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MICROBIAL IMBALANCE: A KEY TO PERIODONTITIS-A REVIEW

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ABSTRACT

The microbial etiology of periodontal disease has been in the focus of researchers since long. An emerging concept is the tight relationship between dysbiosis and disease. The increase in knowledge about alterations in microbial communities that reside within the host has made a strong impact not only on dental science, but also on immunology and microbiology as well as on our understanding of several diseases. Periodontitis is a biofilm-associated inflammatory disease of the periodontium and appears to be multifactorial in origin with multiple etiology, microbial factor contributing to disease. This paper entititles about the concept of, "Microbial Dysbiosis" and how this dysbiotic microbiota leads to Periodontitis, with the scope of traditional and emerging technologies for treating this disease.

KEYWORDS: Biofilm, Dysbiosis, Periodontitis.

KEY MESSAGES

Periodontal disease has been in focus by many researchers since long time. Mouth has been considered as a home for bacteria to survive. About 700 microbial species resides in the oral cavity with 200 present in one person. Periodontitis has been considered as a biofilm associated multifactorial inflammatory disease of the periodontium. It appears to have multiple aetiologies with microbial factor that contribute to the progression of disease. This

paper entitles about the microbial imbalance and how the disease associated biofilm contribute to the initiation and progression of periodontal disease i.e. Periodontitis and the scope of traditional and emerging technologies for treating this disease.

INTRODUCTION

The mouth is a habitat for bacteria and other microorganisms. It is evaluated that over 700 microorganisms reside in the oral cavity,[1] with 200 present in an one individual and about 50 present at any one site.[2] Oral cavity is an open growth system and removal of microbes and their nutrients. It offers diverse habitats where-in-different species of microorganisms can prosper. The aggregation of microbes on tooth surfaces has been commonly referred to as 'Plaque'. It represents a true biofilm consisting of a different variety of micro-organisms involved in a wide range of physical, metabolic and molecular interactions.[3]

Wilderer and Charaklis in 1989 described 'Biofilms' the relatively indefinable microbial community associated with a tooth surface or any other hard non-shedding material, randomly distributed in a shaped matrix or glycocalyx.[3] It has been suspected as the chief culprit in the etiopathogenesis of dental caries and periodontal disease. Bacteria grow in complex polymicrobial associations. Biofilms attached to biotic or abiotic surfaces. As a surface becomes colonized with single cells, the bacteria form microcolonies, secretes a sticky extracellular polymeric substance that helps the bacteria to adhere to the surface, and to each other. By the secretion of the extracellular polymeric substance, the biofilm matures by taking a distinctive architecture. This structure includes distinct regions of fast and slow growing cells, water channels that circulate metabolites, and the establishment of nutrient gradients. Such complex structural organization allows the biofilm to exhibit functional heterogeneity which allows biofilm to demonstrate tremendous metabolic and phenotypic flexibility.[1] Periodontal disease is one of the most common afflictions faced by the human beings. It is a biofilm-associated inflammatory disease of the periodontium, a disease affecting more than 30% of the humans, and is a major cause of tooth loss in the world.[4]

Periodontitis means "inflammation around the tooth" - it is subjected as a gum infection that damages the soft tissue and alveolar bone that supports the tooth. Microorganisms, such as bacteria, stick to the surface of the tooth and multiply - an overactive immune system reacts with inflammation. The exact etiology of periodontal disease is still an enigma. The primary microbial factor contributing to periodontitis is a shift in the content of the oral microflora, while the primary immunological factor is the destructive host inflammatory response.[4]

A decrease in oral hygiene builds home for oral biofilms on tooth surfaces and, if left untreated will progress to gingival inflammation and possibly to periodontitis. The differences in the host defence mechanisms, including anti-microbial proteins profiles, determine whether bacterial colonization progresses to manifest disease. It is indicated that dietary changes combined with poor oral hygiene can cause a shift in the composition of the oral bacteria.[5] Inadequate oral hygiene results in deposition of plaque which further leads to gingivitis and in later stage periodontitis. It is characterized by the breakdown of junctional epithelium, loss of connective tissue attachment, and alveolar bone resorption.[6]

There have been several techniques implicated clinically to treat periodontitis, but the most successful ones appear to address both the bacterial and inflammatory components of the condition. In this article will discuss about the possible role of bacteria leading to dysbiosis and causing periodontitis.

Biofilm microbiology

Oral biofilms play a major role in the etiology of periodontal diseases and health. Hypothesis regarding the mechanisms by which oral biofilms exert their pathogenic potential have evolved over time. Early speculation in which oral diseases were associated truely with the quantity of colonizing biofilms have been replaced by understanding the emergence of specific pathogenic microorganisms may lead to diseases.[7] A small sample of dental plaque contains, about 27 species of bacteria.[8] Biofilm can be considered as a dense compressed accumulations of cells, and is believe to be responsible for many of the novel properties of biofilm.[9]

The first description about biofilm was discussed earlier in 17th century, When, Anton Von Leeuwenhoek - the inventor of the Microscope,[10] discovered the approach of studying biofilms by direct microscopic observation when he reported on the diversity and high numbers of ‘animalcules’ present in ‘scrapings’ taken from human teeth.[9]

Bill Costerton in 1978 given the term “Biofilm”

Donlan and Costerton, in 2002, stated that biofilm is *“a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription.*”[10]

Biofilm behaviour may affect the properties of an organism in different ways. Firstly, by direct effect, the attachment of cells to a surface, triggers 'sensors' on the cell surface, and inducing the specific expression of a subset of genes. And secondly, by the growth environment within the biofilm may differ significantly in respect to key factors such as- pH, oxygen and nutrient concentration compared with planktonic culture. This may result in altered gene expression, and hence an altered phenotype, but as an indirect effect of growing in a biofilm.[9]

Quorum sensing, or cell density mediated gene expression involves the regulation of expression of specific genes through the accumulation of signalling compounds that mediate intercellular communication. Quorum sensing is thought to give biofilms their distinct properties.

In S. mutans, quorum sensing is mediated by competence stimulating peptide, wherein genes are responsible for multiple functions - biofilm formation, competence and acid tolerance. This structural and functional heterogeneity allows biofilms to establish formidable metabolic and phenotypic flexibility.[4] Biofilm related regulation of gene expression has been shown in certain bacteria. eg. Exposure of S. gordonii to saliva results in induction of genes that mediate host surface binding and coaggregation with P. gingivalis and Actinomyces. Similarly, genes encoding glucan and fructan synthesis are differentially regulated in Biofilm-associated S. mutans.[10]

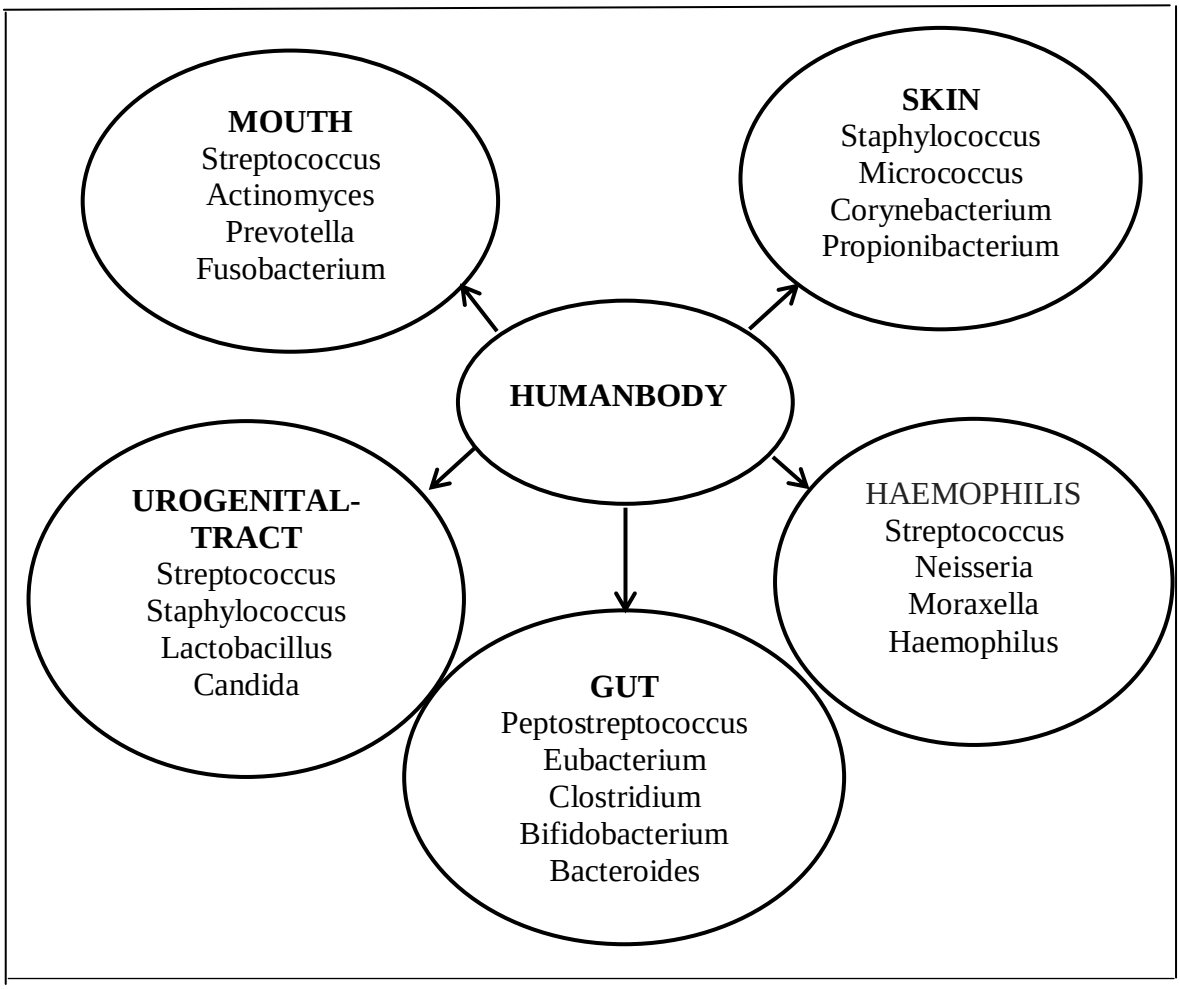

Fig.1. Distribution of resident oral micro-flora in various parts of human body.

Biofilm and oral health

Years back having a passive relationship with the host various researches' has demonstrated that the resident microflora plays a positive role in the normal development of the host. This resident microflora also plays an active role in the maintenance of the healthy state by contributing to the host defences and preventing colonisation by exogenous microorganisms. Disease can be a consequence of disruption of this resident microflora. The mouth is not a homogeneous environment for microbial colonisation. Distinct habitats exist, for example, the mucosal surfaces (such as the lips, cheek, palate, and tongue) and teeth which, because of their biological features, support the growth of a distinctive microbial community.[11]

In general, these natural microflora's live in harmony with humans and animals and, indeed, all get benefited from the association. Loss or perturbations of this resident microflora can lead to colonization by exogenous (and often pathogenic) microorganisms, thereby predisposing sites to disease. The microbial colonization of all enviromentally accessible

surfaces of the body begins at birth. Such surfaces are exposed to a wide range of microorganisms derived from the environment and from other persons. Each surface, however, because of its physical and biological properties, is suitable for colonization by only a proportion of these microbes. This results in the acquisition, selection and natural development of a diverse but characteristic microflora at distinct sites.[9]

Bacteria associated with periodontal health include, various facultative gram-positive bacteria such as Streptococcus *Sanguis, Streptococcus Mitis, Actinomyces Naeslundii and Actinomyces Viscou*s. Whereas, anaerobic gram negative species associated with periodontal disease include, *Prevotella Intermedia, Porphyromonas Gingivalis and Aggregatibacter Actinomycetemcomitans*. Healthy periodontal tissue is maintained through symbiosis between the host immune system and bacterial plaques. This symbiosis is dependent on the capability of the host immune system and the quality and quantity of the bacterial plaque.[12] It is perfectly clear that the health of the host is inextricably tied to the nature of the oral microflora.[11]

Table.1. Principal Bacterial Genera Found In the Healthy Oral Cavity[11]

Gram Positive		**Gram Negative**	
Cocci	***Rods***	**Cocci**	**Rods**
Abiotrophia *Peptostreptococcus* *Streptococcus* *Stomatococcus*	*Actinomyces* *Bifidobacterium* *Corynebacterium* *Eubacterium* *Lactobacillus* *Propionibacterium* *Rothia*	*Moraxella* *Veillonella*	*Campylobacter* *Capnocytophaga* *Desulfobacter* *Desulfovibrio* *Eikenella* *Fusobacterium* *Haemophilus* *Leptotrichia* *Prevotella* *Selenomonas* *Simonsiella* *Treponema* *Wolinella*

A number of other genera are isolated infrequently and/or in low numbers in health, but increase markedly in disease (e.g. Actinobacillus, Porphyromonas).

Table.2. Distinct Microbial Habitats within the Healthy Mouth [11]

Habitat	Predominant Microbial Groups	Comments
Lips, palate, cheek	Streptococci Neisseria, Veillonella	desquamation restricts biomass; surfaces have distinct cell types; *Candida* act as opportunistic pathogens; staphylococci may be present.
Tongue	Streptococci, Actinomyces, Veillonella, Obligate Anaerobes Simonsiella	highly papillated surface—reservoir for anaerobes
Teeth	Streptococci, Actinomyces, Veillonella, Eubacterium, Obligate Anaerobes, Spirochaetes, Haemophili	Non-shedding surfaces—promote biofilm formation (dental plaque). Distinct surfaces for colonisation (fissures, approximal, and gingival crevice) which support a characteristic flora due to their intrinsic biological properties. Teeth harbour the most diverse oral microbial communities.

Dysbiosis Leading to Periodontitis

With the knowledge that the human oral micro-flora is complex the role it plays in contributing to oral health or disease is quite daunting and equally complex. In some cases there are good bacteria that contribute to health and bad bacteria that contribute to disease.[12] Just as entire microbial communities can be associated with health; recent research also indicates that entire microbial communities can be associated with disease.[4] As more than one bacterial species may be associated with a particular disease, the historic concept of "one germ, one disease" may need modification. The lack of a beneficial organism in a biofilm may be just as important as the presence of a pathogen in the contribution to disease, a hypothesis has been developed linking certain diseases to a shift of the local microbiota so called "*Microbial Shift*" hypothesis.[1] Microbial shift, more commonly known as 'Dysbiosis' or 'Dysbacteriosis', 'Microbial Imbalance'. It is the alterations in microbial communities that reside within the host have made a strong impact not only on dental science, but also in immunology and microbiology as well as on our understanding of several diseases. Dysbiosis is largely dependent on cooperative and competitive interactions among oral microbes during the formation of the pathogenic biofilm community at gingival sites.[13] This cite to the concept that some diseases are due to a decrease in the number of beneficial symbionts, and an increase in the number of pathogens.[14] The development of oral dysbiosis, gradually changes the symbiotic host–microbe relationship to a pathogenic one. During this period, the oral health of the host deteriorates until a state of clinical disease occurs.

Simultaneously, a succession of microbial complexes develops. The first such Complex, been associated with disease is the so‑called socransky's orange complex, which consists of gram‑negative anaerobic species such as Prevotella Intermedia, P. Nigrescens, P. micros, and Fusobacterium Nucleatum. As the disease progresses, the microbiota shifts, to the so‑called red complex, which consists of the periodontal pathogens P. Gingivalis, Tannerella Forsythia, and Treponema Denticola,[1] this indicates that in oral cavity dysbiosis leads to periodontitis. Changes in the relative abundance of individual components of the microbiota compared to their abundancies in health can lead to alterations in the host-microbial crosstalk sufficient to initiate inflammatory disease.[15] Riep et al., established that periodontal pathogens for example, *P. gingivalis* and *T. forsythia* could also be isolated from healthy controls. Kumar et al., contradicted the existing pattern when they observed that the gram-negative bacterium *Veillonella* was associated with periodontal health, while the gram-positive anaerobe *Filifactor alocis* was associated with the disease. Even more daunting is the likelihood that other pathogens associated with Periodontitis have never been isolated.

Uncultivated clones from the phyla *Deferribacteres, Bacteroidetes* etc. was also investigated by cloning and sequencing 16S rRNA genes, were associated with chronic periodontitis. Further, it has also been proposed that herpesvirus species, Epstein-Barr virus and human cytomegalovirus, act synergistically with bacteria in the pathogenesis of periodontitis.[4] Molecular approaches have been developed to compare the diversity of oral microbial communities from different sites in health and disease. These approaches include microbial community profiling using denaturing gradient gel electrophoresis (DGGE).[9] Total genomic DNA is extracted from samples, amplified by PCR using universal primers for bacterial 16S rRNA genes, and products resolved on polyacrylamide gels with a denaturing gradient. These culture-independent approaches are radically changing our perception of the diversity of the resident oral microflora in health and disease. DNA microarray chips with the ability to detect each component or specific subsets of the resident oral microflora, enabling clinicians to rapidly screen for the presence or absence of micro-organisms may develop in future, this may lead to the promise of improved diagnostic and treatment opportunities in future.

The host–microbe homeostasis can also be agitated by congenital or acquired host immunodeficiencies, systemic diseases such as diabetes, obesity, environmental factors, such as smoking, diet, and stress, and epigenetic modifications in response to environmental changes, which alone or in combination can contribute to the homeostatic balance. Aging is

also a major factor associated with a disturbance in immune regulation and function, which in turn can predispose to increased susceptibility to periodontitis.[16]

Re-Thinking Koch's Postulates

Periodontitis affects the majority of adults, whereas an estimated range of 10–15% develops severe periodontitis, which increases the patients' risk for atherosclerosis, aspiration pneumonia, diabetes, adverse pregnancy outcomes, and rheumatoid arthritis (Genco and Van Dyke, 2010; Lalla and Papapanou, 2011; Lundberg et al., 2010; Pihlstrom, et al., 2005; Tonetti et al., 2007; Xiong et al., 2006).[15] The primary goal is determining which of the 700 microbial species or more found in the oral cavity is/are responsible. While Koch's postulates served medical microbiologists for determining the explanation of many human diseases, their limitations have been brought to light in the study of chronic infections. However, to resolve this, two different concept has been postulated. The first is the concept of a "pathogenic microbial community"[17] and the second concept is "Hill's criteria of causality" (Table-3).

Table.3. Hill's Criteria Of Causality Applied To Periodontitis

(Adapted from Lowe et al.[18])

	Questions	**Questions Specific For Periodontitis**
Biological plausablity	Does a hypothesized effect make sense in the current biological knowledge?	Could dysbiosis be associated with chronic human disease?
Dose response Natural Interventional	Does disease occur more in individuals colder to the source? Does disease recede with antimicrobial treatment?	Are high levels of pathogenic bacteria associated with Periodontitis? Does therapy reduce the number of suspected agents and improve the oral health of the patient?
Strength of association	What is the risk of disease after infection?	Do most patients who have these pathogens develop periodontitis? Are most patients with periodontitis colonized with the same bacterial pathogens (eg: Red complex bacteria)?
Specificity of association	Is the agent associated with only one clinical syndrome?	Do "red complex" bacteria cause disease other than periodontitis?
Consistency	Do studies by different groups consistently arrive at the same findings?	Do most laboratories agree upon which bacterial species are associated with periodontitis?
Temporality	Does infection precede disease?	Can infection with suspected pathogens precede the development of periodontitis? Can these pathogens cause periodontal disease in animal models?

Siqueira and Rocas in their review explained first concept. Where the researchers has suggested that enormous variation in the composition of the oral microflora has been observed even between patients with the same disease, it is best to approach the etiology of periodontitis from a *"community‐as‐pathogen"* model, as opposed to the traditional *"single‐pathogen"* model. This approach could be supported by the use of functional gene arrays. Environmental microbiology, just like oral microbiology, copes with the presence of bacteria that cannot be cultivated. In the study of oral microbiology, bacterial communities from healthy and diseased periodontal samples could be quarantined for *"pathogenic genes"* using functional gene arrays, and correlations between the presence of pathogenic genes and periodontitis could be established. Since, in *Hill's criteria* of causality. Because of the austere nature of Koch's postulates, it is difficult or impossible to satisfy them for many chronic conditions. The causal link between infection with Helicobacter pylori and peptic ulcer disease is almost universally accepted not because it fulfills Koch's postulates, but because it fulfills Hill's criteria of causality.[18]

In order for causation to be established, Hill's criteria required most of the following conditions are to be fulfilled:

- Biological plausibility,
- Dose response,
- Strength of association,
- Specificity of association,
- Consistency, and
- Temporality.

Given the current snag, it appears that the etiology of periodontitis might be more readily established if current research combines the pathogenic microbial community concept with Hill's criteria of causality (Table.3).

Although, its difficult in defining the precise etiology, of periodontitis. Substantially, clinically healthy periodontal tissue maintains a highly ordered, mild state of inflammation. Such as, E‐selectin expression.[19] and an established interleukin‐8 gradient,[20] constantly guide neutrophils toward the junctional epithelium that borders the normal oral microflora, which is thought to provide the stimulus for this mild inflammatory response.[21] and on the other hand; clinically diseased periodontal tissue exhibits a marked histopathology.

Expression of inflammatory molecules normally present in small amounts (such as Toll‐like receptor 2) is greatly increased,[12] other inflammatory molecules (such as Toll‐like receptor 4) are also expressed,[12,21] and the highly ordered state of mild inflammation is replaced by a disordered state of severe inflammation. Thus, it is proposed that the shift from a symbiotic to a dysbiotic micro-flora, the pathogenic community triggers the potent host inflammatory response that contributes to the tissue destruction and alveolar bone loss that are characteristic features of periodontitis.[21]

As Periodontitis is a multifactorial disease and is associated with a multiple etiological factors choosing an appropriate treatment option is quite daunting. Despite this, recent advances show tremendous potential to help patients suffering from periodontitis. Various surgical and non-surgical therapies can be performed for patient's maintenance. Gold standard treatment of choice Scaling and root planing is the primary therapy for most clinicians, in treatment of disease. However, scaling and root planning alone often does not produce the clinical outcomes desired in severe cases. Because of the underlying microbial basis of periodontitis, it is good to use antimicrobial therapy as an adjunct in treatment to prevent disease. Different treatment modalities such as the use of antiseptics, Host modulation therapy, Photo Dynamic therapy as an adjunct to scaling and root planning and the use of probiotic therapy is been added as a recent advances in treatment of a disease.

CONCLUSION

As periodontitis is the multifactorial disease its necessary to maintain the harmony between the good and bad bacteria. The transition to periodontitis requires both a dysbiotic microbiota and a susceptible host. However, despite great advances in our knowledge of the underlying microbial basis of the disease, the fact still remains that periodontitis has multiple etiologies which have yet to be fully understood. With dysbiosis, genetic, immunologic and enviromental factors must also be investigated in order for clinicians and researchers to fully understand disease progression. For successful treatment, it is important that the underlying cause of the disease can be identified and addressed. The goal of clinicians should be to find the best treatment. The most successful treatments will need to attack the integrity of the periodontal biofilm and suppress the destructive host inflammatory response. From a clinical perspective, treatment should be simple, affordable, and able to confer a clinically relevant benefit to the patient.

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