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REVIEW

Oral microbial biofilms: an update

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Abstract

Human oral cavity (mouth) hosts a complex microbiome consisting of bacteria, archaea, protozoa, fungi and viruses. These bacteria are responsible for two common diseases of the human mouth including periodontal (gum) and dental caries (tooth decay). Dental caries is caused by plaques, which are a community of microorganisms in biofilm format. Genetic and peripheral factors lead to variations in the oral microbiome. It has known that, in commensalism and coexistence between microorganisms and the host, homeostasis in the oral microbiome is preserved. Nonetheless, under some conditions, a parasitic relationship dominates the existing situation and the rise of cariogenic microorganisms results in dental caries. Utilizing advanced molecular biology techniques, new cariogenic microorganisms species have been discovered. The oral microbiome of each person is quite distinct. Consequently, commonly taken measures for disease prevention cannot be exactly the same for other individuals. The chance for developing tooth decay in individuals is dependent on factors such as immune system and oral microbiome which itself is affected by the environmental and genetic determinants. Early detection of dental caries, assessment of risk factors and designing personalized measure let dentists control the disease and obtain desired results. It is necessary for a dentist to consider dental caries as a result of a biological process to be targeted than treating the consequences of decay cavities. In this research, we critically review the literature and discuss the role of microbial biofilms in dental caries.

Keywords Human oral microbial · Biofilms · Dental caries · Periodontal

Introduction

Like other complicated multicellular eukaryotes, the human being is not an independent organism. Various microbial symbionts and their genomes are in association with the biological functioning of the human body. The microbial population heavily colonizes in the inner and outer surfaces of our bodies and develop an operative organ, which is crucial to our health and physiology. The bacterial populations of the mouth are extremely complex with approximately 1000 species. They have been estimated to be the second most complicated in the body, after the colon [1]. The treatment of dental caries in clinical practice is usually referred to as the restoration of the hard tissues of the teeth according to the functional and aesthetic needs. However, this cannot be considered as disease treatment, but a cure its consequences. Contemporary Dental Clinical (CDC) practice is entirely focused on restorative treatment. In clinical practice, a commonly used method of caries treatment includes removing the infected tissues in order to stop the further progression of the disease. It is well-known that there is no permanent filling, but gradually, the repaired sections develop little spaces for microbial activities. As a result, the small filling should be replaced with the larger one and tissues of the teeth should be removed more and more. Restoration practice is mainly followed by endodontic treatment, which leads to dental prosthetic dentistry. However, this is not the final stage in the pathology of the tooth. If the tooth is extracted by surgery due to some complications, the

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final stage dental implant needs surgery to improve the function of the tooth [2, 3]. It must be admitted that the limited understanding of tooth decay as a biological incidence takes us to such a trap. Given the scientific advances in genetics and particularly molecular biology, the new discoveries allow us to change the old paradigm with a new model, creating new ways to prevent and treat caries. Tooth decay is commonly referred to as a disease that occurs when an ecological balance is disturbed in the oral microflora. These alterations can be impacted by biological and environmental factors. Only with managing the mentioned changes, the process of dental caries can be controlled [4, 5].

The oral microbiome

The phrase microbiome has been introduced by the Human Microbiome Project (HMP). Researchers of the field believe that comprehension of human health and illness is not possible without total realization of the merged microbiome/human entity [6]. The oral microbiome is a complicated ecological environment with up to 750 types of microorganisms recognized [7]. Prevailing microbial populations of mouth includes *Veillonella*, *Actinomyces*, *Streptococcus*, *Neisseria* and another aerobic organism. These living organisms help to maintain oral hygiene by preventing pathogenic kinds from attaching to the mucosal surface [8]. The microorganisms residing in the human mouth cavity have been nominated as the "oral microflora", "oral microbiota" and recently "oral microbiome" [9]. The phrase microbiome was created by Joshua Lederberg to indicate the ecological population of commensal, symbiotic and pathogenic microorganisms that occupy the body environment and have been ignored as determiners of health and illness state [10]. The oral microbial population is one of the most complicated bacterial florae related to the human body [11]. Mouth microflorae are able to generate dental plaques, dental cavities, and gum problems. Health problems of the oral cavity (mouth) in a person can be originated from poor oral hygiene and factors such as nutrition which affect the oral microbial context [12]. The comprehensive understanding of the oral cavity environment and relating microbial interactions reveals the main causatives of mouth-related diseases (Fig. 1).

Composition of the oral microbiome

The human mouth is the host for one of the most diverse microbiomes in the body [13]. The oral microbiome comprises viruses, protozoa, archaea, fungi, and bacteria [14]. Colonization of the human oral microbiome has mostly occurred on tooth surfaces which lead to biofilm (dental plaque) formation. Dental plaque is an extremely active and complicated oral biofilm ecosystem [15]. About 95% of the bacteria found in nature are present in biofilms. Body of evidence indicates that biofilm bacteria are the main causatives of dental problems [16]. Despite dental plaque research has lasted more than a hundred years, consideration of dental plaque as a biofilm and a microbiological ecosystem is almost new. Biofilms are organized societies containing extensive spans of microbes, which are embedded with self-made patterns of extracellular polysaccharides, and are obviously identified as acute factors to various mouth and teeth infections such as dental caries, gingivitis, aggressive periodontitis, peri-implantitis, and periapical periodontitis [16, 17]. Biological macromolecules such as proteins, carbohydrates, and nucleic acids establish the scaffolding of the matrix. Biofilms can be observed practically everywhere. They form on approximately all of the humid surfaces, like oral cavity, natural wet environments, ponds, and lakes. Since the oral biofilm is mainly propagated in the mouth by using saliva ingredients as the initial source, food components are quickly cleared [18]. The complicated microbial network works in a harmonized way to acquire nutrients, sugars, and amino acids, from salivary ingredients containing epithelial mucin, by the function of glycosidic enzymes such as sialidase, N acetylglucosaminidases, β-galactosidase, mannosidases, α-fucosidase, exo-proteolytic, and endo-proteolytic activities [19]. There is a cross-feeding relationship among the species. Oral biofilms grow under a series of various situations and environments. In vitro investigations show that Glycans decomposition happens consecutively and the liberated sugars are rapidly transported [20]. *Streptococcus oralis* is a conspicuous example of bacteria which has the highest capability to deglycosylate both N- and O-linked Glycans and is considered as a model microorganism [21]. New investigations should take the privilege of modern high throughput sequencing methods to discover the biofilm transcriptome of human beings taking a specific diet and also fasting, to determine the response of the biofilm to in vivo situations [22]. A series of viruses can be discovered in the mouth, which is predominantly disease-associated. Viral infections are the main causes of mouth sores [23]. A variety of viruses have been discovered in the human mouth, containing herpesvirus, human papillomavirus, coxsackievirus, mumps, measles or rubeola, and *rubella* or German *measl*es [24]. In addition, *Entamoeba gingivalis* and Trichomonas tenax are the principal protozoans recognized as members of the natural microbiome [25]. The numbers of those organisms are prevalent in cases with reduced oral sanitation and gum diseases and were once recognized as possible pathogens [7]. Some protozoa can be regained from saliva, dental, and periodontal specimens of children and teenagers with an uninjured dentition or with rebuilt teeth [26]. Fungi are omnipresent microorganisms. Candida species are the most frequent genera in the oral fungal microbiome of a healthful person (about 80%) while *Saccharomycetales*, *Fusarium*, *Cryptococcus Cladosporium*, *Aureobasidium*, and *Aspergillus* are the next most occurring fungal microorganisms [27]. Archaea, as a main part of the prokaryotic world, have long been ignored

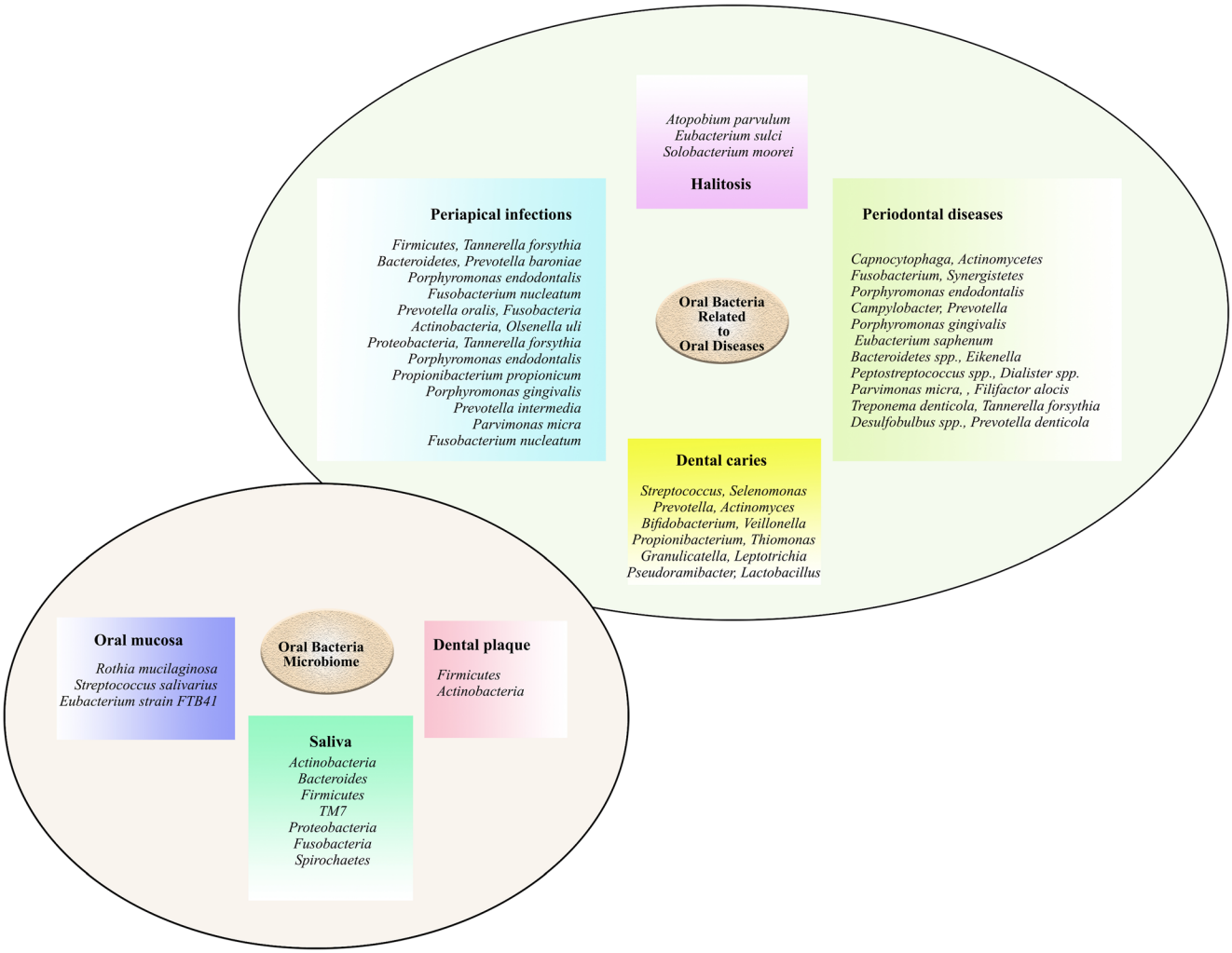

Fig. 1 Oral bacteria microbiome and oral bacteria related to oral diseases

in medical microbiology and the information about the association between this particular taxonomic group with the human host is yet rare. Nevertheless, it is identified that archaea are existing in low amounts within the human body [28, 29]. In the oral microbiome, archaea constitute a small population limited to a few numbers of methanogens phylotypes (methane-producing strict anaerobes) such as *Methanosarcina mazeii*, *Methanobacterium curvum/Congolese*, and *Methanobrevibacter oralis* [30].

The microbiome of the oral cavity

There are two types of surfaces for bacterial residence in the oral cavity, including the hard faces of the teeth and the soft tissues of the oral mucosa, so that the bacteria can colonize in those parts [31]. The teeth, tongue, gingival sulcus, hard and soft palates, cheeks, and tonsils all provide enriching conditions in which microbial colonies can grow [6]. Various sorts of microorganisms favor different racks according to diverse surface structures and purposes [32]. These microbial populations are biofilms that are specified by their composition, their coverage or substratum combination, and the conditioning films covering the surfaces on which they arrange [33]. The arrangement of the resident oral microflora shows regional variations in combination on different surfaces (e.g., teeth, tongue, cheek) because of variations in fundamental environmental situations [34]. Every niche supplies the optimum states and nutritional prerequisites for its resident microbes. To such an extent, jaw, tongue, hard, and soft palates include different bacterial compositions [35]. Moreover, because of the oral cavity's frequent connection with the outside environment, the oral microbiome is remarkably dynamic [36]. Consequently, the oral microbiota has developed abilities to handle difficulties that are not experimented by other microbiotas [37]. Bacterial growth and activity in the oral cavity are influenced by mouth functions including feeding and protecting from diseases [38]. Moreover, the microbial ecosystem is agitated by hygienic practices. Yet, oral microbial colonies that are less sensitive to disturbance experience changes associated to age, diet, and health, as well as stable

changes in pH, redox potential, salinity, climate conditions, and water activity of saliva [39]. Oral cavity bacteria are mainly classified into 13 separate phyla including *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Spirochaetes*, *Fusobacteria*, *Synergistes*, *SR1*, *TM7*, *Chloroflexi*, *Deinococcus*, *Acidobacteria*, and *Cyanobacteria* [40].

Oral microbiome in health

The commensal microbiota plays significant roles in sustaining oral and general health [31]. Immunoglobulin A (IgA), the main category of antibody released from the gut mucosa, is a significant participant to the functioning of the gut barrier [41]. In the germ-free intestine, lymphoid follicles are not developed and mucosal IgA is made just in the microbiota presence [42]. Due to the colonization resistance phenomenon, the presence of the oral microbiota in the mouth prevents the colonization of the pathogens [43]. Since all of the mouth surfaces are occupied by commensal bacteria, there only remain few binding sites for pathogen binding [44]. The extent of this effect can be evident when the commensal microbiota is interrupted by factors like antimicrobial agents [45]. When this occurs, opportunistic pathogens like *Candida* spp. and *Staphylococcus aureus* take the chance to come up. A number of health-related bacteria have been proved to antagonize to oral pathogens [46]. An outstanding feature of the oral microbiome is its linkage with cardiovascular health via nitrate metabolism. About 25% of consumed nitrate is restored to the mouth through an entero-salivary circuit [47]. Oral bacteria in the human mouth convert nitrate to nitrite, which is being moved into the bloodstream via gastric absorption and changed into nitric oxide. Nitric oxide is necessary for vascular health and maintains the elasticity of the blood vessels and prevents hypertensive results [48]. Dietary supplements with nitrate contents lower the blood pressure by a similar mechanism [49]. The plasma nitrite levels following nitrate ingestion are considerably decreased by the use of mouth antimicrobial agents [50]. The blood pressure lowering the impact of nitrate has shown to be abolished by antimicrobial mouth rinse in rats. These are evidence for the essential role of oral bacteria in nitrate metabolism and blood pressure adjustment. It can be deduced that excessive mouth hygiene could negatively impact the natural mechanism of cardiovascular health maintenance [51].

The main genera with the greatest incidence in healthy oral cavities involve *Streptococcus*, *Leptotrichia*, *Eikenella*, *Granulicatella*, *Actinomyces*, *Fusobacterium*, *Corynebacterium*, *Rothia*, *Porphyromonas*, *Prevotella*, *Haemophilus*, *Treponema*, *Neisseria*, *Capnocytophaga*, *Lactobacterium*, *Veillonella*, *Peptostreptococcus*, *Gamella*, *Staphylococcus*, *Eubacteria*, and *Propionibacterium* [38, 52]. Oral health is relying on the preservation of permanent microbial populations and disease happens when this equilibrium is disrupted and pathogenic species dominate the commensals. Health and disease in the mouth are active procedures in which the ecology of communities, but not a single microorganism, is determining [53]. Understanding the definition of health and disease, and recognizing molecular variations between health and illness, may give clinicians the strength to identify and diagnose diseases at an earlier and reversible step [39].

Bacterial infections of the oral cavity

Microorganisms of the mouth are responsible for the creation of a number of oral infectious diseases, including dental cavities [54]. More than 270 species of the oral cavity bacteria have been identified by employing the culture-independent techniques, which mostly rely on 16S rRNA gene sequencing. Bacteria in the human mouth are commonly fastidious and slow-growing, and about 50% of them cannot be grown in normal lab conditions [55, 56]. The acid produced by these bacteria erodes teeth and leads to infections or cavities [57]. These infections have diagnostic values and could be utilized to cure or prevent infections in prophylaxis [58]. The primary adherence of bacteria to surfaces of the teeth is preceded by the configuration of a conditioning layer on the clean dental covers which essentially include some salivary glycoproteins, named acquired pellicle [59, 60]. The diagnosis and characterization of oral bacterial infections are important in conducting suitable treatments and disease inhibition [58].

Relationship between biofilms and saliva

Oral microorganisms evolve to persist in the varying environments in protective biofilms [61]. The microbial colonies not only have essential roles in protecting oral homeostasis but also are notable players in oral diseases [62]. Dental plaque can do two functions in this case. It may catch in and block the proliferation of a current oral pathogen or donate a refuge for a pathogen to conceal from the salivary flow and the host's safe system [63]. Under healthy status, the ecological homeostasis among microbial activity and combination keeps biofilms healthy and stable [64]. However, the removal of biofilms is required because pathogens can also infiltrate within and result in disease [15]. Oral hygiene methods and salivary flow are both important for this separation. Saliva is vital to the oral cavity because it has a big role in preservation homeostasis and preventing disease [65]. Individual's teeth are a component of the oral cavity and saliva is the principal fluid in it. There are cariogenic bacteria which are living in the oral cavity; consequently, saliva has a serious and big influence on their survival and growth [66]. Given the latest findings, that saliva has two different functions. It could neutralize acidic pH in dental plaque and wash the byproducts from microorganisms' metabolism since acids are the principal product of the

bacterial metabolism [67]. The most important role of saliva through its buffer capacity is to deactivate the acidic pH of the plaque. When the flow rate of saliva is increased, its composition changes and the concentration of sodium, protein, chlorine, and bicarbonates increase whereas the condensations of magnesium and phosphorus decrease [68]. Bicarbonates spread out into the plaque and make the acidic condition nullified. The rise of pH in the plaque provoke regeneration of the weakened enamel and dentin [69]. Saliva in natural situations is satiated with calcium and phosphate ions. Therefore, the mentioned ions help to preserve the coherence of teeth composition. There is a theory implying the uniqueness of both saliva's flow rate and composition of the oral microbiome in different people [70]. These results convinced us to accept the truth, which is the combination and creation of the plaque in any person is unique [71]. The distinct plaque biomass, pH, and particular microbial response in each person could be the reasons that how the people take care of their oral hygienic are more sensitive to the disease [72].

Oral microbiome and oral diseases

Mouth and tooth diseases including dental caries and periodontal disease are among the most common diseases all over the world, affecting almost every age and geographic communities [6]. Tooth surface biofilms may generate dental caries, and supra- and subgingival biofilms along and below the gingival area may induce periodontal disorders. Furthermore, peri-implantitis may develop when the dental implant takes the place of the tooth [62]. Accordingly, finding etiological causes and risk factors for disease initiation and advancement could provide novel diagnostic and therapeutic tools [58].

Endodontic infections

Endodontic infections are distinct from other types of oral infection. They arose in closed environments. Consequently, the root channel system which is a closed environment enclosed by solid tissues could be a suitable milieu for this type of infection [73]. Majority of periradicular and the tooth pulp diseases are correlated with microorganisms [74]. Endodontic infections may initiate and spread when the root canal is opened to the oral cavity and there is a weakened immune system response [40]. At the first place, the microbes are limited to the intra-radicular area when the intrusion from a carious lesion or an injury to the coronal tooth structure [75]. When dental carious lesions are left with no treatment, the lesion could proceed into dentine and inside the pulp and cause harmful infections and pulp destruction. The microbiota correlated with non-treated endodontic infections is mainly composed of anaerobic proteolytic bacteria [59].

Endodontic infections, which continue after treatment mainly, are due to the presence of enterococci that are not commonly observed in the healthy mouth. The origin of these organisms has been debated for a long period. It has been newly confirmed that both pasteurized and unpasteurized slices of cheese are the source of the mentioned microorganisms and they could remain in the mouth for some time after the cheese consumption [76].

Periodontal diseases

Periodontal disease includes all pathological situations of the periodontium. They are bacterial infections which stay on the tooth exterior levels recognized as plaques [77]. Periodontal disease is progressive, generally non-regenerative, and untreatable diseases. Accordingly, it is better to consider prevention rather than treatment. Prevention and treatment of periodontal diseases need comprehensive knowledge about the connection between the etiology of the pathogenesis of the disease [78]. This disease originates from subgingival plaque reposition that makes changes in the microflora composition from healthy to the unhealthy condition [79]. Periodontal disease has arisen from a polymicrobial inflammatory problem of the periodontium. The mildest form of this disease (periodontal disease) is Gingivitis [80]. Microorganisms inside biofilms start to demonstrate pathogenic specifications that worsen and stimulate the gingiva when disrupted by activities like flossing. Fortunately, gingivitis could be reversed with a fine mouth and tooth care [81]. Bacteria which are related to periodontal health contain initial or early colony-forming microorganisms including *Streptococcus sanguinis*, *Gemella* spp., *Streptococcus mitis*, *Fusobacterium nucleatum*, *Atopobium* spp., and *Capnocytopha* [82]. Members of *Veillonella*, *Streptococcus*, and *Capnocytophaga* genera are considered to be helpful to the host [83, 84]. Molecular investigations show a strong linkage between special non-cultivated species like Bacteroides oral clone BU063 and periodontal health [85].

Bacterial biofilm in gingival health

Gingivae with good health is related to a very simple arrangement of supragingival plaque including (1–20) layers of mainly gram-positive bacteria, for instance, *Streptococcus* spp., *Actinomyces viscosus*, and *Parvimonas micra* (*previously Peptostreptococcus micros*), and also gram-negative species like *Campylobacter gracilis*, *F. nucleatum*, *Prevotella intermedia*, and *Veillonella* [86]. In healthful situations, the microflora at the gums groove is scanty and gram-positive streptococci dominate the population [58]. The first colonizers are organisms capable to endure the high oxygen condensations and to resist the different elimination mechanisms of the oral cavity like swallowing, chewing, nose-blowing, salivary, nasal mucosa, and crevicular fluid outpour [87]. Their proliferation allows the subsequent adhesion of other bacterial sorts,

which, however, are incapable to attach to hard surfaces of teeth and can only attach to previously existed microorganisms. This is so-called "Secondary Colonization". As the quantity of plaque layers rises, nutritional and atmospheric slopes are generated. As the amount of oxygen decreases, the anaerobes can continue living [88, 89].

Clinical gingivitis is accompanied by the evolution of more arranged dental plaque. Before-mentioned biofilms are distinguished by multiple cell layers, with bacterial layers located by metabolism and aerotolerance [90, 91]. Moreover, the gram-positive cocci, rods, and filaments connected with healthful gingivae, the amount of gram-negative cocci, rods, and filaments raises, and anaerobic bacteria such as *F. nucleatum*, *C. gracilis*, *B. forsythus*, *Capnocytophaga* spp. become evident [92]. Gingivitis generally happens before periodontitis. The change to periodontitis from gingivitis does not take place automatically, in every patient or every site too, but it relies on three principal agents: host sensitivity, bacteria that are pathogenic, and preventative bacteria [93]. Pathogenic bacteria maintain borderline characteristics which reduce the efficacy of the host reply by beginning tissue failure and preventing tissue recovering [94].

Biofilms and periodontitis development

A continual inflammatory disease which is named periodontitis gets involved in the totality of the tooth-supporting tissues, which consist the alveolar bone, gingiva, and periodontal ligament and are as a whole recognized as the periodontium [85]. Periodontitis might not only induce tooth loss, but could also further change physical health by raising the risk of atherosclerosis, rheumatoid arthritis, aspiration pneumonia, adverse pregnancy outcomes, and cancer for sick people [95]. The biofilm-related periodontitis is complex and composed of many cell layers. Three oral anaerobic bacteria including *P. gingivalis*, *Treponema denticola*, and *Tannerella forsythia* have conventionally been regarded as causative factors of periodontitis, based on their virulence characteristics and strong relationship with disease involved parts [96]. The structure of the bacterial community in the dynamic phase and the decomposing phase is slightly different from while the remission period. This supports the hypothesis of the high specificities of pathogenic plaque; a superiority of *P.gingivalis* and *T. denticola* is connected with raising probing depth and bleeding on probing [97].

The data that has been obtained recently contends that various periodontitis-associated microbiota is included in the disease and is more than that previously thought. In the suggested model, the disease not originates from the single pathogen, but from polymicrobial cooperative interaction and dysbiosis, which disturb the ecologically stable biofilm correlated with periodontal tissue homeostasis [98, 99].

Dental caries

Causatives of dental caries

Dental caries is a complicated disease procedure that hurts a great part of the world, unconcerned of gender, age, and nationality, though it seems that the disease affects more on people with a low socioeconomic situation to a greater extent [100]. Dental caries is identified by local demolition of the hard texture of the tooth [101]. At first, the dental enamel destroys and then the dentine. Dental caries is the localized demolition of sensitive dental hard texture by acidic results of bacterial fermentation of patient's consumed carbohydrates [102].

Dental caries is a common disease of childhood; however, individuals of any age are sensitive to the disease during their life [103]. This painful disease can be restricted and possibly inverted in its initial steps, however, is frequently not self-restricting and without adequate care, caries can continue until the tooth is demolished [104]. The disease at first point could be reversed and terminated at each degree, even if dentine or enamel is corrupted, regarding sufficient biofilm can be separated [103]. The latest molecular biology methods assign the microorganisms associated with caries as *Bifidobacterium dentium*, *Bifidobacterium adolescentis*, *Streptococcus mutans*, *Scardovia wiggsiae*, *Bifidobacterium longum*, *Selenomounas* spp., *Prevotella* spp., *Lactobacillus* spp. [105]. Oral streptococci face extremely different kinds of environmental tensions and population densities [60]. These stimuli are perceived by efficacious detection methods that furthermore stimulate the proper adaptive genetic reflexes. A major part of these detection methods utilizes membrane-anchored sensitive proteins that are adjusted directly or indirectly by their sensed stimuli [106]. Before-mentioned systems have an essential role in relieving the possible harms produced by shifts in redox potential, oscillations in local pH, and toxicity from antimicrobials. Moreover, normal life period of the mouth streptococci comprises a shift from growth in an almost low cell density planktonic status to a significantly high cell density biofilm environment [60]. As a result, different sensual systems are devoted to identify this rise in population and control the genetic pathways that are fundamental for durability in extremely competing for multispecies biofilm environments [107]. Despite oral streptococci form two-thirds of the whole commensal microorganisms, only a division which is known as *mutans* streptococci is associated in producing dental caries [60]. The oral streptococci are the preliminary colony-forming microorganisms of the teeth and other mucosal surfaces in the oral cavity and originate plaque

biofilm creation [108]. The double-faced interplay in the forms of collaboration and rivalry defines the composition of the oral microflora [109]. *Streptococcus mutans* colonization takes place by important acidogenicity and aciduricity effects, which is an essential causative of dental caries [110]. New advancements in nucleotide sequencing and different high throughput techniques elucidate the biological and gene regulation events of *S. mutans* in community formation [111]. Many treatment strategies are being devised to especially affect the *S. mutans* in the biofilm, without interfering other bacterial kinds. An effectual technique is targeting the interbacterial signaling which replaces cariogenic flora by non-cariogenic flora, especially using targeted antimicrobial peptides (STAMPs) [112, 113]. The disease could be observed on all sides of the teeth including the crown, the root parts of initial and continued teeth, and on soft as well as surfaces with small holes or surfaces with long deep cracks. It could modify enamel, the exterior coatings of the crown; cementum, the exterior layer of the root; and dentine, the tissue below cementum and enamel both. Caries in early teeth of preschool children is generally named as initial childhood caries [114]. The title dental caries could be applied to recognize both the caries proceedings and the carious lesion that is created as a consequence of that process [115]. This is a common disease caused by an environmental disturbance in the physiological balance among tooth minerals and microbial biofilms [116]. In other words, dental caries is a disease with a bacterial origin that is characterized by demineralization of the exterior level of teeth, which might head to cavitation, pain, and tooth loss [117]. Dental caries is related to many factors that begin with microbiological changes inside the biofilm complex and is induced by salivary flow and compounds, exposure to fluoride, using of dietary sugars, and protective behaviors like teeth cleaning (Table 1). In the past few decades, development of dental caries was attributed to only a few gram-positive bacteria in the biofilm, i.e., *Streptococcus mutans* along with some *Lactobacillus* spp. were believed to be the main types of pathogens [118, 119]. Bacteria exist on teeth in micro-colonies that are packed in organic matrixes of proteins, polysaccharides, and DNA exported by the cells. This protects Bacteria from dehydration, host defense, predators, and donates boosted resistance to antimicrobial materials [120]. Teeth provide tight surfaces for bacterial colonization and great amounts of bacteria. Subsequently, their secretory byproducts are gathered in biofilm on tooth exteriors in health and disease situations [121]. The etiological factors ascertaining caries could be classified into two categories: environmental and genetic factors. Based on the "ecological plaque hypothesis", pathological microorganisms start to dominate when the plaque homeostasis is interrupted [122]. The latest epidemiology investigations point out that there are varying levels and prevalence of caries risk in different populations [123]. It can be deduced that genetics is another factors that take part in dental caries formation [124].

Genetics and oral microbiome

The discrepancy in the immune system of different hosts which is defined by the genetic factors influences the composition of host microbiome. Individuals with weak biodiversity of mouth microbiome experience are more susceptible to tooth decays or cavities. Biodiversity of the plaque bacteria confers more resistance and adaptability to harsh environmental conditions and helps them to survive [125]. Genetic factors of individuals could also prepare a beneficial environment only for pathogenic bacteria to remain alive. For example, persons with a very low dose of saliva antibodies or proteins are more sensitive to plaque accumulations and consequently caries formation [38]. While there is acceptable commensalism and also mutualism between the host and oral microorganisms, the microbiome remains balanced and confers a good situation mouth and teeth health [126]. In an individual with the impaired immune system, this kind of relationship starts to abolish and parasitic communications start predominating. As a result, a situation of caries progression and pathogen dominance will be witnessed [127]. For instance, when *Streptococcus mutants* begin to create an acidic situation, *Veillonella* species grow more robustly and in turn help to strengthen growth of *Streptococcus mutans* [110]. As well, the weakened immune system impedes the amount of saliva and then reduces the object flow in them. Loss of object removal by saliva permits the plaque to expand in a facilitated manner [127]. The combination of a microbiome could be contrasted to a biomarker that displays the rate of activeness of the disease. Microbiome in a healthy situation could be preserved by the right oral sanitation and the strong immune system. It is essential for a dentist to know that dental caries is a disease, which is relied on an oral microbiome. Translation of this information to clinical operations when treating a patient's caries and exclusive personalized medication is necessary [128].

Prevention of dental caries

Focus on the preventative measures seems to be more logical in case of dental caries. Since dental caries is a progressive and recurrent disease, the symptoms could not be recovered completely. Table 2 lists the major strategies for dental caries prevention.

Table 1 The effect of pathologic and protective factors on the risk of dental caries

Protective factors (health)	Pathological factors (disease)
Dietary factors	
Tooth healthy diet	Irregular eating habits
Sugar exposures are limited to meal times	Ready access to snacks
Preference for non-cariogenic snacks	Multiple sugar exposures through the day
	availability of cariogenic snacks
	Frequent consumption of dietary sugars
	Available fermentable carbohydrates
	Smoking
	Alcohol consumption
Fluoride	
Presence of continuous, low concentration of-free F-ions around teeth	No exposure to fluoridated drinking water
	No access to professionally applied topical-fluorides
Daily use of a fluoridated dentifrice	
Use of fluoride in drinking water	Inadequate fluoride
Salivary	
Normal salivary function	Salivary dysfunction
Salivary buffers	Poor salivary flow rate
Salivary proteins and lipids	Reduced secretion (xerostomia)
Salivary Calcium & Phosphate ions	
Alkaline pH	
Enzymes such as lactoperoxidase, lysozyme-lactoferrins, glucose oxidase	
Immunoglobulins IgA	
Socioeconomic factors	
Good oral hygiene	High caries risk in family
A regular dental checkup	Poor oral hygiene
Detecting early dental caries	Indifference into early dental caries
Use of fluoridated toothpaste and brush	Socially deprived
Regular brushing (twice a day)	Low knowledge of dental diseases
Fluoridated mouth wash	Irregular attender
	Low dental aspirations
Medical factors	
Preventive and therapeutic sealants	Salivary dysfunction caused by medications
Alternate sugar-free medications	radiation therapy
Absence of cavitated caries lesions or restored-teeth	general systemic conditions
Without dental plaque	Cariogenic medication
Without gingivitis and	Premature extractions
Without active initial caries lesions	Anterior caries/restorations
	Multiple restorations
	No fissure sealants
	Orthodontic treatment
	Partial dentures
Microorganisms	
Antibacterial substances	Acidogenic bacteria in plaque
	Biofilm homeostatic imbalance
	Dental plaque accumulation
Remineralization	Demineralization

Table 2 Prevention strategies of dental caries

Treatment	Result	Ref
A fluoride gel, toothpaste and mouth rinse	The use of fluoride gel is associated with a significant reduction in tooth decay in children and teenagers.	[129]
	the use of topical systems (without discriminating between mouthwash gels, toothpaste and varnishes) for fluoride therapy is obviously correlated with the reduction of caries in children	[130]
	Fluoride mouthwash is associated with a significant reduction in the prevention of tooth decay in children and adolescents.	[131]
	Toothpaste containing fluoride to prevent tooth decay in children and adolescents	[132]
	The combination of topical fluorides with fluoride toothpaste reduces dental caries more than 10%.	[133]
	Combinations of topical fluoride (toothpaste, mouth rinse, gels, varnishes) versus single topical fluoride for preventing dental caries.	[134]
	The effectiveness of several local fluoride agents for preventing tooth decay	[135]
	A statistically significant difference between the higher concentrations of fluoride toothpaste compared with placebo in preventing dental decays. (Improved the prevention of caries from 23% to 36% compared to placebo).	[136]
	toothpaste with Low fluoride contents significantly increase the risk of decay in the primary teeth and do not reduce the risk of aesthetical fluorosis in the permanent teeth.	[137]
	The use of supplemental and professionally applied fluoride in moderate and high caries risk adults is effective in preventing and/or remineralizing dental caries.	[138]
	The application of xylitol in existing fluoride diets can help prevent caries.	[139]
	The caries-preventive effect of fluoride gel on the primary dentition	[129]
	Products with Xylitol-contents for prevention of tooth caries in adults and children.	[140]
	Chlorhexidine treatment for the prevention of dental caries	[141]
Pit and fissure sealants	Sealing is an effectual way to prevent decay of the occlusal surfaces of permanent molars.	[142]
	The effectiveness of pit and fissure sealants in preventing dental caries.	[143]
	The application of sealants on the occlusal surfaces of permanent molars in high-risk children to prevent and control decays.	[144]
Varnishes	The advantage of sealant over varnish utilization in the prevention of tooth decay	[145]
	preventive effect of chlorhexidine varnish on Caries is unconvincing for adolescents and children with daily fluoride exposure	[146]
	2–4 times application of fluoride varnishes in a year is associated with a significant reduction of decays in population with varying levels of caries risk and exposure to other related details such as possible side effects and those related to the suitability of treatment.	[147]
Fluoridated supplements, water and milk	-The potential correlation between early childhood consumption of fluoride supplements and the possibility of enamel fluorosis is typically slight.	[148]
	Application of fluoride varnish to reduce caries in high-risk children.	
	The consumption of vitamin D in childhood, before the age of 13, to prevent dental caries.	[149]
	The effect of chewing gums containing polyol on dental caries: the consumption of polyols as part of usual oral hygiene thanks to their influence on S. mutans and on salivary dynamics initiated by chewing	[150]
	Fluoride supplements including drops, tablets, lozenges or chewing gums to prevent dental caries in children: there was no differential effect on permanent or deciduous teeth when fluoride supplements were compared with topical fluorides or with other protective actions.	[151]
	There is inadequate evidence to determine the impact of ending water fluoridation programs on caries incident.	[152]
	The fluoridate milk seems to provide profits to children in school age, particularly for the permanent dentition.	[153]

Diet and oral hygiene

Genetic factors are of major causes in dental caries. However, alongside the research progression, the role of different factors such as epigenetic factors may have an impact either on one or the other gene expression [154]**.** These basic factors could be further affected by environmental factors. There have been recognized some important environmental effects like inflammation, bacteria, smoking, and diet; they might have a great influence on oral health via epigenetic modifications happening in the genes that are associated in the immune response in the oral mucosa. A great consequence of the diet side effects because of acid generation from carbohydrates in the dental plaque is tooth demineralization. There is not any direct side effect from carbohydrates to hurt the teeth [155]. The demineralization is caused by the production of organic acids by carbohydrate-fermenting bacteria, which catabolize monosaccharides and disaccharides from the ingested foods [156]. The indications of the carious demineralization are noticed on the rigid dental tissues, but the disease procedure is started inside the bacterial biofilm-dental plaque that covers surfaces throughout the tooth and oral cavity. Furthermore, the very

initial transformations in the enamel are not detected with traditional clinical observations and need a clinical evaluation supported by radiograph examination methods [157]. Dental caries is a result of a degradation of tooth structure due to acid creation as a consequence of the food carbohydrate fermentation by mouth cavity bacteria [158]. While sugar and other carbohydrates leak to the oral cavity, microorganisms begin to convert them to acids. A pH decrease in the plaque results in hydroxyapatite solubility and demineralization start [159]. This acid shifts the local pH amounts to drop under critical amounts causing in demineralization of dental tissues. Demineralization of teeth happens when pH in the biofilm on the tooth exterior is under 5.5 which is known as the critical pH value [160]. In people with high carbohydrate consumption, the continuous acid creation causes the failure of the protective potential of saliva and frequent and persistent decreases in pH. In turn, this alters the composition of the oral microbiota to one that favors aciduric kinds [161]. The balance between demineralization and remineralization causes the persistence, termination, or relapse of dental caries. The action of demineralization and remineralization happens often during the day in most individuals [117]. During the time, this procedure will head to both cavitations in the tooth or restoration and reversal of the injury or keeping of the situation [162]. Dental caries is an active flow that relies on environmental acidification. Therefore, molecular microbiology investigations should include both numeral parameters of microbiome structure and metabolic dynamism. Dental caries emerges from gradual interaction between acid-producing bacteria, a layer that the bacteria could metabolize, and various host factors which involve teeth and saliva [122]. Remineralization can be increased by reducing levels of calcium and phosphate levels in association with minimum quantities of fluoride. Interestingly, a trace amount of fluoride (< 1 ppm) has profound effects on demineralization and remineralization [163]. Since fluoride plays as a catalyst and impacts reaction rates with dissolution and transformation of different calcium phosphate phases inside tooth combination and Millie within plaque next to tooth surfaces [163]. It has been confirmed by several investigations that sugar in nutrition is one of the earliest causes of caries agents, which is related to the oral microflora. Traditional diets with a lowered concentration of sugar lead to a lower prevalence of dental caries even when using a starch-rich nutritional plan [3]. The surplus sugar of the diet, apportion of these carbohydrates (especially sucrose) could be polymerized to extracellular glucans and fructans by the action of by bacterial glycosyl- or fructosyltransferases [164]. These polysaccharides associate to the extracellular matrix of the biofilm and facilitate the attachment of the bacteria to the enamel or dentin surface. Moreover, polysaccharides placed out of the cell surface create a nutrient supply for the biofilm bacteria, during the starving situations letting the acid formation and continuous degeneration of dental rigid tissues [165]. Endogenous bacteria like *Lactobacillus* spp., *Streptococcus mutans*, and *Streptococcus obrinus* in the biofilm generate weak organic acids as a carbohydrate fermentation byproduct [110]. Liquid nourishment and especially drinks move over the oral cavity rapidly and have a small contact with the teeth exterior layer. However, if a soft drink is kept in the mouth for a long period of time or drunk continuously, there is a higher risk of caries formation. When hard candies like mints, lollipops and … stay in the mouth for an extended time, sugar is released slowly and the demineralization time becomes extended [166]. Individuals ought to prevent consuming food that may adhere to their teeth (like caramel or adhesive food like potato chips). Irritating foods (especially avitaminosis) may have an especial influence on oral well-being [154]. Given the theories, vitamin D is the mineral, which is related to the calcium's metabolism and hard teeth structure mineralization. It has been discovered that because of the lack of vitamin D, enamel hypoplasia happens [167]. Hypoplasia of dental enamel is associated with the enhanced risk of caries. It must be noticed that vitamin D is also vital to the immune system [168]. Vitamin D provokes the distinct gene, which controls the generation of antimicrobial peptides such as cathelicidin, which fight bacteria, viruses, and fungi. Furthermore, antimicrobial peptides include immune supervisory features too: having a role in chemotaxis, cell reproduction, sore improving, cytokine, and chemokine generation, enhance vascular penetrance, affecting shifts in the mouth microbiome homeostasis [149]. Currently, a reasonable nutrition regime that functions as a prevention method turns into secondary priority; furthermore, fluoride could neutralize the harmful effect of carbohydrates [169]. However, from the biological viewpoint, an unreasonable, full in processed carbohydrate nutritional regime is one of the principal causes that interrupt microbiome homeostasis; its bad effect on the patients with high caries probability may be crucial [170].

Fluoride

Fluoride is the most important caries preventive criterion. It can be received either systemically in various via drinking fluoridated liquids, using fluoridated salt and pills. Fluoride also could be used locally [171]. Studies on fluoridated and non-fluoridated zones show that in industrialized counties, systemic fluoridation of water is not critical to preventing tooth caries [152]. Since an acceptable level of the mouth, hygiene and using toothpaste with fluoride has been useful in caries management [172]. The impact of the locally utilized fluoride relies on concentration, frequency of the use, application interval, and specificities of the fluoride composition. Using higher concentrations of fluoride in toothpaste and repeated application lowers the chance for caries formation [173]. Through three key mechanisms by which fluoride

manages to prevent or restrict tooth decay including (a) diffusion of hydrogen fluoride (HF) inside the plaque bacteria and arresting their metabolism when acidic conditions, (b) inhibition of demineralization on the dental crystal surfaces in acidic environment, and (c) improved remineralization and creation of a low solubility layer of defensive fluorapatite on the remineralized crystals [120]. Fluoride has an important effect on the acid-producing bacteria in a biofilm. While hydrogen fluoride defuses into the cell, it decomposes to hydrogen and fluoride. Consequently, the alkaline pH of cell interiors becomes acidic and an imbalanced proton gradient occurs. At the same time, key enzymes like enolase lose their activity due to the acidic pH [174]. This is identified as the antimicrobial mechanism of the fluoride in tooth care. Accordingly, two times of brushing for about 2 min by the toothpaste including fluoride is suggested. The majority of people believe that they can protect their teeth from caries by only regularly brushing. But brushing is not solely enough, particularly for children who are more susceptible to caries. It is fundamental using toothpaste with fluoride for brushing teeth [175].

Conclusion

Utilization of molecular techniques has recently developed our knowledge about the composition and functioning of the oral microbiome in health and disease conditions. However, it still remains much to be done. The bacterial species are not highly homogeneous, and the comparison of the genomes of isolates from similar species represents a highly diverse composition especially in case of the presence or absence of genes involved in pathogenicity. In past decades, consideration of dental caries as a biological phenomenon has been developed. It is now proven that tooth decay is due to the effect of environmental factors that affect humans' microbial balance. Molecular microbiology technologies give us the opportunity to observe the microbial composition in healthy people and those with caries for identification of new species of pathogenic microorganisms, their interaction, synergistic abilities, and the cross-reactivity of these factors on the process of decay and development of decay. Since each person has a distinct oral microbiome, which relies on the interaction of genetic factors and the immune system, consequently, the most effective outcome for caries prevention and planning a treatment method seems to be specific for the individual. As a result, it can be deduced that dentists should firstly be therapists who know about the disease and its etiology, then begin to treat it.

Future direction

Dental caries is mainly caused by the imbalance in the composition of oral microorganisms. Since the oral microbiome is unique to an individual, it seems that the treatment strategy should be specific to each person. Having a comprehensive knowledge about the composition of oral microbiome could help dentists to predict the possible disease in the future and take the preventative measures. It seems that, by the advent of advanced molecular techniques such as next generation sequencing (NGS), which can simultaneously detect the presence of DNAs from multitudes of organisms, the existing differences between the microbial composition in health and disease revealed and the acquired information provide the opportunity to predict and treat the oral diseases. Last but not least, identification and re-engineering the microbiome composition could be an effectual step toward individualized medicine in dentistry.

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