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REVIEW ARTICLE

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Dental caries and their microbiomes in children: what do we do now?

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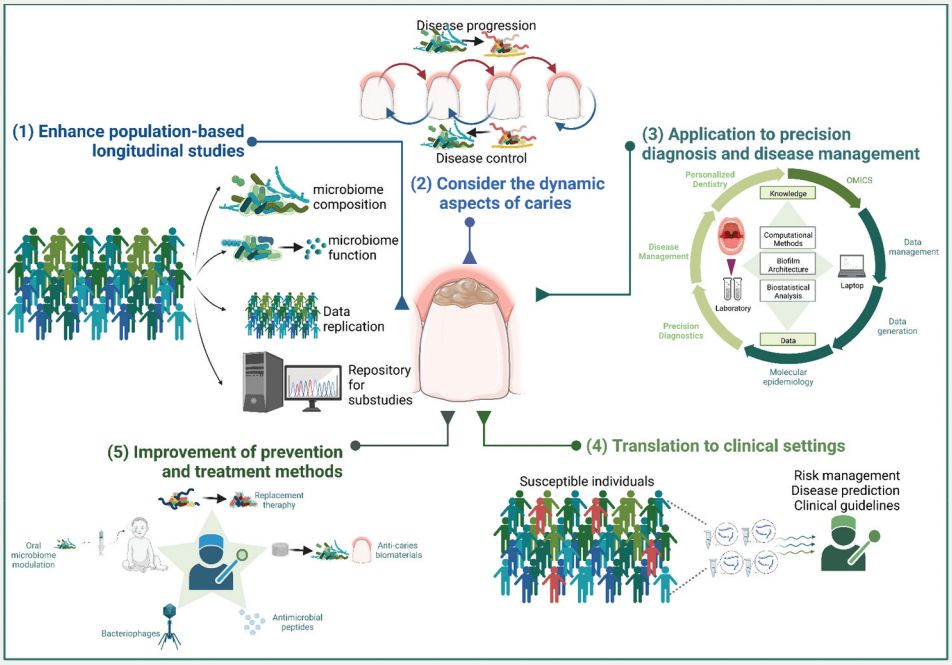
ABSTRACT

The oral cavity is an unique ecosystem formed by different structures, tissues, and a complex microbial community formed by hundreds of different species of bacteria, fungi, viruses, phages, and the candidate phyla radiation (CPR) group, all living in symbiosis with healthy individuals. In an opposite state, dental caries is a biofilm-mediated dysbiosis that involves changes in the core microbiome composition and function, which leads to the demineralization of tooth tissues due to the fermentation of dietary carbohydrates, producing acid by select oral bacteria. The cariogenic biofilm is typically characterized by bacterial species with the ability of adhering to the saliva-coated tooth surface, production of exopolysaccharides-rich matrix (which will limit the diffusion of acidic products of carbohydrate fermentation), and the ability of surviving in this acidic environment. Besides years of research and dental treatment, dental caries remains the most common chronic disease in children worldwide. This article aims to bring an insightful discussion about important questions that remain unanswered in the Cariology and Oral Microbiology fields, to move Science forward, characterize the interrelationships of these communities, and understand mechanistic functions between microorganisms and the host, therefore leading to translatable knowledge that benefits the provision of care to our pediatric patients.



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Dental caries remains the most prevalent disease in humans [1,2–4], and is the most common chronic disease in children between the ages of 5 and 17 years in the US, as well as worldwide [5–7]. The prevalence of untreated caries among US children remains high (between 41.4 and 45.7% among 1- to 9-year-olds) [8]. It is estimated that over 51 million school hours are lost each year due to oral health problems [5].

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This issue especially affects the most vulnerable groups, typically low-income children, many of whom are from racial or ethnic minorities, enhancing oral health disparities. It is a recognized challenge in public health, and efforts to address the inequity are urgent [7,9–11].

Decades of research have led to the development of new materials, new equipment and technologies focused on operative treatment, for advancing our understanding about the biology of the disease and to efficiently prevent or manage it. The contemporary understanding of caries is that it is a biofilm-mediated disease, where a diverse microbial community promotes disease initiation and progression strongly influenced by fermentable carbohydrate frequency, quality and availability [12].

However, dental caries is a very complex disease. In most cases, caries can be controlled by good oral hygiene practices, focusing on biofilm control, frequent exposure to fluorides, and a balanced diet with low intake of fermentable carbohydrates, especially between meals. But there are exceptions in that some individuals with poor oral hygiene practices, and/or frequent consumption of sugary snacks, do not necessarily develop the disease. Also, individuals with dental caries have different progression rates, and some are more susceptible or predisposed to the disease than others. Consequently, several key questions are raised. How can these differences be explained? Are there important differences in the virulence of the bacterial strains that compose the oral microbiome? Is there a genetic predisposition of the host to caries susceptibility? In helping to answer these questions, this review will address the following four topics: (1) The oral microbiome and its association with health in children; (2) Dysbiosis in the oral microbiome and dental caries development in children; (3) Dental caries is more complex than previously believed; and (4) What's left?

The oral microbiome and its association with health in children

The composition of the oral microbiome is in a state of dynamic flux that is driven by the unique environment of the oral cavity and the interplay between microorganisms, environmental exposures and host factors. The oral cavity is formed by different structures and tissues, such as saliva, gingival fluid, and keratinized/non-keratinized epithelial or mineralized tooth surfaces, including the tongue, gingiva and teeth. These structures form micro-environments with unique characteristics for bacterial colonization and community development [13–17]. As a result, the mouth is the second most heavily colonized part of our bodies and the commensal microbiome of the oral structures consists of microorganisms that live in symbiosis with healthy individuals. This balance is possibly due to the diverse commensal microbial community that prevents the colonization of foreign pathogens and contributes to host ecology and physiology [14]. The oral microbial community collectively comprises more than 700 bacterial species, of which about 30% still have yet to be cultivated [12,18,19,20]; https://www.homd.org/), with distinct subsets populating discrete niches in the oral cavity [21].

The unique features of the oral cavity and the environment shape the oral microbiome from birth to adult age. The early oral environment for initial microbial colonization is strongly shaped by exposure to the mother and delivery mode (vaginal *vs* caesarean) [22]. This initial exposure also shapes the diversity of the oral microbiome later in the infant's life as vaginally born children show a proportionally significant difference in oral taxa at 3 months that discriminates them from children born by caesarean section [22]. Although the delivery mode does not appear to have an impact on species richness, infants born by C-section have higher bacterial diversity at 12 months of age [23,24]. Metabolic products of pioneer colonizers, such as species of *Streptococcus* and *Actinomyces*, acquired at birth and the following hours, can alter the environment and benefit other species (including more strictly anaerobic genera like *Veillonella* and *Fusobacterium*) [19,25]. Furthermore, the nature of feeding also has demonstrable effects on oral microbiome composition with 3-month-old: significantly higher proportions of lactobacilli was observed among breast-fed infants, compared with infants fed exclusively by formula [26], while children not being breastfed through 12 months of age have higher bacterial species diversity [23]. As the baby ages, microbial communities develop further and increase in microbial diversity. With the eruption of teeth, non-shedding surfaces are now available for selective colonization of a new community of bacterial species, known as obligate, hard surface colonizers [27]. Furthermore, new retention sites at the epithelia-tooth interface (gingival crevice) form a new habitat for yet another bacterial consortium [19,28]. The emergence of teeth is also accompanied by a steady increase in diversity and richness of the oral microbiome, especially between the first and second years of age [23]. Current knowledge indicates that the oral microbiome reaches adult-like stability around 2 years of age, and by the age of 3, it is characterized by high variability [25]. By the age of 6, the beginning of the mixed dentition (with loss of primary teeth and the eruption of permanent teeth) represents a new phase of major

dynamic alterations in oral microhabitats, marked by a shift in the composition of the oral microbial communities [29].

Once established, the stability of the pioneer species of the oral microbiome is sustained. However, this dynamic community is in constant flux, fighting against host-protection mechanisms. For the commensal microbiome, the oral cavity is characterized by a variety of microhabitats that challenge microbial colonization and persistence, such as the constant salivary bathing of all accessible surfaces of the oral cavity and the mechanical disruptive activities of eating, swallowing and speech [13,21]. Crucial to colonization are key salivary components that bind to tooth surfaces forming the acquired pellicle on tooth enamel – this is required for the initiation of a selective bacterial attachment to tooth surfaces and further biofilm development. For this reason, dynamic ecological landscapes are formed by the teeth and epithelial surroundings that are reflected in different microbial communities that have successfully colonized all of these sites [29]. The stability of soft surface sites for colonization and biofilm development represents another challenge faced by the oral microbiome. Thus, despite the rapid turnover of oral mucosal epithelial cells, the newly exposed host cells are continuously repopulated with microbial colonizers. In contrast, the teeth provide the only natural, non-shedding surface in the human body and provide unique microhabitats that permit persistent and extensive biofilm development [30,31].

Dysbiosis in the oral microbiome and dental caries development in children

Dysbiosis is defined as a disruption to the commensal microbiome homeostasis caused by an imbalance in the microbiome, changes in their functional composition and metabolic activities, or a shift in their local distribution. The most prevalent diseases in the oral cavity, dental caries and periodontal diseases, are mainly caused by dysbiosis, which may also play an important role in altering the homeostasis of systemic conditions, including the gut microbiome and gastrointestinal diseases [16,32].

Dental caries can be defined as a biofilm-mediated dysbiosis that involves changes in the microbiome composition and function, which leads to the dissolution of tooth tissues (enamel and dentin) by acid produced by select oral bacteria, as a result of the fermentation of dietary carbohydrates [16]. When the fermentation process is enhanced by the excessive and/or frequent ingestion of fermentable sugars, saliva's buffering capacity is not enough to neutralize the acidic challenge, and the constant reduction of the pH (typically below 5.5) leads to the demineralization of enamel, cementum, and dentin. The frequent access to sugars also leads to a massive production of exopolysaccharides (EPS) that, due to its hydrophobic properties, maintain an acidic environment that results in selecting for aciduric microorganisms and creates a barrier for saliva to penetrate in the environment [33]. Due to the highly dynamic nature of the disease, resulting from continuous physical-chemical interactions between the saliva-coated tooth surface and biofilm that covers the surface, multiple pH fluctuations in the biofilm lead to episodes of mineral loss (demineralization) and mineral gain (remineralization) of the teeth. If equilibrium of these episodes is not achieved over time, demineralization will reach the level when an incipient lesion, known as active white spot lesions, can be visually detected by a trained professional [12,16].

The cariogenic biofilm is typically characterized by bacterial species with the ability of the following characteristics: (1) adhere to the saliva-coated tooth surface, (2) produce exopolysaccharides (EPS)-rich matrix, which will limit the diffusion of acidic end products of carbohydrate fermentation and, (3) survive in this acidic environment. Ultimately, these traits result in an enamel acid-dissolution due to the localized acidic pH microenvironments across the biofilm structure and at the tooth–biofilm interface (Xiao et al. 2016 [34]).

In addition to *Streptococcus mutans*, bacterial species associated with caries initiation and progression include *Actinomyces* and *Bifidobacterium* spp., respectively [35,36]. Several studies have characterized the oral microbiome in caries-active children in general, and additional species were also associated with dental caries, including *Streptococcus salivarius, Streptococcus sobrinus, Streptococcus parasanguinis, Scardovia wiggsiae, Slackia exigua, Lactobacillus salivarius, Parascardovia denticolens*, and species of *Porphyromonas, Actinomyces*, and *Veillonella* [37–41]. The species associated with caries initiation and development (including species of *Streptococcus, Actinomyces*, and *Lactobacillus*) are saccharolytic bacteria – hence they can metabolize dietary carbohydrates and some sugar alcohols to initiate lactic acid production resulting in potential enamel demineralization [42]. More specifically, during mealtime when sugar is often in excess supply, lactate dehydrogenase is activated, resulting in the dominant production of lactate. Furthermore, pyruvate kinase is activated, which accelerates glycolysis and lactate production (Abbe et al. [43]). Conversely, *Veillonella* species utilize lactate as an essential carbon and energy source, converting it to pyruvate and succinate by enzymatic reactions, respectively, by a series of enzymatic reactions [42]. The excess of carbohydrates also promotes the accumulation of intracellular polysaccharides, which can be used when the extracellular sugar

supply is limited (as in-between meals) as energy sources [42]. *Scardovia wiggsiae* and species of *Bifidobacterium* exhibit high acetic and lactic acid production from glucose through a unique metabolic pathway different than the glycolytic pathway of *Streptococcus, Actinomyces*, and *Lactobacillus*. *Scardovia wiggsiae*, as well as *S. mutans* and species of *Lactobacillus*, are very tolerant to lactic acid and low pH [44].

Children under the age of 6 years have primary dentition and the presence of one or more caries lesions at this phase is defined as Early Childhood Caries (ECC, Bangkok declaration [45]). The prevalence of ECC increases with age, ranging from 12 to 27% among 2- to 3-year-old children [46–48] to 27 to 48% among 4- to 6-year-old children [49,50]. The incidence of ECC in the United States ranges from 3 to 28% [10,40,51]. The characteristics of the oral microbiome in patients with or without ECC were analyzed using molecular assays and revealed that *S. mutans* was strongly associated with the severe form of ECC (S-ECC) [52], which affects children younger than 36 months of age, and is characterized by any sign of dental caries in smooth tooth surfaces [53]. Other species significantly associated with S-ECC included *Scardovia wiggsiae* [54], and *Bifidobacteriaceae* [55]. Another study evaluated the bacterial profiles and potential biomarkers in saliva and supra-gingival biofilm samples from children with S-ECC by using HOMIM (the Human Oral Microbe Identification Microarray) and verified that several genera, including *Streptococcus, Porphyromonas*, and *Actinomyces*, are strongly associated with S-ECC and can be potential biomarkers of dental caries in the primary dentition [40]. Interkingdom associations, especially between *S. mutans* and *Candida albicans*, have an important role in ECC. *C. albicans* is often detected at higher levels in the oral cavity of children with severe forms of ECC as compared with caries-free children, and fungal presence positively correlated with caries severity and *Streptococcus mutans* carriage [56].

Significant changes at genera levels were observed by Du et al. [57] in the transition from primary to mixed dentition. At the age of 6 and beginning of mixed dentition, 17 genera were observed to increase in mixed dentition and two decreased, including one predominant genus in the primary dentition, *Haemophilus*. The caries children harbored a significantly higher proportion of *Corynebacterium* in the mixed dentition group, while samples from caries lesions showed a significant decrease in *Fusobacterium* and *TM7*. However, although slight differences between the microbiome in the primary and mixed dentitions have been reported [12,57,58], no 'specific bacterium', unique to a particular dentition, can be listed which supports the concept that once the microbiome is established, the disease development will be characterized by the shift in abundance of specific groups in response to changing environmental variables, leading to dysbiosis, rather than the 'appearance' of novel cariogenic species or the pathogenicity of a single species that will enable differentiating dental caries in primary or mixed dentitions.

Dental caries is more complex than previously believed

The oral cavity is a complex and heterogenous ecosystem with many variables influencing the microbial composition and function. Early studies on caries development were primarily based on culture-dependent methods and, consequently, *S. mutans* and its virulence characteristics were considered the primary etiologic agents of caries. However, more recent studies have employed culture-independent methods based on molecular analysis of the conserved 16S rRNA genes. By using molecular techniques, it has been shown that the bacterial microbiome associated with dental caries is polymicrobial, as well as tissue-dependent, since the bacterial composition and biochemical profile are specific to the tissue affected, e.g. the microbial composition and activity at the initial enamel lesions are significantly different from cavitated enamel and dentinal lesions [17,59]. It is noteworthy that *S. mutans*, the most studied caries-associated species, represents only 0.02–0.73% of the total bacterial community [60] and that approximately 15% of the individuals who develop caries do not have *S. mutans* [61,62,], [63,64]. Other caries-associated bacteria, in addition to *S. mutans*, include species of *Actinomyces, Abiotrophia, Atopobium, Bifidobacterium, Lactobacillus, Olsenella, Pseudoramibacter, Scardovia, Selenomonas,* and *Veillonella* [35,60,64–69]. The contribution of some of these species to the disease, in terms of metabolic activity, was reviewed deeply by Takashi in 2015 [41].

The microbial communities found on sound surfaces are significantly different from the communities at surfaces with active white spot lesions [66,68,69] and open or closed dentinal lesions [17]. In particular, *Actinomyces gerencseriae, A. naeslundii, A. israelii, A. viscosus, Prevotella nigrescens, Dialister micraerophilus, Eubacterium_XI G 1 infirmum, Streptococcus sp_Oral_Taxon_065,* and *Corynebacterium matruchotii* were more abundant on surfaces with initial enamel lesions (active white spot lesions) [60,66,68,69]. Furthermore, many studies support the findings that bacterial species, either alone or as a group, other than *S. mutans*, may also play major roles in caries development [36,59,62,66,69,70]. A diverse community, mainly represented by the genera *Lactobacillus, Shlegelella,*

Pseudoramibacter, and *Atopobium* appeared to be clearly associated with dentinal caries lesions. In some studies, lactobacilli were commonly found in dentin cavities [59,60].

In a small cohort study, we showed that there were significant differences in the microbiome of white spot lesions (early stage) of caries-active children compared to control children. Eighteen species were associated with caries activity or health [68]. Our results corroborated previous findings showing that *S. mutans* could be found in healthy and diseased sites and suggested that other species of *Lactobacillus, Prevotella, Propionibacterium*, non-mutans streptococci and *Actinomyces* also played important roles in caries initiation and biofilm community interactions [59,60]. We also showed that high consumption of fermentable carbohydrates was associated with a shift in bacterial microbiome composition and a reduction in bacterial diversity. Among patients with high frequency of carbohydrate consumption (i.e. more than two times between meals), statistically significant differences in the relative abundances were observed with two taxa not normally associated with caries (*Yersinia mollaretti* and *Streptococcus sp._Oral_Taxon_487*). In addition to traditional bacterial taxa, the oral microbiome represents a multi-kingdom environment, including fungi, the 'Candidate Phyla Radiation' (CPR) bacterial group and human and bacterial viruses. To date, knowledge regarding the cariogenic potential of fungi and 'tiny' (CPR group bacteria known as TM7 and viruses/phages) residents is lagging behind [71,72]. Recent studies observed that *Candida albicans* does play an active role in the pathogenesis of dental caries, being more prevalent in the oral cavity of children with Early Childhood Caries (ECC) compared to children without caries [73,74]. A study showed that the presence of *C. albicans* in the oral cavity of preschool children increases the risk of developing dental caries by nearly seven times [56]. The presence of oral *C. albicans* is also associated with changes in oral bacterial composition such as highly acidogenic and acid-tolerant bacterial community, abundance of *S. mutans*, and species of *Lactobacillus, Prevotella*, and *Scardovia*. *Candida* species from dental biofilms of HIV+ children can cause demineralization of primary enamel *in vitro* [75]. Biofilms of *C. albicans* and *S. mutans* are more voluminous than *S. mutans* biofilms without *C. albicans* in enamel [76] and dentinal cavities [72]. Apparently, *Candida albicans* enhances the cariogenic potential of *S. mutans* biofilms by the interaction of the cell wall of *C. albicans* and the EPS matrix formed by *S. mutans*, increasing dentin demineralization [72]. Recent studies also showed that *Candida* species are frequently detected with heavy infection of *S. mutans* in biofilms from children affected with ECC [56,77,34].

The diversity of the human oral phageome is astounding. It is estimated that there are over 2,000 oral phages known to infect species mostly in the phyla Actinomycetota (formerly Actinobacteria, that contains cariogenic species of *Scardovia* and *Actinomyces*), Bacteroidota, Fusobacteriota, Pseudomonadota and Bacillota (formerly Firmicutes, which contains cariogenic species of *Streptococcus* and *Lactobacillus*) [78]. The mechanisms by which phages impact the ecology of oral biofilms are largely unknown. Phages have their own virulence factors and defense mechanisms against their bacterial host. However, it has been suggested that phages may be important ecological factors in determining the establishment, maintenance, and pathogenicity of cariogenic bacteria. For example, it has been shown that *Streptococcus*-specific phages may increase bacterial virulence [79]. Furthermore, phages that infect species of *Actinomyces* promote biofilm development [80].

These advances, e.g. via Next-Generation Sequencing and bioinformatic tools, helped us to begin to understand the complex oral microbiome profiles containing bacteria, fungi and viruses associated with caries disease. However, even with these molecular bioinformatic advances, new questions arose; What are they actually doing in the community? Is there any downstream effect of caries? How can we prevent them from doing it? What are the mechanisms that can be used to interfere with what are they doing? Can we develop new efficacious and safe means of therapy? Is prevention enough? In other words … what else can we do – What's left?

What's left?

As discussed above, there is substantial literature available describing the oral microbiome and its role in the development of caries. Although this review does provide a basic understanding of the disease process, we want to focus more on what we can do next with the microbiological information that has been amassed over the last 50 years.

The evidence shows that the human microbiome comprises 10 trillion bacterial cells, as opposed to 1 trillion human cells that make up the body itself. Microbial and viral genes outnumber human genes dramatically: the Human Microbiome Project collected ~4.5 trillion bases of DNA (1500 times the human genome). While these numbers show that humans are more microbes than human, considerably more funding goes to human genome sequencing, although evidence has shown the impact of the human microbiome in maintaining health or driving systemic diseases (https://hmpdacc.org/).

Among the different body sites, 26% of the whole bacterial community colonizes the oral cavity. Knowledge gathered over the recent years indicates the importance and influence of the oral microbiome community in oral and systemic diseases, such as cardiac arrest, lung disease, colorectal cancer, and Alzheimer's disease [81–84]. The development of PCR-based techniques and NGS sequencing for the characterization and quantification of bacterial, viral, and fungal compositions caused a paradigm shift in the research of biofilm microbiome associated with health and disease. However, it is time to migrate from pure annotation to characterization of the interrelationships within these communities and understanding of mechanistic functions between microorganisms and the host. For instance, the use a metatranscriptomic approach to evaluate the influence of dietary habits on the oral microbiome in a single caries-free individual showed that there were no changes in the active bacterial microbiome before and after a meal with carbohydrate intake. Consequently, this homeostasis indicates that the microbiome of some individuals is not affected by food ingestion, potentially reducing the risk of acidic pH and promoting dental health [85]. Another important consideration is that the oral microbial community (and thus, activity) changes with aging. For example, members of the phylum Firmicutes decreased and phylum Proteobacteria increased in children, while the opposite was observed in young and older adults [86]. In the elderly, the caries-active and caries-free patients showed similar community structure, suggesting that disease status may not markedly influence bacterial composition but rather cause a disruption of the activity of specific microorganisms as a result in dysbiosis in the caries process [87].

Therefore, what important questions remain unanswered in the Cariology and Oral Microbiology fields that can lead to applications to be translated to benefit the way we provide care to our pediatric patients? In our opinion, listed below is what we need to do now to move forward into future dental caries and oral microbiome research:

1. Large-scale, population-based longitudinal studies

To date, no prospective, large-scale, epidemiological studies investigating the role of the oral microbiome in dental caries have been published. Epidemiological studies from which we can draw inferences require a large number of participants, followed by accurate and reproducible assays, with the inclusion of investigations for potential factors affecting the microbiome and an understanding of its metabolic functions. Moreover, we will need to replicate findings across multiple populations and, ideally, pool data from many different study designs. Multiple analyses will have to account for variations at each step in the research pipeline: sample collection, storage, DNA extraction techniques, polymerase chain reaction (PCR) amplifications, DNA sequencing, bioinformatics pipeline, and statistical analyses [88]. Ideally, readily accessible databases complete with sequencing and metadata can be a repository of information to be used for all future research for cumulative purposes or for mining of existing data.

2. Dynamic aspects of caries progression and arrest

Studies with biological samples collected at one-time point have showed some associations between caries presence and the microbiome composition and activity. However, they depict only a static moment in a very dynamic disease, which, at the tooth surface level, dental caries is characterized by periods of demineralization and remineralization. The acidic environment resulting from biofilm metabolism selects for specific bacterial consortia, with unique virulence factors. This shift in the microbiome composition during lesion development and arrest was first studied *in vivo* by our group, using 16S rRNA gene sequencing and ^{1}H NMR spectroscopy [66]. We showed that caries lesion progression is associated with biofilm maturation characterized by an increase of Gram-negative anaerobes, including *Veillonella* and *Prevotella*, and bacterial species such as *Kingella oralis, Rothia dentocariosa, Gemella* sp. and Alloprevotella sp., marked by an increase in concentrations of lactate, acetate, pyruvate, alanine, valine, and fermentable carbohydrates. Since our study followed the continuous process of caries lesion progression and arrest *in vivo*, the experimental design used mimics what occurs *in situ*. Unlike other *in vitro* and *in situ models, t*hese data provided insights of the actual mechanisms by which biofilms play in caries progression and arrest. Furthermore, this knowledge will help identify patients who require strategies for caries prevention, as well as determine chairside risk assessment and effective disease management. Ultimately, valid biomarkers can be identified for use in clinical settings.

3. Precision diagnosis and disease management

Inter-individual differences in the composition of biofilm microbiome have been shown to result in differentiation between caries and caries-free children. The analysis of biofilm microbial composition and metabolic activity could be used as a tool for disease diagnosis by identifying individuals who are colonized by more virulent cariogenic species. It is

important to understand dental caries as an ecological change of the oral ecosystem that has led to the dysbiosis of highly individualized beneficial resident microbial communities. Specific species can harbor different abilities in key virulent aspects for caries disease, such as biofilm formation, glucans and acid production. These key factors can be used in the differential diagnosis to drive precise disease intervention. A promising future approach could use commensal microbiome that compete against *S. mutans* by producing H_2O_2 and ammonia in the prevention and treatment of dental caries. However, prospective follow-up studies that use oral microbiome as a strategy to identify individuals who would benefit from a certain probiotic intervention are still lacking, to provide evidence for the effectiveness of this approach. A further challenge will be to perform large, long-term randomized controlled trials to investigate the effect of single vs multiple personalized probiotic strains on biofilm control. Use of probiotics may be a potential adjunct therapy with standard caries preventive and management methods. Furthermore, a probiotic approach will elucidate how such strategies may mediate short- and long-term changes in oral microbiome ecology.

4. Translational research

Clinical guidelines based on targeted identification of susceptible individuals (prevention, caries risk modulation/management) before clinical symptoms appear, i.e. determine risk, are highly desirable. One avenue would be to conduct microbial metagenomic GWAS studies of pathogenic and commensal microorganisms, which have a tremendous role in caries pathogenesis. Although analytical advances are necessary to handle the unique features of microbial genomics, such endeavors could provide exciting new insights into the effects of microbial variants and, if integrated with human genomic data, how they may be dependent on the human genetic background [89].

5. Newer forms of treatment or prevention of caries

- Targeted therapy for cariogenic bacteria

Traditional therapies for preventing or managing dental caries include a combination of non-invasive methods (oral health education, mechanical control of cariogenic biofilms, application of fluoride, diet counseling and use of antibacterial drugs), paired with minimally invasive methods when necessary. Although evidence has shown that these methods are effective and economical, genetic, behavioral and cultural factors may limit the effectiveness of these therapies in different individuals or populations and be associated with the alarming prevalence of the disease worldwide. Contemporary treatment strategies that target cariogenic microbiome with high specificity represent promising alternatives without disrupting the balance of the surrounding oral niche. It is worth noting that these strategies may indeed be a viable therapy, although it may not be looked upon with favor by some, e.g. those who do not wish their children to be vaccinated. Nevertheless, recent advances in modulation of cariogenic biofilm are as follows:

a. Replacement therapy The overall concept is to replace a disease-associated strain with a modified, non-pathogenic version of that strain, termed an effector strain. Thus, a successful effector strain a bacterial disease must not cause disease itself nor predispose the host to other disease states by disrupting the ecosystem in which it resides. An excellent example of an effector strain involved construction of a mutant of *S. mutans* did not make acid. Since acid production by mutans streptococci was essential to the pathogenic process of dental caries, effector strains, lactate dehydrogenase (LDH)-deficient mutants of *Streptococcus rattus*, were shown to have little or no cariogenic potential with low acid-producing capabilities *in vitro* and in various rodent models [90]. Consequently, this LDH mutant was a candidate for replacement therapy. Another effector strain called BCS3-L1 was constructed to produce an LDH deficiency with mutacin 1140 production, which is capable of killing virtually all strains of mutans streptococci. This genetically stabile effector strain in reduced pathogenic potential by, selectively colonizing the tissues/teeth at risk for disease [91,92]. Additional clinical trials in humans are needed for validation and safety for the prevention of caries [92].

b. STAMP - Specifically Targeted Antimicrobial Peptides. Synthesized STAMPs represent a novel therapy that showed inhibitory effects on the growth of *S. mutans* by selectively killing *S. mutans* without effects on other species of oral Streptococcus, such as *S. gordonii* and *S. sanguinis* [93,94]. STAMP C16G2 was tested in a saliva-derived in vitro model containing over 100 oral species to simulate the diversity and overall metabolic functionality of the human oral microbiome and showed a selective antimicrobial activity against *S. mutans*. Concomitantly, there was an increase in the abundance of several commensal species of Streptococcus, including *S. mitis, S. cristatus, S. oralis*, and *S. sanguinis* [93]. STAMPs have also been developed against *Candida albicans*, a fungal species that many consider to be important in dental caries [94].

c. Oral microbiome modulation The oral microbiome is shaped in the beginning of human life, and its composition is influenced during pregnancy [95], by mode of delivery [22,28], feeding habits [26,96], mothers and caretaker's oral health [19], and by the environment [28]. Previous studies [97–99] documented the difficulty of persistently introducing new strains into the mouths of humans if they already harbored an indigenous strain of the organism, or if the commensal community is already established. However, since the oral microbiome is being shaped in the first months, the introduction of probiotics in infants could be facilitated to allow new strains, without cariogenic properties, or strains that are natural competitors to cariogenic species, to persistently colonize the host tissue at risk and thereby prevent colonization or outgrowth of putative pathogens.

d. Bacteriophages Phages, the viruses of bacteria, can penetrate into biofilms and represent an innovative form of biocontrol that is specific and non-toxic to humans. It has been suggested that some *Streptococcus* phages can be used for dental caries prevention and treatment [100]. Phages for *Actinomyces naeslundii, Aggregatibacter actinomycetemcomitans, Enterococcus faecalis, Fusobacterium nucleatum, Lactobacillus* spp., *Neisseria* spp., *Streptococcus* spp., and *Veillonella* spp. have been isolated and characterized. Enzymes (lysins) of recombinant phage are able to lyse *A. naeslundii* and *Streptococcus* spp. However, only a tiny fraction of known phages and their lysins have been explored so far. For example, phages can be engineered to express the antimicrobial peptide C16G2220, which specifically targets *S. mutans* as an anti-caries therapy. ClyR lysin is active against cariogenic *S. mutans* and *S. sobrinus*, but not active against commensal *Streptococcus* such as *S. sanguinis, S. oralis*, and *S. salivarius*. [101]

Anti-caries restorative biomaterials

Extensive research in biomaterials have provided the development of new materials that can be 'supporting actors' in the fight between health and disease. The emerging materials developed in recent years can be divided into two main categories: antifouling materials and antibacterial materials.

Antifouling materials include those agents with properties that protect the tooth surface from early biofilm formation and can include protein repulsion and bacterial anti-adhesion. Examples are polymeric agents, biomolecules and metal oxides. For example, polymeric agents with hydrophilic properties can create a water barrier with shielding effect, inhibiting the absorption of bacteria and protein on the material surfaces. Currently, polyethylene glycol (PEG) and zwitterionic polymers are the two most widely used hydrophilic anti-fouling materials. Therefore, they can represent a new avenue for the development of alternative anti-caries biomaterials [102,103].

Antibacterial materials (including covering metals and metal oxides, inorganic non-metallic materials, organic small molecules, polymers and antimicrobial peptides) are indicated once bacteria are attached on the surfaces of teeth and dental materials to form the biofilm, to kill those attached bacteria or destroying EPS. Nanoparticulate silver (NanoAg) is an effective microbicidic agent as it affects cell permeability and induces structural changes in the cell leading to cell death. In addition, NanoAg does not cause resistant microbial species to develop. It has been applied to medical devices [104] and plastic materials [105]. In dentistry, its usage has been investigated in experimental orthodontic adhesives [106], prosthetic acrylic resins [107,108]. and implants [109]. A randomized clinical trial showed the antimicrobial activity and anti-biofilm effect of NanoAg against *S. mutans*, *S. sobrinus* and *Lactobacillus casei* when incorporated into Polymethyl-methacrylate (PMMA) baseplates of orthodontic appliances [110]. Another example is the novel graphene oxide-copper nanocomposites (GO-Cu), which maintain a long-term release of copper nanoparticles that disrupt the exopolysaccharide matrix assembly and further impairs optimal biofilm development with minimal cytotoxicity [102].

Considering the complexity of microorganisms in the oral cavity, it is difficult to predict the effect of a biomaterial applied to oral cavity. Materials possessing antimicrobial effects usually have cytotoxicity as well. An innovative concept might be the development of smart pH-responsive materials that selectively inhibit acid-producing bacteria for caries prevention and treatment, without disrupting the commensal microbiome.

In summary for 'What's left', there is still much to do. Progress will likely require a change in the mindset of oral microbiome researchers in order to develop microbiome-based personalized treatments. Nevertheless, with our current technologies, we can already achieve a comprehensive integration of oral microbiome data (such as virulence investigations at strain-level variation, transcriptomics, proteomics and metabolomics) with human multi-omic data (such as GWAS, transcriptomics, epigenetics, proteomics and metabolomics) in large, longitudinal cohorts is required, to advance the knowledge that will bridge the gap between basic research and clinical application. Our focus needs to combine oral microbiome research with preventive/treatment strategies to restore health-related ecosystems rather than simply killing pathogens – a homeostasis that is not conducive to the development of caries. These interventions should be able to change acidic environmental factors with limited destruction of beneficial bacterial communities. Consequently, translational

research should result in real and affordable products and therapies, which requires the joint cooperation and efforts of researchers, dentists, and patients.

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