introduced species now recognized as *Aa, P. gingivalis, Prevotella intermedia, Campylobacter rectus, Campylobacter gracilis*, and *Tannerella forsythia*.
Costerton et al. 1987 (University of Calgary, Alberta, Canada)	The biofilm concept: microorganisms are predominantly found in the attached "sessile" state on surfaces, inserted in an extracellular glycocalyx, rather than in the free planktonic state. Cells growing within a biofilm structure are phenotypically distinct from planktonic cells and communicate with one another by chemical signaling.
Mombelli et al. 1987 (University of Bern, Switzerland)	The microbiota associated with unsuccessful implants show similarities with the microbiota of periodontitis.
Kolenbrander et al. 1993 (NIDR, Bethesda, MD, USA)	Coaggregation of oral bacteria in dental biofilm is specific.
Marsh 1994 (PHLS Centre for Applied Microbiology and Research, Salisbury, UK)	Ecologic plaque hypothesis: environmental factors will favor the overgrowth of more pathogenic communities, which will lead to disease.
Moore and Moore 1994 (Virginia Commonwealth University/VPI, Richmond, VA, USA)	This review presents the major bacterial species isolated from subgingival biofilm of various periodontal clinical conditions. Approximately 500 species were identified.
Van Houte et al. 1996 (Forsyth Dental Center, Boston, MA, USA)	Acidogenic and acid-tolerant *Bifidobacterium* spp., *Actinomyces* spp., and nonmutans streptococci are predominant in root caries.
Socransky et al. 1998 (Forsyth Dental Center, Boston, MA, USA)	Description of the microbial complexes of subgingival plaque associated with periodontal health and disease.
Paster et al. 2001 (Forsyth Dental Center, Boston, MA, USA)	High species diversity of the oral microbiota is determined through 16S rRNA sequencing and cloning.
Munson et al. 2004 (King's College London, London, UK)	Combined cultural and molecular analyses revealed a diverse bacterial community in dentinal caries. Actinobacteria were underrepresented by the molecular analysis.
Haubek et al. 2008 (University of Aarhus, Aarhus, Denmark)	Carriage of the JP2 clone of *Aa* is associated with a high risk for periodontal attachment loss in children and adolescents.
Zaura et al. 2009 (University of Amsterdam and Free University Amsterdam, Amsterdam, the Netherlands)	Next-generation sequencing methodology was used to define the microbiome diversity of several intraoral niches. The concept of a core microbiome in health is proposed.
Dewhirst et al. 2010 (The Forsyth Institute, Cambridge, MA, USA)	The Human Oral Microbiome Database curated description of species/taxa found in the oral cavity and nasopharynx.

(continued)

Table 1. (continued)

Reference (Institution)	Key Findings
Tanner et al. 2011 (The Forsyth Institute, Cambridge, MA, USA)	Anaerobic culture of severe early childhood caries identified *S. mutans* and *Scardovia wiggsiae* as major caries-associated species.
Belda-Ferre et al. 2012 (Center for Advanced Research in Public Health, Valencia, Spain)	Metagenome and functional analysis of supragingival biofilm of caries lesions showed a complex microbial community.
Hajishengallis et al. 2012 (University of Pennsylvania, Philadelphia, PA, USA)	The "keystone pathogen" hypothesis: low-abundance microbial pathogens can orchestrate inflammatory disease by remodeling a benign microbiota into a dysbiotic one.
Frias-Lopez and Duran-Pinedo 2012 (The Forsyth Institute, Cambridge, MA, USA)	With metatranscriptomic analysis, periodontal pathogens have been shown to drastically influence the gene expression profiles of health-associated biofilm.
Lamont and Hajishengallis 2015 (University of Louisville, Louisville, KY, USA; University of Pennsylvania, Philadelphia, PA, USA)	Polymicrobial synergy and dysbiosis model of pathogenesis for periodontal diseases.
Eriksson et al. 2018 (Umeå University, Sweden)	*S. mutans* with *S. wiggsiae* were associated with more aggressive caries in Swedish adolescents. Low or undetectable levels of *S. mutans* were associated with a larger panel of saccharolytic species in subjects with lower levels of caries.

Aa, Aggregatibacter actinomycetemcomitans; NIDR, National Institute of Dental Research; NUG, necrotizing ulcerative gingivitis.

Computer modeling of 40 oral species identified with DNA probes from gingival and subgingival samples showed that these bacteria cluster into microbial complexes that were labeled by different colors (Socransky et al. 1998). Further analysis indicated the co-occurrence of the color complexes of bacteria related to one another within the microbiomes of periodontal and peri-implant diseases (Socransky and Haffajee 2005; Shibli et al. 2008). The composition of the clusters of microorganisms was found to resemble the sequence of plaque development. Early colonizers belonged in blue, yellow, and green complexes and secondary colonizers in the orange complex, whereas a climax community of the periodontal anaerobes *P. gingivalis, Treponema denticola*, and *Tannerella forsythia* belonged to the red complex (Socransky and Haffajee 2005).

For dental caries, in response to dietary carbohydrates, the increasing acidogenic and acid-tolerant environment leads to suppression of acid-sensitive species, including those that counterbalance local acidity. Secondary acidogenic caries-associated colonizers become predominant (*S. mutans, Streptococcus sobrinus, Lactobacillus, Bifidobacterium*, and *Scardovia* species; Tanner et al. 2011; Takahashi and Nyvad 2016).

Bacteria within biofilms communicate to one another through nutritional interactions, genetic exchange, and quorum sensing signaling (Marsh 2005), and investigators realized that assessing the role of oral bacteria in health or disease required evaluation of the whole community rather than individual species. The ecologic concept of an amphibiontic oral microbiota described by Rosebury 30 y ago reemerged with the ecologic plaque hypothesis (Marsh 1994), which suggested that an imbalance (dysbiosis) of the oral microbial community was caused by environmental stresses, including diet, inflammation, saliva flow, and composition. Maturation of the biofilm favors enrichment by periodontal pathogens that in turn may induce an altered host response favoring the persistence of more virulent species (Löe et al. 1965; Kolenbrander et al. 1993; Marsh 2005; Socransky and Haffajee 2005). These reciprocal interactions among microorganisms, host, and environment lead to tissue destruction, as described in the expanded ecologic plaque hypothesis for dental caries (Takahashi and Nyvad 2016), and associations of microbial complexes with loss of alveolar bone in periodontal and peri-implant diseases (Socransky et al. 1998; Shibli et al. 2008). These findings emphasized the sophisticated structure of dental biofilm and its role in oral diseases.

Periodontal, Peri-implant-, and Caries-Associated Biofilms

Characterizing the oral microbiotas has evolved from culture- and antibody-based studies of the 20th century to gene-based techniques, initially on 16S rRNA sequences and subsequently by community-based sequencing. The major cultivable species of health, gingivitis, periodontitis, and peri-implantitis revealed disease and host-compatible species (Tanner et al. 1979; Mombelli et al. 1987; Moore and Moore 1994). The microbiotas of initial white spot carious lesions (Hardie et al. 1977; Van Houte et al. 1991), advanced forms of severe early childhood, (nursing bottle) caries (Marchant et al. 2001), and root caries (Van Houte et al. 1996; Brailsford et al. 2001) were found to include increasing proportions of acidogenic and acid-tolerant microbiotas.

Gene probe techniques expanded the number of species that could be detected without cultivation. DNA checkerboard studies were used to evaluate the proportions of the microbial complexes in health and periodontal/peri-implant diseases and the impact of different periodontal therapies on these groups of bacteria (Socransky and Haffajee 2005; Haffajee al. 2006). Red and orange microbial complexes were predominant in disease, and successful treatments were associated with the reduction of these complexes and the increase of host-compatible microorganisms. Other pathogens not considered members of the usual oral microbiota, including staphylococci, *Acinetobacter, Pseudomonas*, and enteric bacteria, were detected in relative

high frequency in periodontitis/peri-implantitis sites (Colombo et al. 1998; Fritschi et al. 2008; Persson and Renvert 2014). Furthermore, these unusual species were more prevalent around healthy and diseased implants as compared with teeth (Charalampakis and Belibasakis 2015), as well as in immunosuppressed patients or those after antibiotic therapy (Slots et al. 1988).

Genetic methods incorporating 16S rRNA sequencing led to reorganization of bacterial taxonomy, and amplification of microbial DNA by polymerase chain reaction (PCR) was introduced to study selected oral species. In particular, PCR data indicated that herpes virus was highly prevalent in aggressive forms of periodontitis, although the mechanisms by which these viruses may contribute to disease remain unknown (Slots 2015).

Use of 16S rRNA probes in a "reverse capture checkerboard" format could detect cultured and uncultivated species recognized from cloning and sequencing analyses. This approach was miniaturized to a microarray in the Human Oral Microbe Identification Microarray, which could assay around 300 oral species (Colombo et al. 2009). These assays showed that many new taxa were associated with periodontal and peri-implant diseases, suggesting new potential pathogens (Colombo et al. 2009; Romanos et al. 2016). Other approaches combined next-generation sequencing methods with in silico hybridization with specific probe sequences (ProbeSeq), allowing identification of an extended number of oral taxa (Mougeot et al. 2016).

Gene probe approaches have also been used extensively in caries studies including children (Aas et al. 2008; Gross et al. 2012), root caries (Preza et al. 2009), and initial white spot lesions (Torlakovic et al. 2012). PCR–denaturing gradient gel electrophoresis methods were used in several studies, clearly demonstrating similar microbial profiles of oral biofilms from mothers and their children (Li et al. 2007), *Lactobacillus* sp. diversity in young women (Caufield et al. 2007), and biofilm composition that included *Scardovia* and *Lactobacillus* species in association with hydroxyapatite demineralization (Thomas et al. 2012).

Distinguishing the microbiotas of periodontitis or caries from nondisease has required identifications to the species level, since different species within the same genus can have a pathogenic or beneficial profile. The 16S rRNA approaches have been replaced by next-generation sequencing–based methods, with the advent of faster and less costly methods for sample analyses. Genetic methods have not, however, superseded cultural approaches. Comparisons of anaerobic culture with cloning and sequencing indicated limitations and advantages to each approach (Munson et al. 2004), with culture being more sensitive to detection of *Actinobacteria* (Schulze-Schweifing et al. 2014), providing isolates for pathogenicity testing and evaluation of new therapeutic approaches. Molecular methods are faster to perform, however, and can evaluate greater depth of the microbiome in samples than feasible by culture.

Composition of the Oral Microbiota Based on Open-Ended Technology

The Human Microbiome Project has been a revolutionary milestone in medical microbiology, revealing that the microbiome is critical in the biology and physiology of humans (Cho and Blaser 2012). High-throughput sequencing of the 16S rRNA gene, the development of bioinformatic analytic tools, and curated 16S rDNA databases revealed a much greater diversity of the oral microbiome than what was previously apparent (Paster et al. 2001; Kumar et al. 2005; Dewhirst et al. 2010; Griffen et al. 2012; Simón-Soro et al. 2013; Eriksson et al. 2018; Xu et al. 2018). Of the approximately 700 species/phylotypes known to colonize the oral cavity, about a third remain uncultivated and include phylotypes prevalent in periodontitis (Dewhirst et al. 2010) that may play a role in the periodontopathogenesis. Some of those microorganisms were recently cultivated (Vartoukian et al. 2016), including Saccharibacteria (TM7) isolates, which were shown to have an interbacterial lifestyle and thus require host species for growth (McLean et al. 2018).

A small core microbiome has been described that is resilient to environmental modifiers. Conversely, a large proportion of less resilient microorganisms, often detected in low abundance, contributes to the high diversity and interindividual variability of the oral microbiome (Zaura et al. 2009). These species may have a protective role by providing functional redundancy. Fluctuations of these microorganisms in biofilms often make interpretation of microbial data difficult, pointing to the value of the ongoing studies in microbiome function by metatranscriptomics and metabolomics (Frias-Lopez and Duran-Pinedo 2012; Simón-Soro et al. 2013; Simón-Soro et al. 2014; Takahashi 2015; Yost et al. 2015).

Shifts in the subgingival microbiome from a healthy state to gingivitis or perimucositis (Kistler et al. 2013; Schincaglia et al. 2017) and posterior destructive disease have been demonstrated (Liu et al. 2012; Boutin et al. 2017). Differences in the composition of the subgingival microbiome between periodontal and peri-implant diseases are discrete, with increased prevalence of staphylococci, Gram-negative bacilli, and *Candida* spp. in peri-implantitis (Shibli et al. 2008; Persson and Renvert 2014; Romanos et al. 2016; Schincaglia et al. 2017). During oral hygiene abstention, implants accumulated less plaque than teeth, whereas the gingivitis microbiome is more diverse than that of peri-implant mucositis (Schincaglia et al. 2017).

Predominant species/phylotypes in the microbiomes associated with distinct periodontal clinical conditions are summarized in Figure 1 and Table 2. The microorganisms shown are not definitive, and distinct microbial biotypes likely exist within each clinical condition, which can further discriminate various forms of disease (Boutin et al. 2017). Based on next-generation sequencing, novel microorganisms have been strongly associated with disease in addition to putative periodontal pathogens.

The caries-associated microbiome varies by lesion site (Simón-Soro et al. 2013), with reduced diversity in many

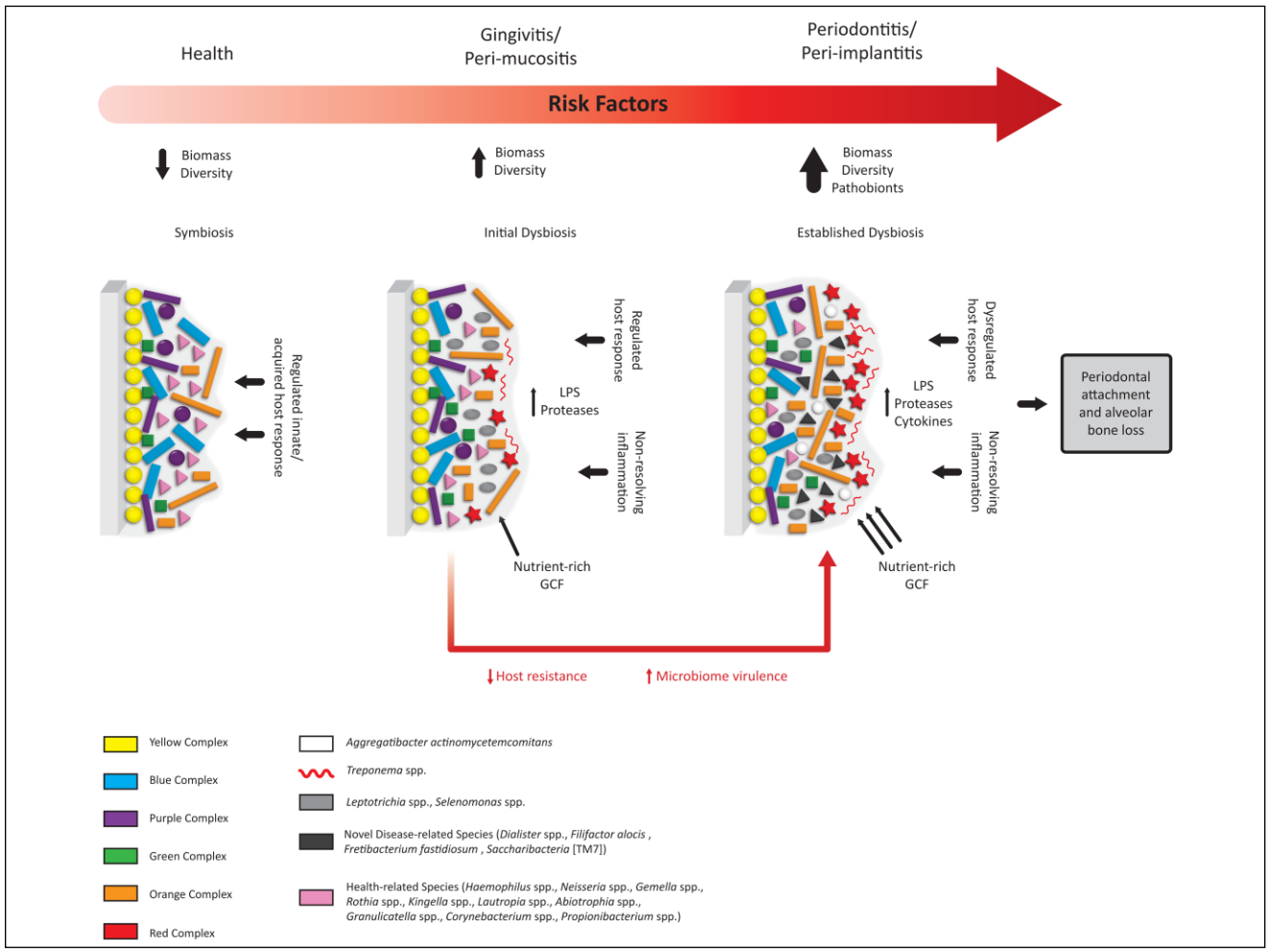

Figure 1. Predominant microbiomes in different periodontal states. In a healthy periodontium, a low biomass of gingival and subgingival biofilm, comprised mainly of symbionts, is controlled by an efficient and self-limiting host response. Biofilm accumulation leads to increased but self-limited chronic inflammation that favors the emergence of periodontal pathogenic microorganisms, including members of the orange and red complexes (Socransky et al. 1998). Depending on host susceptibility and the presence of various risk factors, a complex pathogenic periodontal microbiota composed of high proportions of putative and novel pathogens is established. This dysbiotic microbiota promotes a dysregulated immune/inflammatory response that will result in loss of periodontal supporting tissues. GCF, gingival crevicular fluid; LPS, lipopolysaccharide.

lesions following suppression of acid-sensitive species. The health-associated microbiome plays a significant role in caries by buffering the acidity, for example, by production of ammonia from *Streptococcus sanguinis* with arginine deiminase and/or from *Actinomyces naeslundii* via urease activity (Burne and Marquis 2000). Suppression of these pH-buffering taxa favors growth of secondary colonizers, which include members of the same genera, particularly *Streptococcus* and *Actinomyces*, as those in the health-associated microbiome. While an association of *S. mutans* with caries has been observed since the 1920s, many studies have not detected this species in caries (Hardie et al. 1977; Gross et al. 2012; Simón-Soro et al. 2013). Beyond technical issues, including insensitivity of earlier molecular methods to detect *S. mutans*, it seems likely that this highly cariogenic species is more frequently detected in rapidly progressing lesions, including children (Krasse et al. 1968) and populations of adolescents with differing disease experience (Eriksson et al. 2018). Less aggressive cariogenic microbiomes appear to include other acidogenic species, such as *Streptococcus, Actinomyces*, and other taxa in Actinobacteria (Belda-Ferre et al. 2012; Eriksson et al. 2018). Caries in dentin, including root caries, can be characterized by *Lactobacillus, Bifidobacterium*, and *Scardovia* species in addition to *S. mutans* (Mantzourani et al. 2009; Kianoush et al. 2014), suggesting rapid disease progression, although proteolytic species may be cariogenic in deep dentin. Shifts in predominant caries-associated microbiomes in different stages and sites of caries lesions are determined by environmental ecologic factors (Takahashi and Nyvad 2016; Fig. 2).

Microbial Community–Based Etiopathogenesis of Oral Diseases

For many decades, the pathogenesis of periodontal diseases was based on the premise that untreated gingivitis would inevitably progress to periodontitis. With the demonstration of

Table 2. Species/Phylotypes Frequently Detected in Subgingival Biofilms Associated with Periodontal Health, Gingivitis, Peri-mucositis, Periodontitis, and Peri-implantitis by High-Throughput 16S rRNA Gene Sequencing.

Core	
Campylobacter concisus	*Prevotella* sp. HMT317[2]
Campylobacter gracilis[3]	*Pseudomonas pseudoalcaligenes*
Capnocytophaga gingivalis	*Rothia aeriae*
Cardiobacterium hominis	*Rothia dentocariosa*[2]
Catonella morbi[2]	*Selenomonas noxia*
Corynebacterium matruchotii[2]	*Streptococcus australis*
Eikenella corrodens	*Streptococcus cristatus*
Fusobacterium naviforme	*Streptococcus infantis*
Fusobacterium necrophorum	*Streptococcus mitis*[2]
Fusobacterium nucleatum subsp. *animalis*[3]	*Streptococcus mitis* bv 2
Fusobacterium nucleatum subsp. *canifelinum*	*Streptococcus oligofermentans*
Fusobacterium nucleatum subsp. *fusiforme*	*Streptococcus oralis*
Fusobacterium nucleatum subsp. *nucleatum*[3]	*Streptococcus oralis* subsp. *dentisani* clade 058
Fusobacterium nucleatum subsp. *polymorphum*	*Streptococcus oralis* subsp. *tigurinus* clades 070 / 071
Fusobacterium nucleatum subsp. *vincentii*[2]	*Streptococcus parasanguinis*
Fusobacterium periodonticum	*Streptococcus peroris*
Fusobacterium simae	*Streptococcus pneumoniae*
Fusobacterium sp. HMT203 / HMT205 / HMT370	*Streptococcus pseudopneumonia*
Gemella morbillorum	*Streptococcus salivarius*
Granulicatella adjacens	*Streptococcus sinensis*
Haemophilus sp. HMT826	*Streptococcus* sp. HMT055 / HMT056 / HMT057 / HMT061 / HMT065 / HMT066 / HMT067 / HMT068 / HMT069 / HMT074 / HMT423 / HMT486
Haemophilus parainfluenzae[2]	*Streptococcus vestibularis*
Lautropia mirabilis	*Veillonella dispar*
Peptidiphaga sp. HMT183	*Veillonella parvula*[2]
Prevotella nigrescens[s2]	
Periodontal health	
Abiotrophia defectiva	*Moraxella osloensis*
Acinetobacter junii	*Mycobacterium neoaurum*
Actinomyces gerencseriae	*Neisseria elongata*[2]
Actinomyces massiliensis[2]	*Neisseria mucosa*
Actinomyces naeslundii[6]	*Neisseria pharyngis*
Actinomyces odontolyticus[2]	*Neisseria sicca*
Actinomyces oris[2]	*Olsenella uli*
Actinomyces sp. HMT169[2] / HMT170[2] / HMT171[4] / HMT175[2] / HMT177[3] / HMT180 / HMT448 / HMT525	*Peptidiphaga* sp. HMT183
Aggregatibacter sp. HMT458	*Porphyromonas catoniae*[3]
Bergeyella sp. HMT322[3]	*Prevotella maculosa*[2]
Brachybacterium rhamnosum	*Prevotella oris*
Burkholderia cepacia	*Prevotella oulorum*
Campylobacter concisus[2]	*Prevotella* sp. HMT300 / HMT309
Campylobacter gracilis	*Prevotella tannerae*
Capnocytophaga gingivalis[2]	*Propionibacterium propionicum*[3]
Capnocytophaga leadbetteri	*Pseudopropionibacterium* sp. HMT194
Capnocytophaga sputigena	*Rothia aeria*[4]
Cardiobacterium hominis [2]	*Rothia dentocariosa*[6]
Corynebacterium durum [4]	*Selenomonas flueggei*
Corynebacterium matruchotii [2]	*Selenomonas* sp. HMT138
Eikenella corrodens [2]	*Simonsiella muelleri*
Eikenella sp. HMT011	*Streptococcus cristatus*[2]
Enterococcus sp. HMTA78	*Streptococcus gordonii*[2]
Fusobacterium nucleatum subsp. *polymorphum*	*Streptococcus infantis*[2]
Fusobacterium nucleatum subsp. *vincentii*	*Streptococcus intermedius*[3]
Fusobacterium periodonticum	*Streptococcus mitis*[3]
Gemella morbillorum[3]	*Streptococcus mitis* bv 2
Gemella haemolysans[2]	*Streptococcus oralis*[3]
Granulicatella adjacens[3]	*Streptococcus oralis* subsp. *dentisani* clade 058
Granulicatella elegans[2]	*Streptococcus oralis* subsp. *tigurinus* clade 071[2]
Haemophilus parahaemolyticus	*Streptococcus pneumoniae*[2]
Haemophilus parainfluenzae[3]	*Streptococcus pyogenes*
Halomonas hamiltonii	*Streptococcus sanguinis*[6]
Kingella denitrificans	*Streptococcus* sp. HMT055 / HMT058 / HMT064[2] / HMT066[2] / HMT074
Kingella oralis[3]	*Streptococcus vestibularis*
Lautropia mirabilis[7]	*Veillonella atypica*[2]
Leptotrichia hongkongensis	*Veillonella dispar*[3]

(continued)

Table 2. (continued)

Leptotrichia sp. HMT212 / HMT217 / HMT219 / HMT225 [2] / HMT392	*Veillonella parvula*[3]
Listeria monocytogenes	
Gingivitis / perimucositis	
Acetobacter indonesiensis	*Neisseria menigintidis**
Absconditabacteria (SR1) [G-1] bacterium HMT345[3]	*Ottowia* sp. HMT894[3]
Actinomyces odontolyticus	*Peptostreptococcaceae* [XI] [G-7] *[Eubacterium] yurii subsps. yurii* and *margaretiae*
Actinomyces oris	*Peptostreptococcaceae* [XI] [G-7] bacterium HMT081
*Aggregatibacter*sp. HMT898	*Peptostreptococcus stomatis*[2]
*Alloprevotella*sp. HMT914	*Prevotella denticola*
Capnocytophaga gingivalis	*Porphyromonas* sp. HMT275*
Capnocytophaga granulosa	*Prevotella oulorum*
Cardiobacterium valvarum	*Streptococcus sanguinis*
Catonella morbi	*Saccharibacteria* (TM7) [G-1] bacterium HMT346 / HMT437
Corynebacterium matruchotii	*Selenomonas infelix*[2]
*Eikenella corrodens**	*Selenomonas noxia*
Filifactor alocis[2]	*Selenomonas artemidis**
Fusobacterium nucleatum	*Selenomonas* sp. HMT136 / HMT149* / HMT892*
Fusobacterium nucleatum polymorphum[2]	*Selenomonas sputigena*[2]
Haemophilus parainfluenzae	*Staphylococcus warneri*
Lachnospiraceae [G-2] bacterium HMT096 / HMT100[3]	*Streptococcus anginosus*
Lautropia mirabilis	*Streptococcus cristatus*
Leptotrichia hongkongensis	*Streptococcus mitis*
Leptotrichia sp. HMT212[3] / HMT223[2] / HMT498[2] / HMT909	*Streptococcus pseudopneumoniae*
Leptotrichia trevisanii	*Tannerella* sp. HMT286[2]
Neisseria oralis	*Treponema socranskii**
Neisseria perflava	
Periodontitis / peri-implantitis	
Achromobacter xylosoxidans	*Peptostreptococcaceae* [XI] [G-1] *[Eubacterium] infirmum*[2]
Acidipropionibacterium acidifaciens	*Peptostreptococcaceae* [XI] [G-1] *[Eubacterium] sulci*
Actinomyces johnsonii	*Peptostreptococcaceae* [XI] [G-2] bacterium HMT091[2]
Actinomyces massiliensis	*Peptostreptococcaceae* [XI] [G-4] bacterium HMT103 / HOT369[2]
Actinomyces oricola	*Peptostreptococcaceae* [XI] [G-5] *[Eubacterium] saphenum*[6]
Aggregatibacter actinomycetemcomitans	*Peptostreptococcaceae* [XI] [G-6] *[Eubacterium] minutum*[4]
*Aggregatibacter aphrophilus**	*Peptostreptococcaceae* [XI] [G-6] *[Eubacterium] nodatum*[3]
Aggregatibacter sp. HMT458	*Peptostreptococcaceae* [XI] [G-7] *[Eubacterium] yurii subsps. yurii* and *margaretiae*[2]
Alloprevotella rava	*Peptostreptococcaceae* [XI] [G-9] *[Eubacterium] brachy*[3]
Alloprevotella sp. HMT308	*Peptostreptococcaceae* [XI] [G-1] bacterium HMT383
Alloprevotella tannerae	*Peptostreptococcaceae* [XI] [G-3] bacterium HMT495
Anaeroglobus geminatus[3]	*Peptostreptococcus stomatis*[4]
Anaerolineae [G-1] bacterium HMT439[4]	*Porphyromonas endodontalis*[8]
Atopobium rimae[2]	*Porphyromonas gingivalis*[7]
Bacteroidaceae [G-1] bacterium HMT272[2]	*Porphyromonas* sp. HMT285
Bacteroidales [G-2] bacterium HMT274[3]	*Prevotella baroniae*
Bacteroides heparinolyticus	*Prevotella buccae*[2]
Bacteroidetes [G-3] bacterium HMT280[2] / HMT281 / HMT365	*Prevotella dentalis*
Bacteroidetes [G-6] bacterium HMT516[2]	*Prevotella denticola*[2]
Bacteroidetes [G-1] bacterium HMT272	*Prevotella enoeca*
Bacteroidetes [G-2] bacterium HMT274	*Prevotella histicola*
Bacteroidetes [G-4] bacterium HMT509	*Prevotella intermedia*[4]
Campylobacter concisus	*Prevotella maculosa*
Campylobacter gracilis	*Prevotella melaninogenica*
Campylobacter rectus	*Prevotella multiformis*
Capnocytophaga sp. HMT335	*Prevotella loescheii**
*Campylobacter showae**	*Prevotella marshii**
*Campylobacter sputorum**	*Prevotella nigrescens*[2]
Catonella morbi	*Prevotella oralis*[3]
Catonella sp. HMT451 / HMT164*	*Prevotella oris*
Centipeda periodontii	*Prevotella oulorum*
*Clostridium botulinum**	*Prevotella pallens*
Clostridiales [F-1] [G-1] bacterium HMT093	*Prevotella pleuritidis*
Corynebacterium matruchotii	*Prevotella* sp. HMT292 / HMT296 / HMT300 / HMT301 / HMT305 / HMT315 / HMT317 / HMT376 / HMT443 / HMT475 / HMT526[3]
Desulfobulbus sp. HMT041[6]	*Prevotella tannerae*
Desulfomicrobium orale	*Prevotella veroralis*

(continued)

Table 2. (continued)

Dialister invisus[3]	*Pseudoramibacter alactolyticus*[5]
Dialister pneumosintes	*Pyramidobacter piscolens*
Dialister sp. HMT119	*Rothia aeria*[2]
Filifactor alocis[8]	*Rothia dentocariosa*
Fretibacterium sp. HMT361[6] / HMT452[2] / HMT453[3] / HMT359 / HMT360[7] / HMT362[3]	*Saccharibacteria* (TM7) [G-1] bacterium HMT346[4] / HMT349[3] / HMT351 / HMT356[3] / HMT437[2]
Fretibacterium fastidiosum[7]	*Selenomona flueggei*
Fusobacterium naviforme	*Selenomonas dianae*[2]
Fusobacterium nucleatum subsp. *animalis*[3]	*Selenomonas noxia*
Fusobacterium nucleatum subsp. *nucleatum*[2]	*Selenomonas* sp. HMT126 / HMT134 / HMT149 / HMT478
Fusobacterium nucleatum subsp. *polymorphum*	*Selenomonas sputigena*[5]
Fusobacterium nucleatum subsp. *vincentii*[2]	*Shuttleworthia satelles*
Fusobacterium sp. HMT203[3]	*Slackia exigua*
Johnsonella sp. HMT166[2]	*Solobacterium moorei*
Lachnoanaerobaculum saburreum	*Stomatobaculum* sp. HMT373[3]
Lachnospiraceae [G-8] bacterium HMT500[4]	*Streptococcus intermedius**
Lactobacillus salivarius	*Streptococcus anginosus*
*Lactobacillus psittaci**	*Streptococcus constellatus*[4]
Leptotrichia sp. HMT498 / HMT215 / HMT417	*Streptococcus cristatus*
Leptotrichia buccalis	*Streptococcus gordonii*
Leptotrichia shahii	*Streptococcus infantis*
Leptotrichia wadei	*Streptococcus equis**
Leptotrichiaceae [G-1] sp. HMT210	*Streptococcus mitis* bv 2
*Megasphaera elsdenii**	*Streptococcus oralis*
Mitsuokella multacida	*Streptococcus oralis* subsp. *tigurinus* clade 071
Mogibacterium diversum	*Streptocuccus parasanguinis* II
Mogibacterium neglectum	*Streptococcus agalactiae**
Mogibacterium pumilum	*Staphylococcus pettenkoferi**
Mogibacterium timidum[4]	*Staphylococcus hominis**
Mollicutes [G-2] bacterium HMT906	*Staphylococcus castoreus**
Mycoplasma faucium[2]	*Tannerella forsythia*[8]
Mycoplasma salivarium[2]	*Tannerella* sp. HMT808[2] / HMT286*
Neisseria sicca	*Treponema amylovorum*[2]
*Neisseria elongata**	*Treponema denticola*[7]
Neisseria subflava	*Treponema lecithinolyticum*[3]
Olsenella sp. HMT809	*Treponema maltophilum*[5]
Oribacterium sp. HMT078 / HMT102	*Treponema medium*[4]
Oribacterium sp.	*Treponema parvum*[2]
Ottowia sp. HMT894	*Treponema putidum*
Parvimonas micra[4]	*Treponema socranskii*[10]
Parvimonas sp. HMT110 / HMT393	*Treponema* sp. HMT230[2] / HMT231 / HMT235 / HMT236 / HMT237[3] / HMT238 / HMT246 / HMT257[2] / HMT268 / HMT490[2] / HMT664 / HMT769 / HMT258* / HMT249* / HMT269*
Peptococcus sp. HMT167	*Treponema vincentii*
Peptoniphilaceae [G-1] bacterium HMT113[3]	*Veillonellaceae* [G-1] bacterium HMT129[2] / HMT132[2] / HMT135[3] / HMT145[3] / HMT148 / HMT150[3] / HMT155[2] / HMT483*

Summarized data were obtained from studies using high-throughput 16S rRNA gene sequencing as the microbiome analysis method, including the Life Sciences 454 pyrosequencing platform (Huang et al. 2011; Griffen et al. 2012; Abusleme et al. 2013; Dabdoub et al. 2013; Ge et al. 2013; Kistler et al. 2013; Maruyama et al. 2014; Hong et al. 2015; Kirst et al. 2015; Park et al. 2015) and Illumina platform (Apatzidou et al. 2017; Sanz-Martin et al. 2017; Schincaglia et al. 2017). In addition, unpublished data from Colombo and coworkers were included (Illumina platform). Only studies analyzing subgingival dental plaque at the species/phylotype level were considered. The species/phylotypes shown in each clinical condition were detected at significantly high abundance and/or prevalence in that clinical status as reported in the studies. Some species/phylotypes were abundant in >1 clinical condition. Core bacteria are those detected in high frequency in health and disease. Taxonomic status of the species/phylotype nomenclature was based on the expanded Human Oral Microbiome Database (http:// www.homd.org).

[1–10]Superscript numbers refer to the number of studies in which those species/phylotypes were related to the same clinical condition.

Underlined names refer to microorganisms also abundant in mucositis and peri-implantitis.

*Asterisks refer to bacteria detected in high abundance only in peri-implant diseases.

periodontitis-associated microbiotas, investigations focused on how bacteria could trigger immune/inflammatory responses in the host, and models of etiopathogenesis were proposed (Marsh 1994; Page et al. 1997; Hajishengallis et al. 2012). Page et al. (1997) described the multifactorial concept of destructive periodontal diseases, combining the microbial challenge, the host response, the microenvironmental changes, and the modulating local, genetic, and environmental risk/indicator factors. The host, bacteria, and clinical outcomes in enamel and dentin caries were outlined by Takahashi and Nyvad (2016), with the

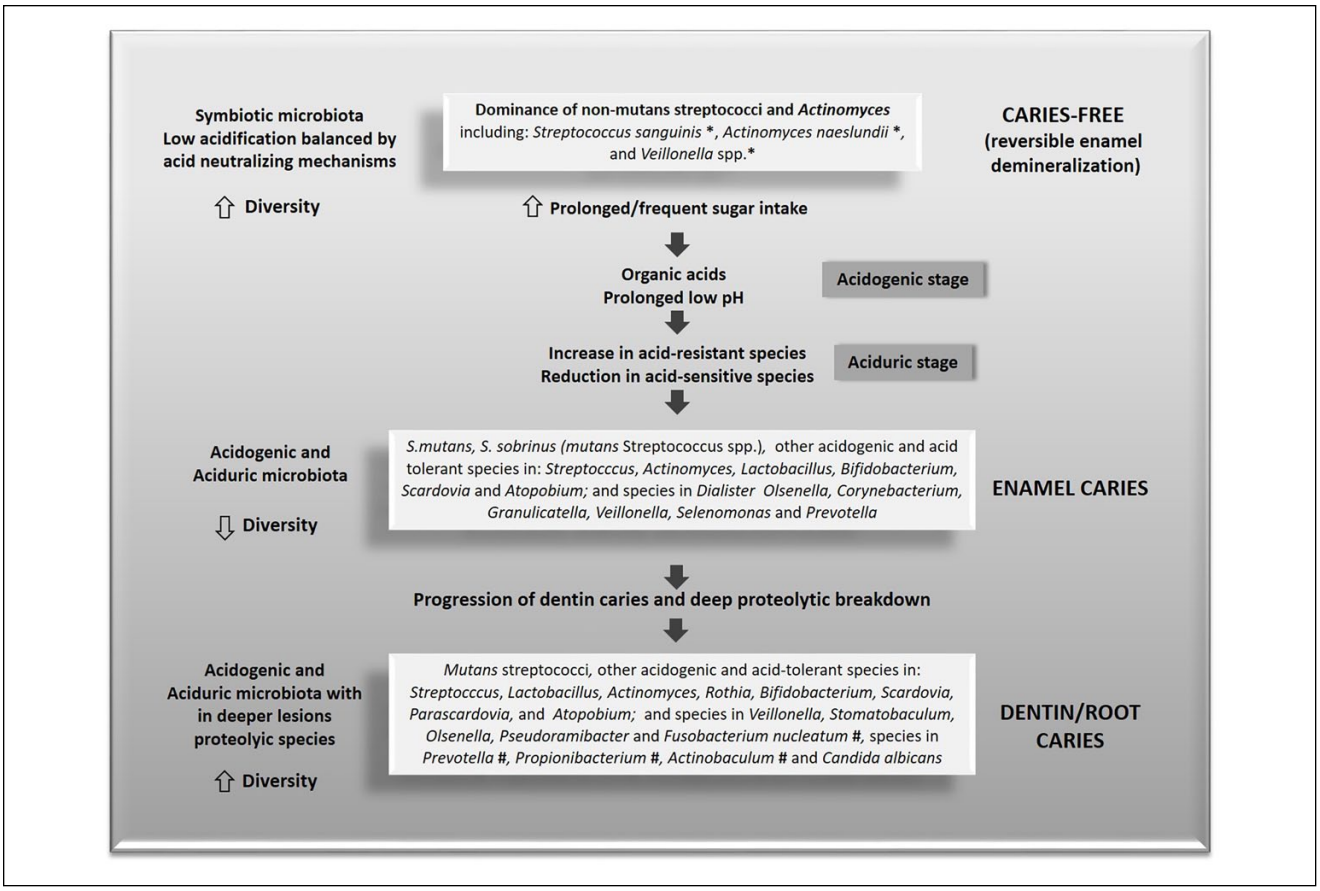

Figure 2. Outline of microbiotas associated with dental caries, taken in part from Takahashi and Nyvad (2016). Caries-free microbiotas are characterized by a diverse community that responds to dietary carbohydrate challenge by acid production that is neutralized by community activity, including ammonia deiminase and urease activity. With increasing acidic environments, acid-sensitive species are suppressed with enrichment for acid-tolerant and highly acidogenic species and with reduction in community diversity. Caries progression into dentin can be associated with proteolytic species. At this stage, the diversity of the microbiota increases but with different species than those of caries-free sites. Different species within genera are associated with either health or disease (for speciation, see text referencing the following: Burne and Marquis 2000; Brailsford et al. 2001; Marchant et al. 2001; Munson et al. 2004; Caufield et al. 2007; Mantzourani et al. 2009; Tanner et al. 2011; Belda-Ferre et al. 2012; Gross et al. 2012; Thomas et al. 2012; Torlakovic et al. 2012; Eriksson et al. 2018). Each stage is likely to be colonized by core microbiome species and, if associated with gingival inflammation, gingivitis-associated species. *Species that can counter local acidity. #Species with proteolytic activity.

proposal that function of the bacterial community would better describe cariogenesis than detection of individual species (Takahashi 2015). More recent studies incorporating molecular biology and physiology of the microbiome, as well as the host's innate and acquired immune responses, continue to reveal that multiple elements play a role in disease.

The complex mechanisms of microbiota-environment-host interactions indicate that periodontal diseases and caries are not classical infections caused by one or few "true" pathogens. The dental microbial communities are diverse with considerable variation, and it becomes important to understand how each member or combinations contribute to pathology. Studies have focused on specific species, including *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* for periodontitis and *S. mutans* and *Lactobacillus, Actinomyces, Bifidobacterium*, and *Scardovia* species for caries. Recognition of novel health- and disease-related species/phylotypes should lead to investigations to unravel additional mechanisms of interactions (Kumar et al. 2005). Furthermore, the importance of the oral microbiome as 1 complex entity has led to development of metagenomic, metatranscriptomic, and metaproteomic approaches to explore community composition and activity in situ. Metagenomics examines the genetic potential and relative abundance of metabolic capabilities of dental plaque and has been used in periodontal and caries studies (Simón-Soro et al. 2014; Takahashi 2015; Yost et al. 2015).

Although oral microorganisms involved in disease are likely commensal members of the microbiota, not all bacteria have the same pathogenic potential. While certain virulence factors are intrinsic to specific species, others may be acquired during development of disease (Vayssier-Taussat et al. 2014). Low numbers of more virulent microorganisms may be sufficient to cause disease-related dysbiosis. This "infective dose" can differ for individual species and strains of the same species within the oral microbiome (Haubek et al. 2008).

The biological events from health to disease at periodontal or tooth sites are dynamic. In the ecologic model of pathogenesis, the selective pressure of the microenvironment shapes the

microbiota, favoring flourishing of a pathogenic microbiome (Marsh 1994). Maturation of disease-associated biofilms occurs in a sequence of interactions with pathogenic climax communities building on existing primed bacterial complexes, rather than blooms of single pathogens in diseased sites (Socransky et al. 1998; Takahashi and Nyvad 2016). Early stages of plaque development with changes in the metabolic activities and composition of the microbiomes precede the clinical manifestations of gingivitis or enamel demineralization. For instance, healthy sites from patients with periodontitis or caries frequently have a disease-like microbiome even in the absence of inflammation or demineralization (Aas et al. 2008; Liu et al. 2012). It can take time for dental biofilms to change sufficiently to induce a significant host inflammatory response (Löe et al. 1965; Page et al. 1997).

In periodontology, low-abundance "keystone pathogens" such as *P. gingivalis* combined to synergistic/antagonistic interactions among commensals and pathobionts of the subgingival biofilm may trigger dysbiosis of the microbial community (the polymicrobial synergy and dysbiosis model) by sophisticated strategies to evade or subvert the host immune system (Hajishengallis et al. 2012; Lamont and Hajishengallis 2015). Members of subgingival microbiota can impair innate immunity, alter the composition of the microbiota, and increase the virulence of the entire community. This community model of disease is reminiscent of the mixed anaerobic infection models of the 1960s (Macdonald et al. 1963; Socransky and Gibbons 1965).

A refined model of pathogenesis of periodontal/peri-implant diseases combines multiple concepts (Meyle and Chapple 2015) that overcome the natural colonization resistance of the health-associated microbiome. The transition from a host-compatible symbiotic to an incipient dysbiosic microbiota of gingivitis and perimucositis involves an acute inflammatory immune response, which induces tissue breakdown-derived nutrients for the bacteria, creating a self-perpetuating pathogenic cycle. Some individuals may tolerate a dysbiotic microbiota by eliciting a self-resolving inflammatory response. This cyclic interaction may persist for years in nonsusceptible individuals or evolve more rapidly to a frank dysbiosis associated with an ineffective nonresolving inflammatory/immune response that culminates in periodontal tissue destruction. In dental caries, beneficial species in the health-associated community have resilience mechanisms to counterbalance local acidity produced after a carbohydrate challenge (Rosier et al. 2018). For instance, *Veillonella* species use lactic acid as an energy source, whereas *A. naeslundii* by urease activity and *S. sanguinis* with arginine deiminase, produce ammonia (Burne and Marquis 2000). Limiting local acidity can reduce risk of demineralization and allow remineralization to occur without pathology. This cycle gets broken, however, with prolonged acidity from frequent dietary carbohydrate or reduced saliva flow induced by systemic diseases or certain medications. Thus, disturbance of the healthy balance of the microbiome, host, and environment can lead to development of a microbiome that contains pathogenic traits and increase susceptibility to disease.

Concluding Remarks: Lessons to Be Learned in the Next Century

Our concepts of microorganisms as disease-causing agents have evolved as we understand the crucial role of bacteria in maintaining human homeostasis. Oral health represents a balance among a coevolved and adapted indigenous microbiota, an adequate and efficient host response, and an undisturbed microenvironment. From the era incorporating microscopic and culture studies to the current heavy reliance on molecular approaches, oral biofilms are recognized to be sophisticated structures created by microorganisms for their survival. These microbial entities vary in composition and metabolism and consequently in levels of pathogenicity in health and disease.

Contemporary microbiome studies indicate that individual pathogens are not always obvious etiologic agents in caries or periodontitis/peri-implantitis and that these diseases manifest as a result of a disequilibrium in the dynamic relationships among biofilm, host, and microenvironment. Translating the nuances of interactions among microbial species, including the ones not yet grown and potential coinfecting viruses and yeasts, and how they interact to deregulate host tissues constitutes major research challenges. Therapies focused on restoring and maintaining the host-microbiome balance and controlling for a range of modulating risk factors will drive personalized management of oral diseases. Contemporary hypotheses and concepts will be probably discarded, refined, or consolidated. To successfully address these exciting issues, it is essential to ensure that we remain open to new ideas, facilitating valuable translational collaborations and exchanges among researchers, clinicians, and patients.

Author Contributions

A.P.V. Colombo, contributed to conception and design, drafted and critically revised the manuscript, A.C.R. Tanner, contributed to conception and design, critically revised the manuscript. Both authors gave final approval and agree to be accountable for all aspects of the work.

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