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Review Article

Oral microbial habitat a dynamic entity

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ABSTRACT

Oral microbial habitat is composed of wide variety of species. These species play a significant role in maintaining the well being of the oral cavity by contributing in various ways. However the proper functioning of these oral microbes can be detrimental for the human oral cavity if the conditions are not suitable such as redox potential (Eh), pH of a site, the activity of the host defenses, and the presence of antimicrobial agents. The oral microbial community represents the best-characterized group associated with the human host. There are strong correlations between the qualitative composition of the oral microbiota and clinically healthy or diseased states. Amongst the bacteria of more than 700 species now identified within the human oral microbiota, it is the streptococci that are numerically predominant. Interactions between mucosal surfaces and microbial microbiota are key to host defense, health, and disease. These surfaces are exposed to high numbers of microbes and must be capable of distinguishing between those that are beneficial or avirulent and those that will invade and cause disease. Our understanding of the mechanisms involved in these discriminatory processes has recently begun to expand as new studies bring to light the importance of epithelial cells and novel immune cell subsets such as T(h)17 T cells in these processes. In this review article we have tried to find out the factors responsible for maintaining oral microbial habitat intact and the reasons which cause changes in its composition.

Keywords: *Human oral micro biome, Metagenomics, Anaerobiosis, Probiotics, Salivary micro biome*

INTRODUCTION

The characteristics of mouth are ecologically distinct from all other surfaces of the body and determine the types of microbes that are able to persist, so that not all of the microorganisms that enter the mouth are able to colonize.[1] Moreover, distinct habitat exists even within the mouth, each of which will support the growth of a particular microbial community because of their precise biological features. Habitats with obviously different ecological conditions include mucosal surfaces (such as lips, cheeks, palate, tongue) and teeth. The properties of mouth as a microbial habitat are dynamic and will change during the life of an individual.[1] Initially during the first few months of life the mouth consists only of mucosal surfaces for microbial colonization but later on eruption of teeth provides a unique, hard non-shedding surface which enables much larger masses of microorganisms (dental plaque) to accumulate as biofilms. The gingival crevicular fluid produced in the gingival sulcus provides additional nutrients for sub-gingival microorganism. The ecology of mouth will change over time due to eruption or extraction of teeth, the insertion of dentures, placement of orthodontics bands and any dental treatment including scaling and restoration. Temporary fluctuations in the stability of the oral ecosystem may be induced by the frequency and type of food ingested, variation in saliva flow and courses of antibiotic therapy.[1]

The human oral micro biome is complex in nature. It is composed of bacteria and fungi. It has been found that

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around 700 species of bacteria are found in the oral cavity, a large number of which are still uncultivable and needs to be identified.[2] Besides the bacteria, fungi also constitute a major population of oral micro biome called as oral mycobiome. The oral cavity contains 74 cultivable and 11 non-cultivable fungal genera.[3] The saliva also contains microbes which are associated with the epithelium of the oral mucosa. These microbes are exfoliated with the epithelium and found in the saliva.[4]

The microbial communities are bound to impact the health of individual humans and a better understanding of their dynamic complexity may contribute to the next level in medical diagnostic tools. Ideally, this should also lead to more specific treatment, by providing the potential to manipulate the micro biome to optimize personal health. These goals gave rise to the Human Micro biome Project, which aims to identify a core human micro biome, a common set of commensal species that can be identified as healthy micro biota.[5]

Similar to other biological sciences, microbiology has progressed gradually at various stages of "reductionism" and "holism". It has a long history that the microbiologist adapted reduction approach to study the complexities of microbiological communities by analyzing bacteria species separately.

Smaller components were analyzed to understand the whole organism which became the characteristic of industrial and scientific revolutions.

Today Microbiologist are working on global gene regulation, metagenomics and biofilms. The knowledge acquired from these systems is changing the understanding of microbial physiology and our ability to diagnose/ treat microbial infections which will have great impact on oral microbiology as well.[6]

Recent study indicated that, other than the host habitat effect, an existing microbial community could impose a selective pressure on incoming foreign bacterial species independent of host-mediated selection ("community selection" effect).[7]

BIOFILMS AND ORAL MICRO BIOTA

The oral microbial flora comprises one of the most diverse human-associated biofilms. Its development is heavily influenced by oral streptococci, which are considered the main group of early colonizers. Their initial attachment determines the composition of later colonizers in the oral biofilm and impacts the health or disease status of the host. Thus, the role of streptococci in the development of oral diseases is the best described in the context of bacterial ecology, which itself is further influenced by interactions with host epithelial cells, the immune system, and salivary components.[8]

Traditionally it was considered that bacteria occur in homogeneous planktonic environment but nowadays it is a determined fact that bacteria in natural environment occur in biofilms.[9] The relationship between host and microbial community has been studied in detail and it has been established that this relationship starts by the time one is born and last for lifetime. There is an association found between the oral microbes and the host and in order to be healthy there should be a harmonious relation between the two entities. Understanding the microbial communities that drive sickness or health is a key to combating human oral diseases.[10] In recent years, the development of in vitro and in vivo biofilm methodologies to study sessile populations have demonstrated that there are many physiologic and molecular differences between planktonic and surface-bound bacteria, suggesting that the organisms can acquire a "biofilm phenotype".[11]

The oral cavity is lined by mucous membrane which is continuously being desquamated. The lining of oral cavity can be composed of keratinized or non-keratinized type of epithelium. The tongue acts as a reservoir of gram-negative anaerobes that are implicated in the etiology of periodontal diseases and malodor.[1] The role of probiotics is relatively new in dentistry. It is being investigated that developing microbes of oral origin as specific providers of probiotic solution for oral diseases is more logical. Attempts have been made to develop streptococcal species as potential probiotics.[12] Evidence now suggests that probiotics may function not only by direct inhibition of, or enhanced competition with, pathogenic microorganisms, but also by more subtle mechanisms including modulation of the mucosal immune system. Similarly, prebiotics could promote the growth of beneficial microorganisms that comprise part of the resident microbiota.[13] Sub-gingival community of microbes still needs to be clarified on the basis of main metabolic processes that regulate these microbes, species identification, interaction of microbes, signaling events that leads to community maturation, and relation between microbes and host. Clinical studies that evaluate community structure need to be coupled with biologically relevant models that allow evaluation of ecological determinants of sub-gingival biofilm maturation.[14] Among the several factors determining the dynamics and virulence of the oral microbial flora, metabolic processes represent a ubiquitous key component. Many of the interactions between different species are mediated by metabolic processes, such as the competition for common resources, the exchange of nutrients, and the chemical communication involved in quorum sensing and biofilm formation. In addition, metabolites are often involved in shaping the relationship between microbes and host cells, from the proinflammatory role of lipopolysaccharides (LPS) present

in the bacterial outer membrane to the cytotoxic effect of organic acids secreted as catabolic by-products.[15] A predominant anaerobic species in the sub-gingival biofilm is *Fusobacterium nucleatum* that is prevalent in mature plaque in both health and disease, and thus is considered an opportunistic pathogen. The presence of *Streptococcus gordonii* and *F. nucleatum* favors subsequent colonization by more pathogenic organisms, such as *Porphyromonas gingivalis*, which play a role in the initiation and progression of chronic periodontitis. Another pathogen is *Aggregatibacter actinomycetemcomitans*, a causal agent of the clinically distinct localized aggressive periodontitis (LAP). Nevertheless, many recognized pathogens are frequently present in healthy individuals, and disease ensues when there is a disruption of the normally balanced host−microbe interaction.[16] Because we and our micro biome have evolved together, we rationalize that the equilibrium between us results in health, and disruption of the homeostasis leads to disease. The constituent microbes in a micro biome do not act alone and alliances have evolved with each other, and with host proteins and cells. Recently, Brown & Whitely examined the metabolic relationship between the oral bacteria *Aggregatibacter actinomycetemcomitans* (*Aa*) and streptococci. Because they inhabit the same environmental niche, the gingival pocket, it was reasoned that *Aa* must derive benefits from this coexistence. Streptococci efficiently produce lactic acid from 6-carbon sugars and dietary sucrose, and it was established that *Aa* preferentially utilizes lactic acid as a carbon source over glucose, fructose, mannose, even at the cost of a lowered growth rate.[17] The teeth act as a non-shedding site for microbial colonization. Teeth and dentures allow accumulation of bacteria and dental plaque which can lead to dental caries and periodontal disease. In disease there is a change in the micro flora of biofilm. Teeth do not provide a uniform habitat but posses several distinct surfaces, each of which is optimal for colonization and growth by different population of microorganism. The most diverse population of bacteria is found in the approximal surfaces and gingival crevice due to the presence of gingival crevicular fluid which is nutritionally rich medium. The pits and fissure surfaces of the teeth are sites of largest microbial communities and, in general, the most disease.[1]

The mouth is kept moist by saliva which forms a thin film about 0.1 mm deep all over the internal surface of oral cavity. Saliva has an important role in maintaining the well being of teeth by flushing away the microbes and by neutralizing the acid produced by bacteria as a result of carbohydrate metabolism. Bicarbonate, phosphates, peptides and proteins are involved. Antimicrobial factors such as lysozymes, lactoferrin, and sialoperoxidase are present in saliva .These factors help in controlling bacterial and fungal colonization of the oral cavity. Immunoglobulin such as IgA, IgG and IgM are important in controlling viral attack on the oral cavity. A range of peptides with antimicrobial activity, including histidine-rich polypeptides, cystatins and defensins are also present in saliva.[1] Salivary micro biomes distinguish caries active from healthy human populations. The salivary micro biota plays an important role in preventing colonization and integration of foreign or pathogenic bacterial species such as *Pseudomonas aeruginosa*, an opportunistic bacterial pathogen into forming salivary biofilm.[18] The role of salivary micro biomes in causing diseases of the oral cavity is well established, however little is known about diversity of salivary micro biome. It has also been investigated that there is important interaction found between salivary micro biome and micro biome of intestinal tract.[19] The human saliva has been used recently as a source of identifying bacterial, viral and systemic diseases.[20] In various genetic studies, saliva samples have been used to identify DNA as well.[21] In one study a relationship was drawn between salivary counts of bacteria found in Oral Squamous Cell Carcinoma (OSCC) positive individuals and healthy individuals. It was found that there is a high salivary count of *Capnocytophaga gingivalis*, *Prevotella melaninogenica* and *Streptococcus mitis* in patients suffering from OSCC and they can used as diagnostic indicators of OSCC.[22]

Direct pyrosequencing of eight samples with different oral health status that *Streptococcus mutans* does not dominate caries rather it is a polymicrobial disease. Individuals who have never suffered from caries contain genes for antimicrobial peptides and quorum sensing. They also do not contain mutans streptococci but displayed high population of other bacteria.[23] The mutans streptococci are most important group of bacteria associated with dental caries. There are about 20% of the total bacterial species in the oral cavity.[24] It has been found that close proximity of bacterial cells in the biofilm appears to be an excellent environment for horizontal gene transfer, which can lead to spread of antibiotic resistance genes amongst the biofilm inhabitants.[25] It has been investigated that individuals who are consuming smokeless tobacco are more prone for periodontal diseases and less susceptible to caries because of lesser population of lactobacilli.[26] The composition and gene expression of resident micro biota is decided by host environment. Any change in the host environment will lead to disruption of normal symbiotic relationship between host and its resident microbes.[27]

FACTORS AFFECTING GROWTH OF ORAL MICROORGANISMS

A neutral pH is required for the growth of most bacteria in the oral cavity. Extremes of pH are not suitable for the

growth of microorganisms. The main source of maintaining the pH of oral cavity is the saliva, however it has been found that different areas of oral cavity have different pHs. The pH of plaque is also sensitive to the pH of saliva. After consumption of acidic diet the pH of plaque also drops to around 5.0 due to formation of lactic acid by carbohydrate metabolism. This drop in pH slowly recovers with the passage of time. This drop in pH is lethal for most plaque bacteria. The pH of healthy gingival crevice is around 6.9 which increase to about 7.2–7.4 during disease. This pattern of pH change can lead to alter gene expression in sub-gingival bacteria which favors the growth of pathogenic anaerobes such as *P. gingivalis* that have a pH optimum for growth of around 7.5.[1] It is found that the resting pH in plaque results from a delicate balance between alkali and acid generation, which is in turn dependent both on the bacterial composition of the plaque and on the supply of substrates and buffers from and metabolite clearance into flowing oral fluid. In vivo the resting pH will vary with site-specific changing saliva flows. Urea continuously supplied at concentrations normal for saliva and gingival crevicular fluid can raise the resting pH of microcosm plaque by an amount that in vivo would probably be significant in reducing dental caries.[28] Data indicated that cotinine (substance found in cigarettes) may interfere with *P. gingivalis* ability to associate and invade the epithelial cells. Further studies are needed to investigate whether oral cells might be more susceptible to be colonized by *P. gingivalis* in smokers.[29]

The human mouth is kept at a relatively stable oral temperature i.e. 35–36 °C. This temperature is vital for the growth of various microorganisms. It has been found that periodontal pockets with active disease have a higher temperature i.e. around 39 °C compared with healthy sites.[1] Irritant chemicals are present, which have different effects in the temperature regulation of oral cavity. In recent years, 6 thermo sensitive transient receptor potential (TRP) ion channels have been identified, several of which also respond to irritant chemicals. Experiment was conducted in order to investigate capsaicin, menthol, mustard oil, and cinnamaldehyde because these chemicals have been studied most in relation to their effects on thermo sensitive TRP channels. Capsaicin, mustard oil, and cinnamaldehyde enhanced lingual heat pain elicited by a 49 °C stimulus. Mustard oil and cinnamaldehyde weakly enhanced lingual cold pain (9.5 °C), whereas capsaicin had no effect. Menthol significantly enhanced cold pain and weakly reduced heat pain.[30] Bioactive glasses have been tested as bone substitutes in different clinical situations. In an aqueous environment Ca^{2+}, Na^{+}, PO_4^{3-} and Si^{4+} are released from the glass, resulting in a rise in pH and in osmotic pressure in its vicinity. Since these are factors that potentially influence the viability of oral microorganisms at the dentogingival margin, studies were done on the effects of bioactive glass S53P4 on the oral microorganisms *Actinobacillus actinomycetemcomitans*, *P. gingivalis*, *Actinomyces naeslundii*, *S. mutans*, and *Streptococcus sanguis*. Thus it was found that glasses have antimicrobial properties since they have inhibited the growth of oral microbes and therefore should be added to toothpastes as ingredient for antimicrobial property.[31]

It has been found that mouth can support a microbial community of great diversity and richness thus satisfies the requirements of many nutritionally demanding bacteria. The nutrients can be obtained from endogenous sources i.e. saliva containing (amino acids, peptides, proteins, glycoproteins, vitamins and gases) or exogenous i.e. food ingested periodically in the diet.[1] Diet has a significant influence on the development of bacterial and chemical composition of biofilms in the oral cavity. Studies in experimental animals have shown that feeding either glucose or sucrose diets or fasting has little effect on the initial stages of development of oral biofilms. However, diet can influence the proportions of different bacterial species later in biofilm development. Oral biofilms provide a sequestered habitat, where organisms are protected from removal by saliva and where interactions among cells generate a biofilm environment distinct from that of saliva.[32] Dietary sugars provide readily available substrates for the oral microorganisms, most of which depend on carbohydrates for energy sources. The metabolism of dietary sucrose by *S. sanguis* and *S. mutans* with the productions of acids and intracellular and extracellular polysaccharides has specific influence on the microbial composition, metabolic activities and mass of coronal plaque. The ready availability of dietary carbohydrates undoubtedly influences the micro flora of other parts of the oral cavity as well.[33]

Anaerobiosis is another important feature for the growth of microorganisms in the oral cavity. Although oral cavity is well-aerated environment but only few of microbes present in oral cavity are truly aerobic species. The majorities of organisms are either facultative anaerobes or obligate anaerobes.[1] Most of the oxygen in the oral cavity derives from saliva where it is in solution readily available to bacteria for metabolism. The oxygen level (PO_2) in the freshly secreted saliva is about 65 mmHg which reduces to about 35 mmHg only after 30–60 s. This reduction in oxygen level continues even more with the passage of time as plaque is formed. The major factor responsible for this depletion of oxygen level is bacteria present in the saliva which utilize oxygen for degrading carbohydrate and nitrogenous substrates. Evidently, progressive bacterial succession, PO_2 depletion, Eh lowering, emergence of gram-negative bacteria, oral putrefaction and oral malodor

go hand in hand.[1] One study provides evidence that an oxidizing agent such as H_2O_2 may affect the biology of *P. gingivalis*. Moreover, growth of some members of the oral micro flora can generate oxidizing and reducing conditions, and thus potentially influence the ecology of subgingival sites by affecting strictly anaerobic bacteria such as *P. gingivalis*.[34]

HOST DEFENSES

Host defenses play a major role in maintaining the well being of oral cavity. The primary function of the immune system of the mouth is to protect the teeth, jaws, gingiva and oral mucosa against infection. These host defenses vary in the different oral microenvironments or domains represented by the oral mucosa, saliva and gingival crevice.[35] The oral mucosa is composed of epithelial and lamina propria compartments which play a significant role in the oral immune system. The continuous exfoliation of epithelial cells prevents the colonization by oral microbes and thus prevents infections. The membrane coating granules secreted by the stratum granulosum also has its role in host defenses. Intraepithelial dendritic langerhans cells are peripheral antigen presenting cells which present the antigens complexed with MHC Class II to helper T cells. Cytokines secreted by different cells plays crucial role in maintaining oral immune system intact.[36]

Saliva has an important role to play in maintaining the oral hygiene of oral cavity. Not only it has flushing effects but it also has the antimicrobial constituents in it. Mucin, lysozyme, lactoferrin, salivary peroxidase and histidine rich proteins are components of innate immunity in saliva. Whereas IgA and IgG make up the humoral immunity element of saliva.[1]

Gingival crevicular fluid is a serum like fluid coming from the junctional epithelium of the gingiva. The GCF is usually flowing at a slow pace in healthy gingiva however in advance periodontal diseases it increases by 30 fold. The GCF significantly alters the ecology of gingival crevice by a number of ways i.e. by removing microbial cells, introduction of immune cells, and source of nutrient for resident microorganisms. The increased production of GCF is associated with increase in pH of periodontal pocket. Average pH of periodontal pocket is 6.9 which increases to 7.5 in diseased state. A number of enzymes can be detected in GCF which includes collagenase and elastase which are derived from phagocytic cells. They are responsible for the destruction of gingival tissues. Several of these enzymes are used as diagnostic markers of periodontal diseases.[1]

Mucosal epithelial cells play an integral role in innate immune defense by sensing signals from the external environment, generating various molecules to affect growth, development, function of other cells and maintaining the balance between health and disease. Mucosal epithelial cells express antimicrobial peptides, including the β-defensins, human β-defensin 1 (hBD-1) and hBD-2, as well as chemokines that attract monocytes and neutrophils and cytokines that activate the adaptive immune system. It is now recognized that the antimicrobial peptide hBD-2 also stimulates antigen-presenting dendritic cells that signal the adaptive immune system, in addition to its antimicrobial activity. Therefore, characterization of β-defensin regulation is essential for understanding the role of these peptides in protecting the host by activating both innate and adaptive immune systems and in contributing to the epithelial barrier to inflammatory disease processes.[37]

The components of acquired immunity are present in the mucosa in the form of langerhans cells, intraepithelial lymphocytes, IgA and IgG where they act as barrier to penetrating antigen. These components of immunity work in a synergistic manner and act together. For e.g. mucin or Ig A and salivary peroxidase.[1]

FUTURE CONSIDERATIONS

1. In general, oral antibacterial agents such as antibiotics are commonly used to treat oral bacterial infection. Traditional periodontal surgery is painful and time-consuming. In addition, bacterial resistance and toxicity of antibiotics have become a global pandemic and unavoidable. Recently, vaccines for dental caries and periodontal disease have been developed and applied. Moreover, the use of photodynamic therapy has become an alternative to antibiotic drugs.
2. The collection and sampling of saliva and its application as a diagnostic tool for the identification of bacterial, viral and systemic diseases. Usage of saliva samples as source of a DNA identification in future.
3. The number of products containing probiotics, viable bacteria with proven health benefits, entering the market is increasing. Traditionally, probiotics have been associated with gut health, and most clinical interest has been focused on their use for prevention or treatment of gastrointestinal infections and diseases; however, during the last decade several investigators have also suggested the use of probiotics for oral health purposes. *Streptococcus* species have been associated with development of probiotics.
4. Use of bioactive glasses like S53P4 in toothpastes because of antimicrobial properties.

CONCLUSION

There is a dynamic interaction between the oral environment and the composition and metabolism of the resident oral micro flora. A substantial change in a key environmental parameter that affects microbial growth can disrupt the natural balance of the micro flora and select for organisms that are potentially pathogenic. The host environment dictates the composition and gene expression of the resident microbiota. Changes in oral environmental conditions can disrupt the normal symbiotic relationship between the host and its resident microbes, and increase the risk of disease.

CONFLICTS OF INTEREST

All authors have none to declare.

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