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ORAL MICROBIOME, BEYOND MICROBES: A REVIEW

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ABSTRACT

A personalized microbiome, is available in every human body which is crucial to maintaining health but also capable of causing disease. Various diverse microbiomes such as fungi, viruses, archaea, protozoa, and bacteria are harboured in the human mouth. These microbes form a symbiotic relationship with the human host and thereby flourish in the oral domain. This microbial flora affects the interspecies and host-microbial interactions, which further impacts the health and disease status of the host. In this review, we discuss the interactions between species as well as host and microbes that take place in the oral cavity and evaluate how these interactions change from healthy (eubiotic) to disease (dysbiotic) states. The dysbiotic signatures associated with caries and periodontitis and their sequalae is further discussed. Through recent advances in technology, we have started to untangle the complexities of the oral microbiome and gained new intuitions into its role during both disease and health. We further discuss the advanced computational techniques to evaluate the dysbiotic oral microbiome alterations along with novel techniques for modulation of the dysbiotic oral microbiome that may help in disease treatment and prevention viz; prebiotics, probiotics, use of nano-sized drug delivery systems, extracellular polymeric matrix (EPM) disruption, and host response modulators. In dysbiosis, the finely-tuned equilibrium of the oral ecosystem is disturbed, permiting disease-promoting bacteria to manifest and cause conditions such as gingivitis, periodontitis and caries. For patients and practitioners alike, promoting a balanced microbiome is important to successfully restore or maintain oral health.

KEYWORDS: Microbiome, Microbe, Symbiosis, Dysbiosis, Diagnostic tools, Diagnostic aids.

INTRODUCTION

Anaerobic bacteria were the first life forms on Earth, about 2.9-4 billion years ago.[2] Because Earth started off as a bacterial planet, all eukaryotic life forms, including plants, animals, and humans today, evolved in the presence of bacteria. The human body hosts approximately as many microbial cells as human cells,[3] and the microbial cells on and in the body carry genes that outnumber human genes by at least a factor of 100.[4] The human body has to rely on its microbial symbionts to carry out numerous chemical reactions (eg, nutrient breakdown) as it lacks the specialized enzymes required for this. About a quarter of the human metabolome (ie, the diversity of molecules circulating in blood) has a microbial origin. In return, the microorganisms receive their preferred food from the host and the habitat. There is continuous host-microbiota crosstalk as a result of the mutualistic symbiosis. Commensal microorganisms prevent exogenous microbes from becoming established and thus forms the first line of defence: they train the immune system to recognize a "friend" from a "foe" by upregulating the pro-inflammatory response against invaders and downregulating this response toward commensals. Acquiring the microbial symbionts that correspond to the individual host is therefore of greater importance for the wellbeing of the individual.[1]

What is oral microbiome?

The oral cavity has the second largest and diverse microbiota after the intestine harboring over 700 species of bacteria including viruses, fungi, bacteria, archaea and protozoa. The mouth is an exceptionally complex habitat with its various niches where microbes colonize the soft tissues of the oral mucosa and the hard surfaces of the teeth.[5]

The community of microbial inhabitants in our body is called the microbiome. The term "microbiome" is coined by Joshua Lederberg, a Nobel Prize laureate, to describe the ecological community of commensal, symbiotic, and pathogenic microorganisms.[6] The oral microbiome is particularly vital to health because it can cause both systemic and oral disease. Throughout the oral cavity, the oral microbiome rests within biofilms, forming an ecosystem that maintains health when in equipoise. However, certain ecological changes in the microbiome allow pathogens to manifest and cause disease. Severe forms of oral disease may lead to systemic disease at different body sites.[7]

Oral microbiome, oral microflora or oral microbiota refers to the microorganisms present in the human oral cavity.[8] Oral microbiome was first identified by the Dutchman Antony van Leeuwenhoek who first identified oral microbiome using a microscope built by him. He is

known as the father of microbiology and a pioneer in the discovery of both protozoa and bacteria. In 1674, he noticed his own dental plaque and reported "little living animalcules prettily moving.[5],,

Microbiomics is the emerging field of research that targets the microbiome for therapeutic purposes. Microbiomics aims to understand how microorganisms' interplay with its host's health and physiology by examing their distinct interrelationships and functions. Today, the Human Microbiome Project (HMP) plays a major role in human microbiomics. It explores the role of the human microbiome in health, physiology and disease through metagenomic research, which analyses the genomes of specific microorganisms (Rajendhran and Gunasekaran, 2009).[7]

The emergence of new genomic technologies including next-generation bioinformatics and sequencing has revealed the complexities of the oral microbiome. It has provided a significant means of studying the microbiome.[6] Understanding the oral microbiome in health and disease will give further directions to explore the functional and metabolic changes associated with the diseased states and to identify molecular signatures for targeted therapies and drug development which will ultimately help in providing personalized and precision medicine.[7]

Dysbiosis

The finely-tuned equilibrium of the oral ecosystem is disrupted, in dysbiosis, allowing disease-promoting bacteria to manifest and cause conditions such as gingivitis, periodontitis and caries. For patients and practitioners alike, promoting a balanced microbiome is therefore important to effectively restore or maintain oral health.

"Dysbiosis" is the specific parasitic/pathogenic state wherein microbials promote disease in the host. This is also so-called as an "unbalanced microbiome". According to Peterson *et al*, there are three different scenarios that may occur simultaneously and are not mutually exclusive, by which dysbiosis can be characterized

i) Loss of microbial diversity;
ii) Losing the beneficial microbes; and
iii) Expansion of the pathogenic microbes.[9]

i) Loss of microbial diversity

The general ecological concept of loss of biodiversity within a community indicates a decline in the genetic variability, number, and/or variety of species of a biological community at a determined location. This loss of biodiversity can lead to the disintegration of that ecosystem. In the context of caries, several reports indicate a loss of diversity as the severity of the disease increased, suggesting that increased acidification of the oral micro-environment is accompanied by reduction in the levels, loss of diversity and metabolic activity of beneficial bacteria, leading to the rise of cariogenic bacteria. In terms of periodontal disease, alterations in microbial diversity remain controversial, with some reports indicating loss of microbial diversity with periodontitis, and others indicating the opposite (i.e., that periodontitis is associated with increased levels of microbial diversity compared to healthy control levels; as a consequence of the increased amount of nutrients derived from host's tissue degradation ; and even others reporting no significant difference between the groups (i.e., periodontitis vs. healthy patients). Interestingly, a meta-transcriptomic study of mature oral microbiomes demonstrated an over-expression of genes related to natural genetic transformation, indicating high functional repetition in the oral microbiome[9]. However, under dysbiosis, this repetition may be lost due to growth of pathogenic bacteria or loss of beneficial microbes, suggesting a general frailty in the oral microbiome related to the composition and diversity of its microbiota.[10]

ii) Loss of beneficial microbes

One of the main characteristics of dysbiosis is the loss of some of the benefits obtained from an established healthy oralome. As discussed before, the oral microbiota is important for the development and maturation of an appropriate oral immune response,[11,12] protecting the host against carcinogenic metabolites and from oral pathogens; and are part of the nitrate-nitrite-nitric oxide pathway, thereby contributing several benefits to the host. Thus, losing those beneficial microbes may reduce the host's ability to fight off pathogenic bacteria and respond to an excessive immune response against the host's own tissue, exposing the host to detrimental vascular changes and carcinogenic metabolites. This loss is particularly important in the context of periodontal disease, wherein excessive chronic inflammation results in loss of supporting tissues around teeth, including alveolar bone loss, which leads to tooth loss in the severe forms of the disease.[9]

iii) Expansion of pathogenic microbes

In a eubiotic environment, the oral microbiome accommodates opportunistic pathogens at such low levels that they do not cause any trouble to an immunocompetent host. However, an outgrowth of these pathogens represents a threat for the host. Particularly, this may increase the risk for periodontal disease, dental caries and may even be correlated to several systemic diseases, such as Alzheimer's disease, atherosclerosis and cancer. Specifically, F. nucleatum and/or P. gingivalis have been associated with periodontal disease, head and neck cancer, pancreatic cancer, Alzheimer's disease, colorectal cancer, atherosclerosis and pre-term births.[9]

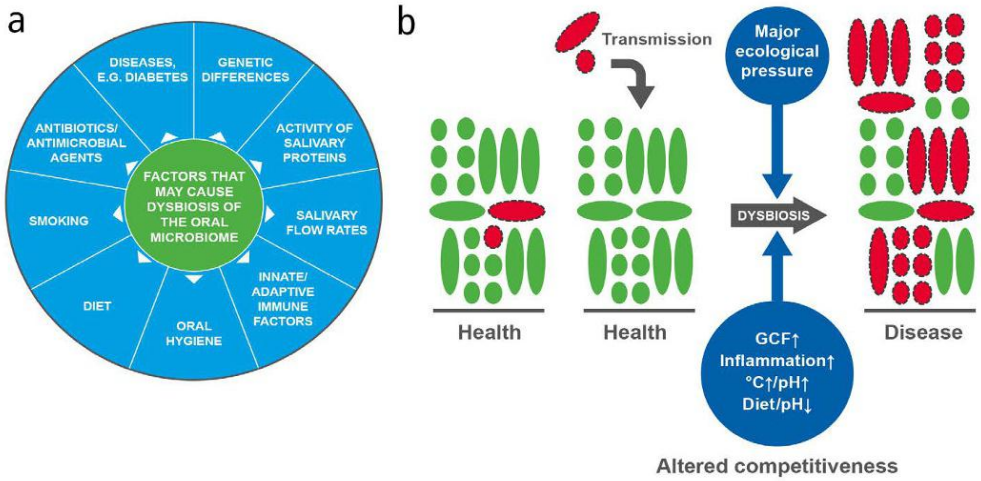


Fig. Showing (a) Causes of dysbiosis; (b) A model of dysbiosis (adapted from Marsh80).[6]

In health, the major part of the bacteria has a symbiotic relationship with the host; these microorganisms are depicted in green. Periodontopathic bacteria or potentially cariogenic (shown in red with dotted outlines) have been identified at healthy sites at low levels that are not clinically relevant; they may also be obtained from closeness (transmission), but again, their levels would be extremely low contrast to the bacteria associated with health. In disease, there is an increase in the proportions and numbers of cariogenic or periodontopathic bacteria, and there may be increased biomass (especially in gingivitis). It is proposed that for this to happen, there has to be an alteration in local environmental conditions (major ecological pressure), which alters the competitiveness of bacteria within the biofilm and selects for those species that are most adapted to the new habitat. The factors driving this selection need to be addressed and recognised for consistent and adequate disease prevention.[6]

Dysbiosis and The associated diseases

Dental caries

The ‘‘chemoparasitic” theory for the origin of caries, was proposed by Willoughby D. Miller in 1890, which described that ‘‘in susceptible hosts who often consumed fermentable carbohydrates, oral microorganisms would change these carbohydrates into acid, which would lead to the demineralization of teeth”; thereby creating the foundation for modern dental research.[9] Based on the work of G. V. Black and Miller, it was believed, at the time, that the quantity and not any specific pathogens were accountable for periodontitis. In this sense, the disease would only progress if the bacteria were able to exceed the threshold capacity of the body to detoxify bacterial products. Thus, plan has been known as the ‘‘non-specific plaque hypothesis.[13] Interestingly, the turn of the century brought new techniques to identify and isolate bacteria. In 1924, James Killian Clarke identified a caries causative agent – Streptococcus mutans. Unfortunately, Clarke was not able to directly demonstrate that S. mutans caused caries; this was later demonstrated by R. J. Fitzgerald and P. H. Keyes in the 1960s.

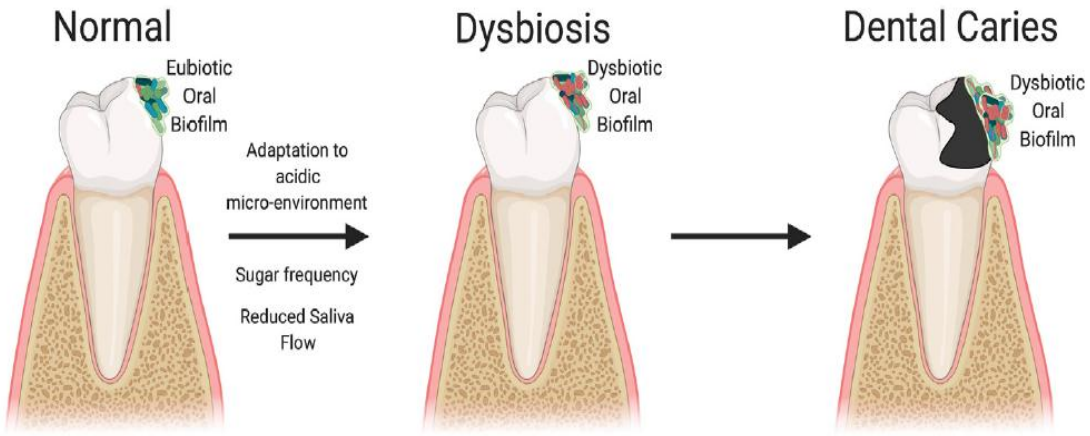
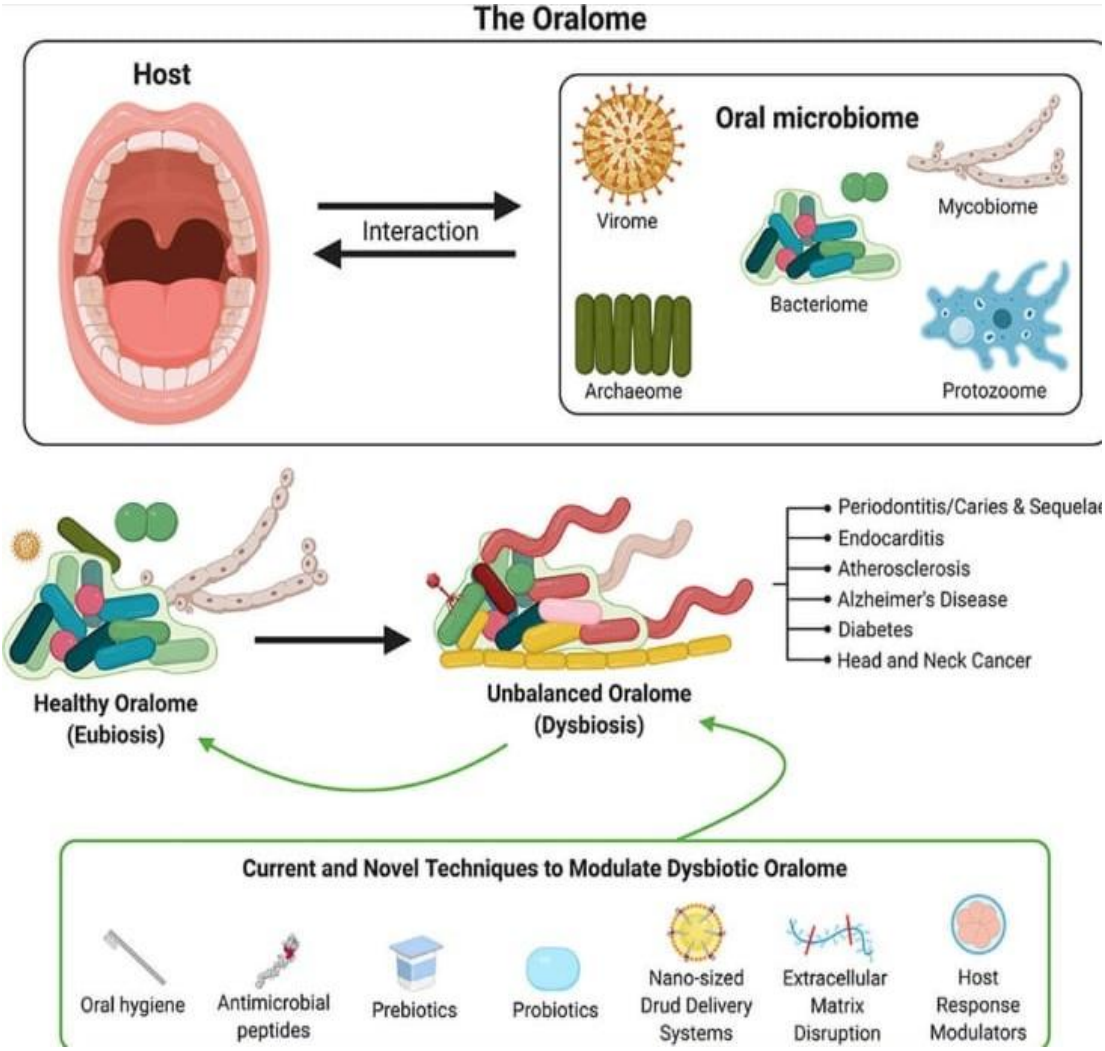


Fig. 2. Oral biofilm dysbiotic signature leading to dental caries.

In the 1970s, W. J. Loesche and colleagues noted that the antibiotic kanamycin was efficient against cariogenic bacteria.[13,14] In 1976, the ‘‘Specific Plaque Hypothesis”, was proposed, which states that dental caries is an infection of specific bacteria present in dental plaque, namely ‘‘mutans streptococci” (including Streptococcus sobrinus and S. mutans) and lactobacilli.[13,14]

In addition to lactobacilli and S. mutans, members of the genera Bifidobacterium, Scardovia and Propionibacterium have been found to be caries-associated. In addition to acid production, some bacteria can raise pH by producing ammonia from arginine and urea, which provides a technique for balancing acid production by other bacteria from dietary sugars and thus maintaining homeostasis.[15] Once inside of the pulp, the dysbiotic bacteria have ingress to the circulatory system, which could result in a transient bacteremia. Interestingly, oral streptococci, especially S. mutans, Streptococcus mitis and Streptococcus sanguinis, are thought to be important causative agents in infective endocarditis.[16,17]



Periodontal diseases

4.3.1. Gingivitis

Gingivitis is perhaps the most familiar bacterial disease of man with a prevalence in adults of over 90%.[18] Dental plaque associated gingivitis is a reversible inflammatory condition caused by persistence and accumulation of microbial biofilms (dental plaque) on the teeth. In

vulnerable individuals, gingivitis may lead to periodontitis with loss of the soft tissue and alveolar bone loss.

Dental plaque forms consistently on tooth surfaces. Salivary pellicle is formed by the selective adsorption of salivary glycoproteins onto the teeth. Oral bacteria then attach to the tooth surface by attaching to epitopes in the pellicle.[19,20] Primary colonisers can then co-aggregate with other bacteria forming the developing biofilm via coaggregation Interactions.[21] In general, primary colonisers tend to be facultative, Gram-positive aerobes such as Actinomyces and streptococci species,[22] while among the predominant anaerobic organisms in mature plaque are Gram-negatives such as Treponema, Fusobacterium and members of the phylum Synergistetes.[23]

Gingivitis is the initial form of periodontal disease. Fortunately, gingivitis is easily reversible with proper oral hygiene. A variety of oral hygiene measures have been used all over human history to remove dental plaque. If tooth cleaning is practised consistently, dental plaque is kept in relatively small amounts and in an immature state. If an individual does not perform oral hygiene, then the proportion of anaerobic and gram-negative species increases and enzymes and other endotoxin pass into the gingivae and cause inflammation and irritation by activating pro-inflammatory pathways.[15] There are no specific bacteria associated with gingivitis but the amount of plaque present, its maturity, and the plaque load, are correlated with disease severity.[24]

Periodontitis

Periodontitis is a chronic inflammatory multifactorial infectious disease which is characterised by loss of attachment as well as alveolar bone loss. The predominant pathogens involved in periodontitis are Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Prevotella intermedia, Tannerella forsythia, and Treponema denticola and Eikenella corredens, (Filoche et al, 2010; Dashiff and Kadouri, 2011).[7]

Role of oral microbiome in systemic diseases

The oral cavity is the primary entrance to the human body; therefore, microorganisms that inhabit the oral cavity are very capable of spreading to different body sites (Dewhirst et al, 2010). Oral bacteria frequently and easily gain entry to the bloodstream via untreated carious lesions and the gingival crevice and are a significant cause of infectious endocarditis,[25] and

liver and brain abscesses.[26,27] Pathogens may enter the blood stream, produce excessive and deregulated amounts of inflammatory mediators, alter proper immune responses, and in turn, cause disease at various body sites (Williams et al, 2008). Frequent bacterial attacks and persistent inflammation not only lead to bacteremia, but also severe systemic diseases and organ abscesses, such as cardiovascular disease and diabetes (Horz and Conrads, 2007; Williams et al, 2008; Meurman, 2010). This connection further supports the importance of the oral microbiome to overall health.[7] oral microbiomic profiles could be used as diagnostic biomarkers for various other diseases.[15]

Biotic oral microbiome

The key to oral health is a diverse and ecologically balanced microbiome that practices mutualism with its host and commensalism within itself (Ruby and Goldner, 2007; Zaura et al, 2009; Filoche et al, 2010). Commensal relationships among microbes allow them to flourish to their co-habitants at no expense and, in turn, maintain biodiversity within the oral cavity. Research has illustrated such biodiversity to be crucial to health.

Furthermore, the relationship between the host and the microbiome during health is mutually beneficial because the host is providing its microbial communities with a habitat in which they can flourish and, in turn, keep their host healthy. In health, microorganisms prevent disease progression in various ways: they can actively prevent a pathogen from occupying a site, they can prevent the adherence of pathogens onto specific surfaces by occupying the niche preferred by a pathogen they can hinder a pathogen's abilities to multiply, and they can degrade a pathogen's virulence factors (Socransky and Haffajee, 1992).[7]

Some biotic bacteria have been shown to be antagonistic to oral pathogens. For example, Streptococcus salivarius strain K12 produces a bacteriocin which prevents the growth of Gram-negative species which are associated with halitosis and periodontitis in vitro,[28] and has been shown to have favourable effects on halitosis in vivo.[29]

Advanced computational techniques to evaluate the dysbiotic oral microbiome alterations

Techniques such as in silico models can be used for the better understanding of biological systems and various bacterial species. Dos Santos-Lima et al[297] analysed peptide epitopes from P. gingivalis virulence proteins using in silico approach for their potential immunogenic host response to periodontitis. With this analysis, the authors were able to identify

immunoreactive peptides from the pathogenic enzymes such as neuraminidase (also known as sialidase) and lysine gingipain (KgP) before synthesizing them and the authors found that the KgP showed a high immunoreactivity, whereas neuraminidase showed very low immunoreactivity.

Valdebenito et al[298] analysed the effects of competition between Streptococcus sanguinis and S. mutans in dental biofilms by using an in-silico approach. The study found that S. sanguinis had competitive advantages over S. mutans, primarily due to glutathione peroxidase activity and by the capacity to undergo gluconeogenesis.

Head et al[141] also used in silico approach to study the relationship between the frequency of and total sugar intake and the growth of cariogenic species within oral biofilms. The authors found that with high sugar intake plaque was cariogenic whereas with low sugar intake plaque was never cariogenic independent of the frequency of sugar intake.

Marsh et al[299] also investigated on how changes in pH after sugar ingestion affects the oral bacterial competitiveness and the results found that these variations in the buffering capacity changed the biofilm composition from a healthy state to a dysbiotic state resulting in increased risk for enamel demineralization.

In addition to in sillico models, computational techniques such as interactomes, metabolic networks and metagenomics, have also been used to identify the oralome.

Novel techniques for modulation of the dysbiotic oral microbiome

The dysbiotic oralome can be modulated to re-establish a eubiotic state by the following techniques such as the use of traditional oral hygiene techniques, nano-sized drug delivery systems, antimicrobial peptides (AMPs), Extracellular polymeric matrix (EPM) disruption techniques, probiotics, prebiotics, and modifiers of host response.[9]

Oral hygiene

Oral hygiene is an important technique for controlling the oral microbial load and thus reduces bacteraemia and risk of periodontitis.[41,42] Tooth brushing with a fluoridated dentrifrice along with the use of anti-plaque mouth rinses significantly improves gingival inflammation and lowers plaque scores.[43,44]

Regular oral hygiene reduces the amount of plaque and keeps the plaque in an immature state and thereby reduces the chances of bacteremias and thus prevents further periodontitis.[15]

The duration and frequency of tooth brushing that is required to remove plaque and prevent periodontal disease is still not clear, in general, it is recommended to brush twice daily with a fluoride-containing toothpaste for 2 min, and the literature seems to affirm this.

In a recently published 11-year-long prospective study conducted on more than 1000 adults revealed a dose–response relationship between toothbrushing frequency and reduction in number of teeth with periodontal pocket depths (PPD)> or equal to 4 mm i.e participants who brushed twice or more a day demonstrated fewer teeth with PPD > or equal to 4 mm than those who brush less.[45]

Antimicrobial peptides (AMP)

Antibiotics can be used for the modulation of oral biofilm dysbiosis; however, antibiotics remove both commensal and pathogenic bacteria and also leads to multi drug resistance. New antimicrobial molecules are effective in order to overcome this. Antimicrobial peptides have shown positive results against antibiotic resistance as well as for modulating the oral biofilm dysbiosis.[46,47]

Antimicrobial peptides, produced by bacteria such as nisin produced by Lactococcus lactis is found to be effective against both gram positive and gram negative bacteria such as S.aureus and Listeria monocytogens. Nisin disrupts oral biofilms without causing cytotoxicity to the oral cells. Nisin (1-50ug/ml) stops the planktonic growth of the oral bacteria, development of multi-species biofilm and retards the bulk and biomass of the biofilm in a dose dependent manner. No signs of apoptotic changes are seen up to 200ug/ml for 24 h as the primary periodontal ligament cells, primary oral keratinocytes, gingival fibroblasts and osteoblast like cells were not affected.[48]

AMP produced by amphibians such as K4-S4 (1-15) is found to be effective against S.mutans, Aggregatibacter actinomycetemcomitans, and F. nucleatum in both biofilm and planktonic state.[49]

AMP seen in humans such a LL-37, which is expressed by the epithelial and immune cells has got host immune response against microbial attacks. LL-37 is a potent AMP. It is found to be anti-microbial, anti-fungal and anti-biofilm and acts as a chemoattractant for human

peripheral blood neutrophils, T-cells, monocytes and even inhibits herpes virus associated with Kaposi sarcoma. It is proven to opsonize A.actinomycetemcomitans and prevents its biofilm formation as well as retards the growth of F.nucleatum.[49,50]

AMP may develop certain challenges such as AMP resistance, which is based on the virulence potential of pathogens. These include proteolytic degradation, inactivation of the biofilm matrix molecule, removal of Amp by efflux pumps, changes in the cell wall or membrane composition via regulatory networks such as LiaR and VanRS. AMPs also generates immunogenicity after repeated exposure which can be avoided to certain extent by combining AMPs with nanoscale drug delivery system.[51]

Nanosized-drug delivery systems

Nanoparticles are colloidal particles of metals, lipids and polymers that range between the size of 1-1000 nm. The antimicrobial activity of these nanoparticles is found to have an inverse relationship compared to their particle size.[52,53] Due to their antimicrobial activity, nanoparticles of copper, titanium oxide and zinc oxide have been incorporated into polymer matrix as filler particles to prevent biofilm growth.[54] These features of nanoparticles have been used in the field of nano medicine, which involves the usage of proteins, low weight molecular agents and genetic materials for the diagnosis and treatment of various diseases with the help of nano-sized drug delivery system. The advantages of nano-sized drug delivery systems include slow release, reduced toxicity, specific targeting and improved bioavailability. Studies have found out that the nano-drug delivery system formulation is found to be more effective than the free drug.[52,53]

Prebiotics

Prebiotics are certain supplements or artificial addictive's that can modulate the microbiome in order to benefit the host. This concept has been earlier used in the case of gastric microbiome and recently it has been applied in the oral microbiome. In this context, arginine prevents dental carries by buffering the acid that is produced by the dysbiotic oral microbiome. Nitrate is considered as a potential prebiotic as it can lead to rapid change in the function and structure of the Poly microbial communities.[55,56] Studies conducted by Rosier et al has found that the nitrate supplementation is found to be effective against both dental caries as well as periodontitis. Other nutritional compounds identified as oral prebiotics include B-methyl-D-galactoside and N-acetyl-D-mannosamine.[57]

Probiotics

Probiotics are live microorganism which when administered orally and in adequate amount offers mucosal immunity and prevents the growth of harmful microbes. Studies have proved that the lactobacilli and streptococci strains isolated from the healthy oralome has got antibacterial activity against the periodontopathic bacteria. Probiotics is found to prevent gingivitis, plaque formation, alveolar bone loss and modulates pro inflammatory effect.[58] Nisin producing probiotic i.e Lactococcus lactis and Lactobacillus reuteri were identified as effective probiotics.[59]

Extracellular polymeric matrix (EPM) disruption

EPM is the matrix that is produced by the oral microbiome that forms the oral biofilm. This matrix protects the microbes from the environmental stresses such as from antibiotics. This matrix it is made up of polysaccharides, proteins, e-DNA and lipids.[60] Studies have found that the compounds such as cyclic-di-GMP or cyclic-di-AMP were found to disrupt the EPS synthesis and secretion as well as it inhibits the adhesion and biofilm formation of various microorganisms such as streptococcus mutans and streptococcus aureus.[61]

Host response modulators

Host response modulators modulate the response of the host against the disease. Periostat is a commercially available product containing doxycycline which downregulates the activity of matrix metalloproteinases (MMPs). MMPs are the main destructive enzymes in periodontal disease which works by inhibiting the collagenase activity. Doxycycline is the first FDA approved drug for host response modulation and it is currently used as an adjunct in the treatment of scaling and root planning in chronic periodontitis patients. Chemically modified tetracycline-3 is also one such HRM.[62] Anti-inflammatory drugs of HRM includes NSAIDs. HRM in spite from modulating the inflammatory host response and suppressing the tissue destruction, do not directly affect the oral dysbiosis, thus the recurrence of periodontitis is always possible.[63] Limited studies have investigated the possible side effects of HRM in humans thus further studies need to be conducted.

CONCLUSION/FUTURISTIC CONSIDERATIONS

The use of recently developed molecular methods has greatly expanded our knowledge of the function and composition of the oral microbiome in disease and health.[15] The diverse community that makes up the oral microbiome is finely tuned by nature to protect from disease, and it is of great significance to maintain its natural diversity. Modern lifestyles can

upset and disturb the natural balance of our oral microbiome, and our clinical goal should be to re-establish its symbiotic equilibrium by whatever means are appropriate and necessary in the individual patient. Thus, it is important that both patients and healthcare professionals embrace the concept of a balanced oral microbiome and its significance in systemic and oral health. Our current understanding of this rapidly evolving scientific field supports the notion that clinical practice needs to shift from its historical focus on management of periodontitis and caries by elimination of the microbiota, to a new focus on proactive management of oral health through an ecological perspective to the holobiont. Future directions may include individual assessment of the microbiome and the host reaction for the early detection of subjects at high risk, and personalised approaches to restore a health-associated oral microbiome after dysbiosis – potentially tantalising developments that would have direct inference for patient management in clinical practice.[6]

REFERENCES

1. Kaan AM, Kahharova D, Zaura E. Acquisition and establishment of the oral microbiota. Periodontology 2000, 2021; 86(1): 123-41.
2. Blaser MJ, Cardon ZG, Cho MK, et al. Toward a predictive understanding of earth's microbiomes to address 21st century challenges. mBio, 2016; 7(3): e00714-e00716.
3. Sender R, Fuchs S, Milo R. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. Cell, 2016; 164(3): 337-340.
4. Cox Laura M, Blaser MJ. Pathways in microbe-induced obesity. Cell Metab, 2013; 17(6): 883-894.
5. Deo PN, Deshmukh R. Oral microbiome: Unveiling the fundamentals. Journal of oral and maxillofacial pathology: JOMFP, 2019; 23(1): 122.
6. Kilian M, Chapple IL, Hannig M, Marsh PD, Meuric V, Pedersen AM, Tonetti MS, Wade WG, Zaura E. The oral microbiome–an update for oral healthcare professionals. British dental journal, 2016; 221(10): 657-66.
7. Zarco MF, Vess TJ, Ginsburg GS. The oral microbiome in health and disease and the potential impact on personalized dental medicine. Oral diseases, 2012; 18(2): 109-20.
8. Oral microbiomes: more and more importance in oral cavity and whole body. *Gao L, Xu T, Huang G, Jiang S, Gu Y, Chen F Protein Cell, 2018; 9(5):488-500.*
9. Radaic A, Kapila YL. The oralome and its dysbiosis: New insights into oral microbiome-host interactions. Computational and Structural Biotechnology Journal, 2021; 27.
10. *Gao L, Xu T, Huang G, Jiang S, Gu Y, Chen F Protein Cell, 2018; 9(5): 488-500.*

11. Ogawa T, Hirose Y, Honda-Ogawa M, Sugimoto M, Sasaki S, Kibi M, Kawabata S, Ikebe K, Maeda Y. Composition of salivary microbiota in elderly subjects. Scientific reports, 2018; 11, 8(1): 1-9.
12. Petersen C, Round JL. Defining dysbiosis and its influence on host immunity and disease. Cellular microbiology, 2014; 16(7): 1024-33.
13. Gao L, Xu T, Huang G, Jiang S, Gu Y, Chen F. Oral microbiomes: more and more importance in oral cavity and whole body. Protein & cell, 2018; 9(5): 488-500.
14. Rosier BT, De Jager M, Zaura E, Krom BP. Historical and contemporary hypotheses on the development of oral diseases: are we there yet?. Frontiers in cellular and infection microbiology, 2014; 16, 4: 92.
15. Loesche WJ. The antimicrobial treatment of periodontal disease: changing the treatment paradigm. Critical Reviews in Oral Biology & Medicine, 1999; 10(3): 245-75.
16. Wade WG. The oral microbiome in health and disease. Pharmacological research, 2013; 1, 69(1): 137-43.
17. Nomura R, Ogaya Y, Nakano K. Contribution of the collagen-binding proteins of Streptococcus mutans to bacterial colonization of inflamed dental pulp. PLoS One, 2016; 21, 11(7): e0159613.
18. Nomura R, Matayoshi S, Otsugu M, Kitamura T, Teramoto N, Nakano K. Contribution of severe dental caries induced by Streptococcus mutans to the pathogenicity of infective endocarditis. Infection and immunity, 2020; 22, 88(7): e00897-19.
19. Coventry J, Griffiths G, Scully C, Tonetti M. Periodontal disease. Bmj, 2000; 1, 321(7252): 36-9.
20. Ahn SJ, Kho HS, Lee SW, Nahm DS. Roles of salivary proteins in the adherence of oral streptococci to various orthodontic brackets. Journal of dental research, 2002; 81(6): 411-5.
21. Murray PA, Prakobphol AK, Lee T, Hoover CI, Fisher SJ. Adherence of oral streptococci to salivary glycoproteins. Infection and immunity, 1992; 60(1): 31-8.
22. Kolenbrander PE, Palmer Jr RJ, Rickard AH, Jakubovics NS, Chalmers NI, Diaz PI. Bacterial interactions and successions during plaque development. Periodontology, 2000, 2006; 42(1): 47-79.
23. Nyvad BE, Kilian MO. Microbiology of the early colonization of human enamel and root surfaces in vivo. Scandinavian journal of dental research, 1987; 1, 95(5): 369-80.
24. Zijnge V, van Leeuwen MB, Degener JE, Abbas F, Thurnheer T, Gmür R, M. Harmsen HJ. Oral biofilm architecture on natural teeth. PloS one, 2010; 24, 5(2): e9321.

25. Socransky SS. Microbiology of periodontal disease—present status and future considerations. Journal of periodontology, 1977; 48(9): 497-504.
26. Mylonakis E, Calderwood SB. Infective endocarditis in adults. New England Journal of Medicine, 2001; 1, 345(18): 1318-30.
27. da Silva RM, Caugant DA, Josefsen R, Tronstad L, Olsen I. Characterization of Streptococcus constellatus strains recovered from a brain abscess and periodontal pockets in an immunocompromised patient. Journal of periodontology, 2004; 75(12): 1720-3.
28. Schiff E, Pick N, Oliven A, Odeh M. Multiple liver abscesses after dental treatment. Journal of clinical gastroenterology, 2003; 1, 36(4): 369-71.
29. Wescombe PA, Heng NC, Burton JP, Chilcott CN, Tagg JR. Streptococcal bacteriocins and the case for Streptococcus salivarius as model oral probiotics. Future microbiology, 2009; 4(7): 819-35.
30. Burton JP, Chilcott CN, Moore CJ, Speiser G, Tagg JR. A preliminary study of the effect of probiotic Streptococcus salivarius K12 on oral malodour parameters. Journal of applied microbiology, 2006; 100(4): 754-64.
31. Nisha S, Samyuktha GS, Shashikumar P, Chandra S. Periodontal disease–Historical and contemporary hypothesis: A review. SRM Journal of Research in Dental Sciences, 2017; 1, 8(3): 121.
32. Bordenstein SR, Theis KR. Host biology in light of the microbiome: ten principles of holobionts and hologenomes. PLoS biology, 2015; 18, 13(8): e1002226.
33. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature, 2007; 449(7164): 804-10.
34. Cho I, Blaser MJ. The human microbiome: at the interface of health and disease. Nature Reviews Genetics, 2012; 13(4): 260-70.
35. Blaser MJ. Who are we? Indigenous microbes and the ecology of human diseases. EMBO reports, 2006; 7(10): 956-60.
36. Rylev M, Kilian M. Prevalence and distribution of principal periodontal pathogens worldwide. Journal of Clinical Periodontology, 2008; 35: 346-61.
37. Gillings MR, Paulsen IT, Tetu SG. Ecology and evolution of the human microbiota: fire, farming and antibiotics. Genes, 2015; 6(3): 841-57.
38. Costalonga M, Herzberg MC. The oral microbiome and the immunobiology of periodontal disease and caries. Immunology letters, 2014; 1, 162(2): 22-38.
39. Marsh PD. Controlling the oral biofilm with antimicrobials. Journal of dentistry, 2010; 1, 38: S11-5.

40. Relman DA. The human microbiome: ecosystem resilience and health. Nutrition reviews, 2012; 1, 70(1): S2-9.
41. Relman DA. The human microbiome and the future practice of medicine. Jama, 2015; 15, 314(11): 1127-8.
42. Han YW, Wang X. Mobile microbiome: oral bacteria in extra-oral infections and inflammation. J Dent Res, 2013; 92: 485–91.
43. Kilian M. The oral microbiome - friend or foe?. Eur J Oral Sci, 2018; 126(1): 5–12.
44. Sälzer S, Graetz C, Dörfer CE, Slot DE, Van der Weijden FA. Contemporary practices for mechanical oral hygiene to prevent periodontal disease. Periodontol, 2000, 2020; 84: 35–44.
45. Chapple ILC, Van der Weijden F, Doerfer C, Herrera D, Shapira L, Polak D, et al. Primary prevention of periodontitis: managing gingivitis. J Clin Periodontol, 2015; 42(l 16): S71–6.
46. Joshi S, Suominen AL, Knuuttila M, Bernabé E. Toothbrushing behaviour and periodontal pocketing: an 11-year longitudinal study. J Clin Periodontol, 2018; 45: 196–203
47. Zhang M, Jiang Z, Li D, Jiang D, Wu Y, Ren H, et al. Oral antibiotic treatment induces skin microbiota dysbiosis and influences wound healing. Microb Ecol, 2015; 69: 415–21.
48. Jin Y, Wu Y, Zeng Z, Jin C, Wu S, Wang Y, et al. From the cover: exposure to oral antibiotics induces gut microbiota dysbiosis associated with lipid metabolism dysfunction and low-grade inflammation in mice. Toxicol Sci, 2016; 154: 140–52.
49. Shin JM, Ateia I, Paulus JR, Liu H, Fenno JC, Rickard AH, et al. Antimicrobial nisin acts against saliva derived multi-species biofilms without cytotoxicity to human oral cells. Front Microbiol, 2015; 6: 617.
50. Altman H, Steinberg D, Porat Y, Mor A, Fridman D, Friedman M, et al. In vitro assessment of antimicrobial peptides as potential agents against several oral bacteria. J Antimicrob Chemother, 2006; 58: 198–201.
51. Sol A, Ginesin O, Chaushu S, Karra L, Coppenhagen-Glazer S, Ginsburg I, et al. LL-37 opsonizes and inhibits biofilm formation of Aggregatibacter actinomycetemcomitans at subbactericidal concentrations. Infect Immun, 2013; 81: 3577–85.
52. Joo H-S, Fu C-I, Otto M. Bacterial strategies of resistance to antimicrobial peptides. Philos Trans R Soc Lond B Biol Sci, 2016; 37.
53. Makowski M, Silva ÍC, Pais do Amaral C, Gonçalves S, Santos NC. Advances in lipid and metal nanoparticles for antimicrobial peptide delivery. Pharmaceutics, 2019; 11.

54. Radaic A, de Jesus MB. Solid lipid nanoparticles release DNA upon endosomal acidification in human embryonic kidney cells. Nanotechnology, 2018; 29: 315102.
55. Jiao Y, Tay FR, Niu L-N, Chen J-H. Advancing antimicrobial strategies for managing oral biofilm infections. Int J Oral Sci, 2019; 11: 28.
56. Nascimento MM. Potential uses of arginine in dentistry. Adv Dent Res, 2018; 29: 98–103.
57. Agnello M, Cen L, Tran NC, Shi W, McLean JS, He X. Arginine improves ph homeostasis via metabolism and microbiome modulation. J Dent Res, 2017; 96: 924–30.
58. Rosier BT, Buetas E, Moya-Gonzalvez EM, Artacho A, Mira A. Nitrate as a potential prebiotic for the oral microbiome. Sci Rep, 2020; 10: 12895.
59. Doran A, Kneist S, Verran J. Ecological control: In vitro inhibition of anaerobic bacteria by oral streptococci. Microb Ecol Health Dis, 2004; 16: 23–7.
60. Radaic A, Ye C, Parks B, Gao L, Kuraji R, Malone E, et al. Modulation of pathogenic oral biofilms towards health with nisin probiotic. J Oral Microbiol, 2020; 12: 1809302.
61. Nadell CD, Drescher K, Wingreen NS, Bassler BL. Extracellular matrix structure governs invasion resistance in bacterial biofilms. ISME J, 2015; 9: 1700–9.
62. Ribbe J, Baker AE, Euler S, O'Toole GA, Maier B Role of Cyclic Di-GMP and Exopolysaccharide in Type IV Pilus Dynamics. J Bacteriol, 2017; 199.
63. Preshaw PM, Hefti AF, Jepsen S, Etienne D, Walker C, Bradshaw MH. Subantimicrobial dose doxycycline as adjunctive treatment for periodontitis. A review. J Clin Periodontol, 2004; 31: 697–707.
64. Cavagni J, Muniz FWMG, Rösing CK. The effect of inflammatory response modulator agents on gingivitis and periodontitis. RGO, Rev Gaúch Odontol, 2016; 64: 312–9.